Appendix (1) Sequence of PCR product of amplification of 16S rDNA by two sets of primer (B16SF and B16SR) of the *Bacillus* isolate B1.

ATGGGAGCTTGCTCCCTGATGTTAGCGGCGGACGGGTGAGTAACACGT
GGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGGGGCTAA
TACCGGATGGTTGTTTGAACCGCATGGTTCAAACATAAAAGGTGGCTTC
GGCTACCACTTACAGATGGACCCGCGGCGCATTAGCTAGTTGGTGAGG
TAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATC
GGCCACACTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCA
GTGGTCATAGCTGTTTCCTGAA

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

GCATGGGAGCTTGCTCCCTGATGTTAGCGGCGGACGGGTGAGTAACAC
GTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGGGGCT
AATACCGGATGCTTGTTTGAACCGCATGGTTCAAACATAAAAGGTGGC
TTCGGCTACCACTTACAGATGGACCCGCGGCGCATTAGCTAGTTGGTGA
GGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGA
TCGGCCACACTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAG
CAGTGGTCATAGCTGTTTCCTGA

Appendix (3) Sequence of PCR products of amplification of 16S rDNA by two sets of primer (B16SF and B16SR) of the *Bacillus* isolate B3.

GAATGGGAGCTTGCTCCCTGATGTTAGCGGCGGACGGGTGAGTAACAC
GTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGGGGCT
AATACCGGATGGTTGTTTGAACCGCATGGTTCAAACATAAAAGGTGGT
TCGGCTACCACTTACAGATGGACCCGCGGCGCATTAGCTAGTTGGTGA
GGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGA
TCGGCCACACTGGGACTGAAACACGGCCCAAACTCCTACGGGAGGCAG
CAGTGGTCATAGCTGTTTCCTGA

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Appendix (4) Sequence of PCR products of amplification of 16S rDNA by two sets of primer (B16SF and B16SR) of the *Bacillus* isolate B4.

GAATGGGAGCTTGCTCCCTGATGTTAGCGGCGGACGGGTGAGTAACAC
GTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGGGGCT
AATACCGGATGGTTGTTTGAACCGCATGGTTCAAACATAAAAGGTGGC
TTCGGCTACCACTTACAGATGGACCCGCGGCGCATTAGCTAGTTGGTGA
GGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGA
TCGGCCACACTGGGACTGAAACACGGCCCAAACTCCTACGGGAGGCAG
CAGTGGTCATAGCTGTTTCCGGAA

Appendix (5) Sequence of PCR products of amplification of 16S rDNA by two sets of primer (B16SF and B16SR) of the *Bacillus* isolate B5.

GAATGGGAGCTTGCTCCCTGATGTTAGCGGCGGACGGGTGAGTAACAC
GTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGGGGCT
AATACCGGATGCTTGTTTGAACCGCATGGTTCAAACATAAAAGGTGGC
TTCGGCTACCACTTACAGATGGACCCGCGGCGCATTAGCTAGTTGGTGA
GGTAATGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGA
TCGGCCACACTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAG
CAGTGGTCATAGCTGTTTCCGGAA

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

GAATGGGAGCTTGCTCCCTGATGTTAGCGGCGGACGGGTGAGTAACAC
GTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGGGGCT
AATACCGGATGCTTGTTTGAACCGCATGGTTCAAACATAAAAGGTGGC
TTCGGCTACCACTTACAGATGGACCCGCGGCGCATTAGCTAGTTGGTGA
GGTAATGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGA
TCGGCCACACTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAG
CAGTGGTCATAGCTGTTTCCGGAA

Appendix (7) Sequence of PCR products of amplification of 16S rDNA by two sets of primer (B16SF and B16SR) of the *Bacillus* isolate B8.

GAATGGGAGCTTGCTCCCTGATGTTAGCGGCGGACGGGTGAGTAACAC
GTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGGGGCT
AATACCGGATGGTTGTTTGAACCGCATGGTTCAAACATAAAAGGTGGC
TTCGGCTACCACTTACAGATGGACCCGCGGCGCATTAGCTAGTTGGTGA
GGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGA
TCGGCCACACTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAG
CAGTGGTCATAGCTGTTTCCGGA

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

GAATGGGAGCTTGCTCCCTGATGTTAGCGGCGGACGGGTGAGTAACAC
GTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGGGGCT
AATACCGGATGCTTGTTTGAACCGCATGGTTCAAACATAAAAGGTGGC
TTCGGCTACCACTTACAGATGGACCCGCGGCGCATTAGCTAGTTGGTGA
GGTAATGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGA
TCGGCCACACTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAG
CAGTGGTCATAGCTGTTTCCGGAA

Appendix (9) Sequence of PCR products of amplification of 16S rDNA by two sets of primer (B16SF and B16SR) of the *Bacillus* isolate B10.

GAATGGGAGCTTGCTCCCTGATGTTAGCGGCGGACGGGTGAGTAACAC
GTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGGGGCT
AATACCGGATGCTTGTTTGAACCGCATGGTTCAAACATAAAAGGTGGC
TTCGGCTACCACTTACAGATGGACCCGCGGCGCATTAGCTAGTTGGTGA
GGTAATGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGA
TCGGCCACACTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAG
CAGTGGTCATAGCTGTTTCCGGAA

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Appendix (10): Sequence of PCR products of amplification of 16S rDNA by two sets of primers (27F and 1492R) of the *Bacillus* isolate B10.

CCTCGCGGTTTCGCTGCCCTTTGTTCTGTCCATTGTAGCACGTGTGTAGC
CCAGGTCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTCCG
GTTTGTCACCGGCAGTCACCTTAGAGTGCCCAACTGAATGCTGGCAACT
AAGATCAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGA
CACGAGCTGACGACAACCATGCACCACCTGTCACTCTGCCCCCGAAGG
GGACGTCCTATCTCTAGGATTGTCAGAGGATGTCAAGACCTGGTAAGG
TTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGG
CCCCCGTCAATTCCTTTGAGTTTCAGTCTTGCGACCGTACTCCCCAGGC

GGAGTGCTTAATGCGTTAGCTGCAGCACTAAGGGGCGGAAACCCCCTA
ACACTTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCC
TGTTCGCTCCCCACGCTTTCGCTCCTCAGCGTCAGTTACAGACCAGAGA
GTCGCCTTCGCCACTGGTGTTCCTCCACATCTCTACGCATTTCACCGCTA
CACGTGGAATTCCACTCTCCTCTTCTGCACTCAAGTTCCCCAGTTTCCAA
TGACCCTCCCCGGTTGAGCCGGGGGCTTTCACATCAGACTTAAGGAACC
GCCTGCGAGCCCTTTACGCCCAATAATTCCGGACAACGCTTGCCACCTA
CGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAG
GTACCGTCAAGGTACCGCCCTATTCGAACGGTACTTGTTCTTCCCTAAC
AACAGAGCTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTC
CGTCAGACTTTCGTCCATTGCGGAAGATTCCCTACTGCTGCCTCCCGTA
GGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCA[illegible]
TCGGCTACGCATCGTTGCCTTGGTGAGCC[illegible]AGCTA
[illegible]TTTTATGT
[illegible]AGCATCC
[illegible]CCCAGTCTTACAGGCAGGTTACCCACGTGTTACTCACCCGTC
CGCCGCTAACATCAGGGAGCAAGCTCCCATCTGTCCGCTCGACTTGCAT
GTATTAGGCA.

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now

Appendix (11) Sequence of PCR products of amplification of 16s rDNA by two sets of primers (27F and 1492R) of the *Bacillus* isolate B9.

CGGCTGGCTCCTAAAAGGTTACCTCACCGACTTCGGGTGTTACAAACTC
TCGTGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCG
CGGCATGCTGATCCGCGATTACTAGCGATTCCAGCTTCACGCAGTCGAG
TTGCAGACTGCGATCCGAACTGAGAACAGATTTGTGGGATTGGCTTAA
CCTCGCGGTTTCGCTGCCCTTTGTTCTGTCCATTGTAGCACGTGTGTAGC
CCAGGTCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTCCG
GTTTGTCACCGGCAGTCACCTTAGAGTGCCCAACTGAATGCTGGCAACT
AAGATCAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGA
CACGAGCTGACGACAACCATGCACCACCTGTCACTCTGCCCCGAAGG
GGAGGTCCTATCTCTAGGATTGTCAGAGGATGTCAAGACCTGGTAAGG
TTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGG
GCCCCCGTCAATTCCTTTGAGTTTCAGTCTTGCGACCGTACTCCCCAG
GCGGTCGACTTAATGCGTTAGCTGCAGCACTAAGGGGCGGAAACCCCCTA
ACACTTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCC

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

TGTTCGCTCCCCACGCTTTCGCTCCTCAGCGTCAGTTACAGACCAGAGA
GTCGCCTTCGCCACTGGTGTTCCTCCACATCTCTACGCATTTCACCGCTA
CACGTGGAATTCCACTCTCCTCTTCTGCACTCAAGTTCCCCAGTTTCCAA
TGACCCTCCCCGGTTGAGCCGGGGGCTTTCACATCAGACTTAAGAAACC
GCCTGCGAGCCCTTTACGCCCAATAATTCCGGACAACGCTTGCCACCTA
CGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAG
GTACCGTCAAGGTACCGCCCTATTCGAACGGTACTTGTTCTTCCCTAAC
AACAGAGCTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTC

CGTCAGACTTTCGTCCATTGCGGAAGATTCCCTACTGCTGCCTCCCGTA
GGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGG
TCGGCTACGCATCGTTGCCTTGGTGAGCCATTACCTCACCAACTAGCTA
ATGCGCCGCGGGTCCATCTGTAAGTGGTAGCCGAAGCCACCTTTTATGT
TTGAACCATGCGGTTCAAACAAGCATCCGGTATTAGCCCCGGTTTCCCG
GAGTTATCCCAGTCTTACAGGCAGGTTACCCACGTGTTACTCACCCGTC
CGCCGCTAACATCAGGGAGCAAGCTCCCATCTGTCCGCTCGACTTGCAT
GTATTAGGC

Appendix (12) Sequence of PCR products of amplification of 16s rDNA by two sets of primers (27F and 1492R) of the *Bacillus* isolate B1.

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

ACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAG
ACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAA
TGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTTTTC
GGATCGTAAAGCTCTGTTGTTAGGGAAGAACAAGTACCGTTCAAATAG
GGCGGTACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTG
CCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATT
GGGCGTAAAGGGCTCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCC
CCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAG
AAGAGGAGAGTGGAATTCCCCGTGTATCGGTGAAATGCGAAGAGATGT

GGAGGAACACCTGTGGCGAAGGCAACTCTCTGGTCTGTAACTGACGCT
GAGGACCTAATGTGTGGGGAGCGTATATGGATTTTATTCCCTGGGTAGT
TTACGGCTTTTACAATA///CGGCTGGCTCCTAAAAGGTTACCTCACCGAC
TTCGGGTGTTACAAACTCTCGTGGTGTGACGGGCGGTGTGTACAAGGCC
CGGGAACGTATTCACCGCGGCATGCTGATCCGCGATTACTAGCGATTCC
AGCTTCACGCAGTCGAGTTGCAGACTGCGATCCGAACTGAGAACAGAT
TTGTGGGATTGGCTTAACCTCGCGGTTTCGCTGCCCTTTGTTCTGTCCAT
TGTAGCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTGACGT
CATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCACCTTAGAGTGCCC
AACTGAATGCTGGCAACTAAGATCAAGGGTTGCGCTCGTTGCGGGACT
TAACCCAACATCTCACGACACGAGCTGACGACAACCATGCACCACCTG
TCACTCTGCCCCCGAAGGGGACGTCCTATCTCTAGGATTGTCAGAGT
GTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGC
TCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAGCCTTGC
GGCCGTACCCCCTAACACTTAGCACTCATCGTTTACGGTGTGGACTA
CCAGGGTATCTAATCCTGTTCGCTCCCCACGCTTTCGCTCCTCAGCGTC

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

AGTTACAGACCAGAGAGTCGCCTTCGCCACTGGTGTTCCTCCACATCTC
TACGCATTTCACCGCTACACGTGGAATTCCACTCGCCTCTTCTGCGTCA
ATGTTCCCCAGTTTCCAATGACCCTCCCCGGTTGAGCTGGGGGTTTTCA
CTTCAACTTAAGAAACCGCCAGCGAGCCTTTACGCCCATATTTGG

Appendix (13) Sequence of PCR products of amplification of 16s rDNA by two sets of primers (27F and 1492R) of the *Bacillus* isolate B2.

GCCTAATACATGCAAGTCGAGCGGACAGATGGGAGCTTGCTCCCTGAT
GTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCTGTAAGAC
TGGGATAACTCCGGGAAACCGGGGCTAATACCGGATGCTTGTTTGAAC
CGCATGGTTCAAACATAAAAGGTGGCTTCGGCTACCACTTACAGATGG
ACCCGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAA
CGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGA
CACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAAT
GGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTTTTCG
GCGGTACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTG
CAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTG
GGAGGAGAGTGGAATTCCACGTGTAGCGGAGAAATGCGTAGAGATGTG

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now

GAGGAACACCAGTGGCGAAGGCGATTCTCTGGTCTGTTTCTGACCCTGA
GGAGCGAAAGCGTGGGGAAGGAACGGGATTAGATACCCTGGGGAGCC
CCACGCTCTCAAACGATGAGTGCTAAGTGTTAGGAGGTTTTCCCCCCCT
CTATTGCTGGCACCTAACGCATTAAGCACCCCCCTCGGGGGGAACGGT
CGGAAGGTGGATAATCCTAATAATTTTATTGGCCCGCTCGCTAAGCTCG
GTGCCCGCGGTTTTATCAAACTACGA///CGGCTGGCTCCATAAAGGTTA
CCTCACCGACTTCGGGTGTTACAAACTCTCGTGGTGTGACGGGCGGTGT
GTACAAGGCCCGGGAACGTATTCACCGCGGCATGCTGATCCGCGATTA
CTAGCGATTCCAGCTTCACGCAGTCGAGTTGCAGACTGCGATCCGAACT

GAGAACAGATTTGTGGGATTGGCTTAACCTCGCGGTTTCGCTGCCCTTT
GTTCTGTCCATTGTAGCACGTGTGTAGCCCAGGTCATAAGGGGCATGAT
GATTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCACCTT
AGAGTGCCCAACTGAATGCTGGCAACTAAGATCAAGGGTTGCGCTCGT
TGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATG
CACCACCTGTCACTCTGCCCCCGAAGGGGACGTCCTATCTCTAGGATTG
TCAGAGGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAA
ACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTT
CAGTCTTGCGACCGTACTCCCCAGGCGGAGTGCTTAATGCGTTAGCTGC
AGCACTAAGGGGCGGAAACCCCCTAACACTTAGCACTCATCGTTTACG
GCGTGGACTACCAGGGTATCTAATCCTGTTCGCTCCCCACGCTTTCGCT
CCTCAGCGTCAGTTACAGACCAGAGAGTCGCCTTCGCCACTGGTGTTC
TCCACATCTCTACGCATTTCACCGCTACACCTGGAATTCTACCCCCTCT
GCTGCACTCAAGGAACCGG
AATTGCCGACACGCTTGCACCTACGTATTACCGCGGCTGCTGGACGTAG
TAGCCGTGCTTTCAGTAAGAACGCAAGCACCGCCTATTCCAACGG

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Wondershare™

Appendix (14) Sequence of PCR products of amplification of 16s rDNA by two sets of primers (27F and 1492R) of the *Bacillus* isolate B3.

TGCCTAATACATGCAAGTCGAGCGGACAGATGGGAGCTTGCTCCCTGA
TGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCTGTAAGA
CTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATGGTTGTTTGAA
CCGCATGGTTCAAACATAAAAGGTGGCTTCGGCTACCACTTACAGATG

GACCCGCGGCGCATTAGCTAGTTGGTGAAGGTAACGGCTCACCAAGGC
AACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGA
GACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCA
ATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTTTT
CGGATCGTAAAGCTCTGTTGTTAGGGAAGAACAAGTACCGTTCGAATA
GGGCGGTACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGT
GCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTAT
TGGGCGTAAAGGGCTCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCC
CCCGGCTCACCGGGGAGGGTCTTTGGAAACTGAGGATTTTGTGCGTAT
////CGGCTGGCTCCTAAAAGGTTACCTCACCGACTTCGGGTGTTACAAAC
TCTCGTGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCAC
CGCGGCATGCTGATCCGCGATTACTAGCGATTCCAGCT[illegible]G
AGTTGCAGACTGCGATCCGAACTGATAA[illegible]GGCTTA
[illegible]TGTTCTC[illegible]
[illegible]ATGATTTG[illegible]
G[illegible]CACCGGCAGTCACCTTAGAGTGCCCAAGTGAATGCTGGCAA
CTATGATCGAGGGGTGGGCTCGTTTCTTGTCGTAACCCAAAAGAAAA
GACGCGCTTTGAATACAACCATGCAAGGCCTGACGTTATGGCTCCAAA
GGGTAGGGGATATGTATAAGTTTTTCCGATTGCGTTTTGGCCTGTGCAG
TCTAGACGCATTGCTGATTTTTTGAGCATTCACTACAGGTATTGGTCCG
GCACCGATTCCTCATAACTGTTATTCCCCTTCTTTACTCCCTGTAAGATC
CCTTCGCGCCACCCGAAC

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now

Appendix (15) Sequence of PCR products of amplification of 16s rDNA by two sets of primers (27F and 1492R) of the *Bacillus* isolate B4.

TGCCTAATACATGCAAGTCGAGCGGACAGATGGGAGCTTGCTCCCTGA
TGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCTGTAAGA
CTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATGGTTGTTTGAA
CCGCATGGTTCAAACATAAAAGGTGGCTTCGGCTACCACTTACAGATG
GACCCGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCA
ACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAG
ACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAA
TGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTTTTC
GGATCGTAAAGCTCTGTTGTTAGCGAAGAAGCTATCGGGTGAGAATAG
GGCGGTACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTG
CCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATT
GGGCGTAAAGCGCGCGTAGGCGGTTTCTTAAGTCTGATGTGAAAGCC
CAGAGGAGAGTGAAACTCCACGTGTACTCGATGAAATGCTTAGAGATG
TGGAGGAACCTAAGTGGATAAACGCGACCTCTCTGT////CGGCTGGCTCC
TAAAAGGTTACCTCACCGACTTCGGGTGTTACAAACTCTCGTGGTGTGA
CGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCATGCTGA
TCCGCGATTACTAGCGATTCCAGCTTCACGCAGGCGAGTTGCAGACTGC
GATCCGAACTGAGAACAGATTTGTGGGATTGGCTTAACCTCGCGGTTTC
GCTGCCCTTTGTTCTGTCCATTGTAACACGTGTGTAGCCCAGGTCATAA
GGTGCATGATGATTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACCG
GCAGTCACCTTACAGTGCCCAATTGAATGCTGGCTACTAAAATCAGGGT
TGGGTTCTTTGTCGGACTAAACCCAACCAGCCACGAGACCATGTAACC

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now
Wondershare™

ACATCTCTGCACAACATGCTACTCTACCCGTGGGGGGGGGGGGGATAT
CACTCAGAAGGCCTAGGATTTAAGGGACCCGATAAGG

Appendix (16) Sequence of PCR products of amplification of 16s rDNA by two sets of primers (27F and 1492R) of the *Bacillus* isolate B5.

TGCCTAATACATGCAAGTCGAGCGGACAGATGGGAGCTTGCTCCCTGA
TGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCTGTAAGA
CTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATGCTTGTTTGAA
CCGCATGGTTCAAACATAAAAGGTGGCTTCGGCTACCACTTACAGATG
ACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGAACTGAG
ACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGAATCTTCCGCAA
GGCGGTACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTG
CCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATT
GGGCGTAAAGGGCTCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCC
CCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAG
AAGAGGAGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGT
GGAGGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGTAACTGACGCT
GAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCCTGTTAGT
CCCCGCCGTAAACGATGAGAGCTAAGTGTAGGGGGTTTCCGCCCCTTA
GCGCTGCAGCTAACACAATAAGAACTCCCGCCTGGGGAGTACGGGTC///
CGGCTGGCTCCTAAAAGGTTACCTCACCGACTTCGGGTGTTACAAACTC

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now
Wondershare™

TCGTGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCG
CGGCATGCTGATCCGCGATTACTAGCGATTCCAGCTTCACGCAGTCGAG
TTGCAGACTGCGATCCGAACTGAGAACAGATTTGTGGGATTGGCTTAA
CCTCGCGGTTTCGCTGCCCTTTGTTCTGTCCATTGTAGCACGTGTGTAGC
CCAGGTCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTCCG
GTTTGTCACCGGCAGTCACCTTAGAGTGCCCAACTGAATGCTGGCAACT
AAGATCAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGA
CACGAGCTGACGACAACCATGCACCACCTGTCACTCTGCCCCCGAAGG
GGACGTCCTATCTCTAGGATTGTCAGAGGATGTCAAGACCTGGTAAGG
TTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGG
CCCCCGTCAATTCCTTTGAGTTTCAGTCTTGCGACCGTACTCCCCAGGC
GGAGTGCTTAATGCGTTAGCTGCAGCACTAAGGGGCGGAAACCCCC
ACACTTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCC
AGAACGTGAATTCCACTCTCCTCTTCTGCACTCAAGTTCCCCAGTTTCA
ATGACCCTCCCGGGGTGAGCCGGGGGCTTTCACATCAGCTTAAAAACC

GCCTGCGAGCCTTTACGCCAATAATTCCGGACAACGTTTGCCACTACGT
ATTACAAGGTGCTGGCACTAG

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Wondershare™

Appendix (18) Sequence of PCR products of amplification of 16s rDNA by two sets of primers (27F and 1492R) of the *Bacillus* isolate B7.

GCCTAATACATGCAAGTCGAGCGGACAGATGGGAGCTTGCTCCCTGAT
GTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCTGTAAGAC
TGGGATAACTCCGGGAAACCGGGGCTAATACCGGATGCTTGTTTGAAC
CGCATGGTTCAAACATAAAAGGTGGCTTCGGCTACCACTTACAGATGG
ACCCGCGGCGCATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCAA
CGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGA
CACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAAT
GGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTTTTCG
GAGGAGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTG
GAGGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGTAACTGACGCTG
AGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCC
ACGCCGTAAACGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGT
GCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAG
ACTGAAAACTCAAAGGAATTAGACGGGGGGCCCGCACAAGCGGTGGA
GCATGTGGTTTATTTCGAAGCAACGCGAAGAACTTTACCAGGTCTTGAC
ATCCTCTGACAATTCTAGAAGATAAGACGTCCCCCTTCGGGGCAGAGT
GACAGGTGGAGCATGGTTGTCGTTCAGCTCGTCGTCGTAGATTGTTGGG
TTTAAGTCCCCGCAACGAAGCACCACCTCCTGAATCCTTAGCTTGTCAA

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now

GCCATTCATGTTTGGCTACTTCTGATGGTGGACTTGGCCGGTTGACACA
ACCCGGAAGGAAGGGAGGAGATGAACGTAGAAATCATCTGTCTCCTTA
TTGATCCTGCGTTCTCTCAGCTCGTCTTTCACAAGTCGCAGCAAGTAAT
CTAGATTG///GCGGCTGGCTCCTAAAAGGTTACCTCACCGACTTCGGGT
GTTACAAACTCTCGTGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAA
CGTATTCACCGCGGCATGCTGATCCGCGATTACTAGCGATTCCAGCTTC
ACGCAGTCGAGTTGCAGACTGCGATCCGAACTGAGAACAGATTTGTGG
GATTGGCTTAACCTCGCGGTTTCGCTGCCCTTTGTTCTGTCCATTGTAGC
ACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTGACGTCATCCCC

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

GTATCTAATCCTGTTCGCTCCCACGCTTTCGCTCCTCAGCGTCAGGTAC
AGACAGAGAGTCGCCTTCGCCACTGGTCGTCTTCACATCTTAACGCATT
TCACCGCACCAGTGAAATGCACTCCTCTCTTCGGCATCAAGTCCCAAAT
CCATGAACCTGCCCGGTGATCGAGCTTCACATCAAACTAAGAACGCCT
GCAGACCTTTAGCCAAAATTCGGAAAAGATTGGCCCTACGATACCGCG
CTGCTGCACGATA

Appendix (19) Sequence of PCR products of amplification of 16s rDNA by two sets of primers (27F and 1492R) of the *Bacillus* isolate B8.

TGCCTAATACATGCAAGTCGAGCGGACAGATGGGAGCTTGCTCCCTGA
TGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCTGTAAGA
CTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATGGTTGTTTGAA
CCGCATGGTTCAAACATAAAAGGTGGCTTCGGCTACCACTTACAGATG
GACCCGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCA
ACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAG
ACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAA
TGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTTTTC
GGATCGTAAAGCTCTGTTGTTAGCGAAGAAGCTATCGGGTATCCGAAG
GGCGGTACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTG
CCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATT
GGGCGTAAAGCGCGCGTAGGCGGTTTCTTAAGTCTGATGTGAAAGCCC
CACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGAGACTTGAGTGCA
GAAGAGGAGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGT
GGAGGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGTAACTGACGCT
GAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTACTC
CACGCCGTAAACGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTCA
GTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGGGCGCA
AGACTGAACTCATAAGAATTTGACGGAGGGCCCGCACAAGCGGTGGAG
CATGTGGTTTTATTTAAAGCAACGAGCAAGAAACTTACTAAGGTCTGG
ACATCCTCTGACATTCCTAGAGATATGTACGGCCCCTTCACGGGCAGAG
TGAACAGGGTAGAGGCAAGCTGTCGTCATCTCTCTGCCATGAAGATGA
TGGTATGAGTCCTAAAACGAGCAACACACATGGACCTAGGTGCCTGCA

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now
Wondershare™

TGACTTGGCATCTGGAAGATATACTCGTCAGTGAAGCACGGAGTATTG
GATTAGGCTGCACTCACATGACGTTGAACCTGCTACACGGGTCGCATAC
GTCATGACTCAGGTA///GCGGCTGGCTCCTAAAAGGTTACCTCACCGAC
TTCGGGTGTTACAAACTCTCGTGGTGTGACGGGCGGTGTGTACAAGGCC
CGGGAACGTATTCACCGCGGCATGCTGATCCGCGATTACTAGCGATTCC
AGCTTCACGCAGTCGAGTTGCAGACTGCGATCCGAACTGAGAACAGAT
TTGTGGGATTGGCTTAACCTCGCGGTTTCGCTGCCCTTTGTTCTGTCCAT
TGTAGCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTGACGT
CATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCACCTTAGAGTGCCC
AACTGAATGCTGGCAACTAAGATCAAGGGTTGCGCTCGTTGCGGGACT
TAACCCAACATCTCACGACACGAGCTGACGACAACCATGCACCACCTG
TCACTCTGCCCCCGAAGGGGACGTCCTATCTCTAGGATTGTGGAAAT
GTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGC
TCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAACCTTGC
GGTCGCGTAACCCCCTAACACTTAGCACTCATCGTTTACGGCGTGGACT
ACCAGGGTATCTAATCCTGTTCGCTCCCCACGCTTTCGCTCCTCAGCGT

CAGTTACAGACCAGAGAGTCACCTTCGCCACTGGTGTTCCTCCACATCT
CTACGCATTTCACCGCTACACGTGGAATTCCACTCTCCTCTTCTGCACTC
AAGTTCCCCAGTTTCAATGACCCTCCCCGGTTGAGCGGGGGCTTTCACA
TCAGACTTAAGAAACCGCCTGCGAGCCCTTTACGCCCATATTCCGGACA
CGATGCCACTACATATTACGCGGCTGCTGGCACGTAGTAGCGTGGCTTC
AGGTAGGTACCGTCAGGACCGCCTATTCGACGGTACTGTCTCGCTACAT
CGAGCTTGCGATCGAAACTTCATCACTCACGCGCGTTGCTCCGTCGGAC
TTTCGTCATTGCGTATATCCTACTGCTGCTCCCGAGGAGCTTGAGCGGG

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

TCAAGCCGATGTGGCCTATCCACTGCTCAGGTCGCCACTGTCGTGCCTA
GGAGGCGTTACCCGACACTAGTATGTCCCGAGTCCTGGGAAGTGAGCC
CATACTCACTTTTATAGTGAACTGT

Appendix (20) Sequence of PCR products of amplification of 16s rDNA by two sets of primers (27F and 1492R) of the *Bacillus* isolate B10.

TGCCTAATACATGCAAGTCGAGCGGACAGATGGGAGCTTGCTCCCTGA
TGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCTGTAAGA
CTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATGCTTGTTTGAA
GACCCGCGGCGCATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCA
ACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAG
GGATCGTAAAGCTCTGTTGTTAGGGAAGAACAAGTACCGTTCGAATAG

GGCGGTACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTG
CCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATT
GGGCGTAAAGGGCTCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCC
CCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAG
AAGAGGAGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGT
GGAGGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGTAACTGACGCT
GAAGAGCGAAAGCGTGGGGAGCGTAACAGGACTAGATACCCTGGTAG
TCCACGCCCTAAACAGATGAGAGCAATGTGATAGGAGGTTTCCGCCCC
CTTAGTGCCGCAGCTAATACATTAATAACTCTGGCCCCGGGATTACGGT

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now
Wondershare™

GCAAGAATGACACTTAAAGGAATTGTCCGTCTGCCCCCAACAGTCAG

CGGATCTTGTGTTTTCTTTAAAACAATTCGACACAACTTACCATCTGTCT

ACATTCATCTGATAATT////CGGCGGCTGGCTCCTAAAAGGTTACCTCAC

CGACTTCGGGTGTTACAAACTCTCGTGGTGTGACGGGCGGTGTGTACAA

GGCCCGGGAACGTATTCACCGCGGCATGCTGATCCGCGATTACTAGCG

ATTCCAGCTTCACGCAGTCGAGTTGCAGACTGCGATCCGAACTGAGAA

CAGATTTGTGGGATTGGCTTAACCTCGCGGTTTCGCTGCCCTTTGTTCTG

TCCATTGTAGCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTG

ACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCACCTTAGAGT

GCCCAACTGAATGCTGGCAACTAAGATCAAGGGTTGCGCTCGTTGCGG

GACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATGCACCA

This is a watermark for the trial version, register to get the full one!

CCTGTCACTCTGCCCCCGAAGGGGACGTCCTATCTCTAG[illegible]

GGATGTCAAGACCTGGTAAGGTTCTTCG[illegible]TTAAACCAC

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now

[illegible]

[illegible]GGCGGAGTGCT[illegible]

[illegible]GGAAACCCCCTAACACTTAGCACTCATCGTTTACGGCGTGG

ACTACCAGGGTATCTAATCCTGTTCGCTCCCCACGCTTTCGCTCCTCAG

CGTCAGTTACAGACCAGAGAGTCGCCTTCGCCACTGGTGTTCCTCCACA

TCTCTACGCATTTCACCGCTACACGTGGAATTCCACTCTCCTCTTCTGCA

CTCAAGTTCCCCAGTTTCCAATGACCTCCCCGGTTGAGCCGGGGGCTTT

CACATCAGACTTAAGAAACCGCCTGCGAGCCCTTTACGCCCAATAATCC

TGACAACGCTTGCCACCTACGAATACCGCGGCTGCTGGTACGTAGTAG

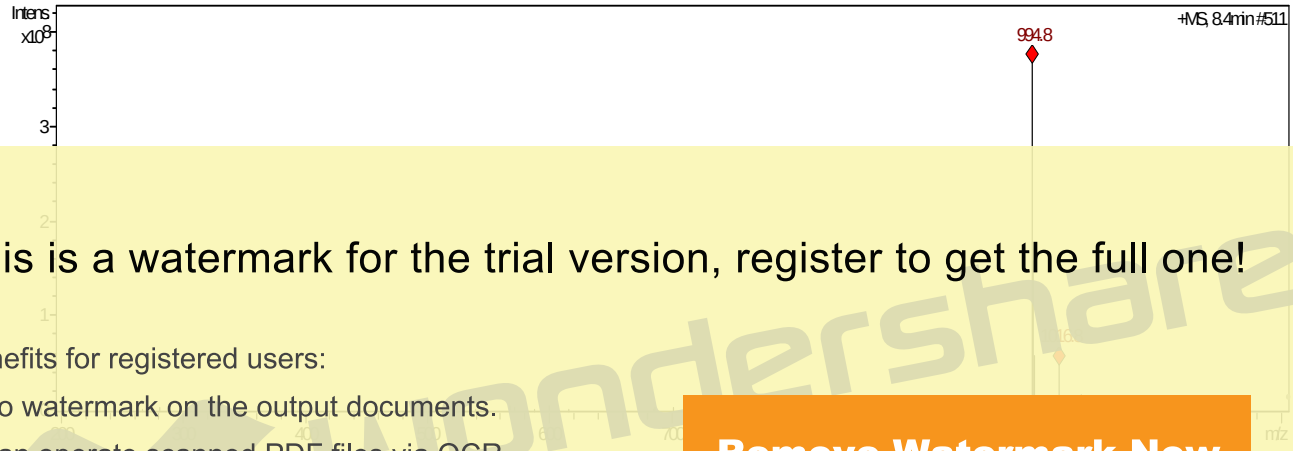
ACGTGGCTTTTCTGGTTAGGTACGTCTAGGTACCGCCCTATCGAACGGA

ATTGTCTTCCTAACACCAGAGTTTTCGATCCAAAACCTCATCACTCCGC

GGCGTTCTTCGTCCAGACTTCTTCATGGGAAAATCCTACTGCTGCTCT

CTCAGTGCTCGGGACGGGGGATATTCCCGGGGGCGTATACCTCTCAGG CGGCTCCCT

Appendix (21) Mass spectrum obtained from the MS1 analysis of peak retained at 8.4min for standard surfactin after HPLC analysis.



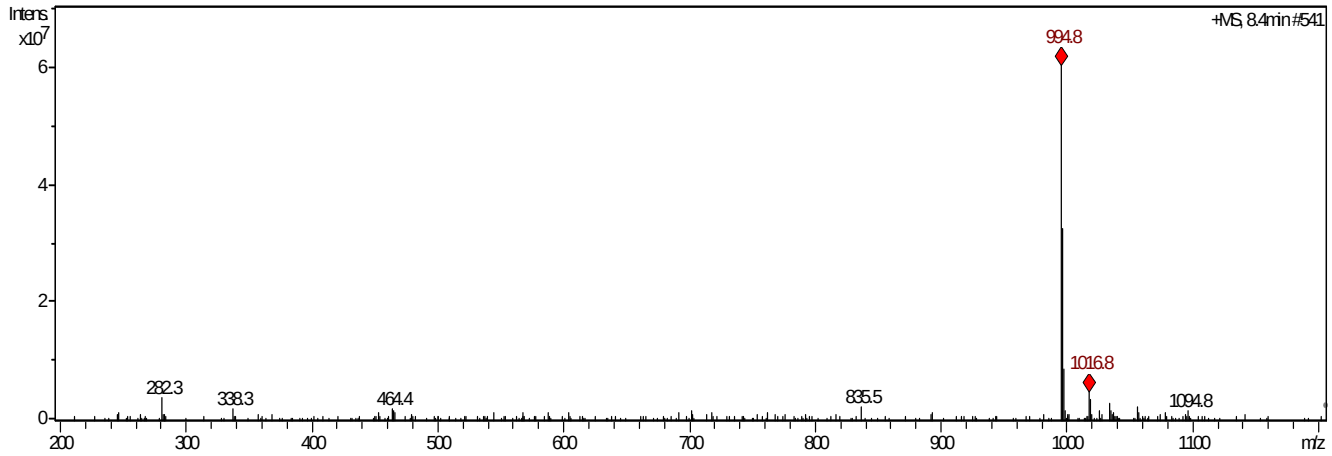
This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Wondershare™

Appendix (22) Mass spectrum obtained from the MS1 analysis of peak retained at 8.4min for *B.* B6 lipopeptide after HPLC analysis.



Appendix (23) Mass spectrum obtained from the MS1 analysis of peak retained at 9.9 min for standard surfactin after HPLC analysis.

This is a watermark for the trial version, register to get the full one!

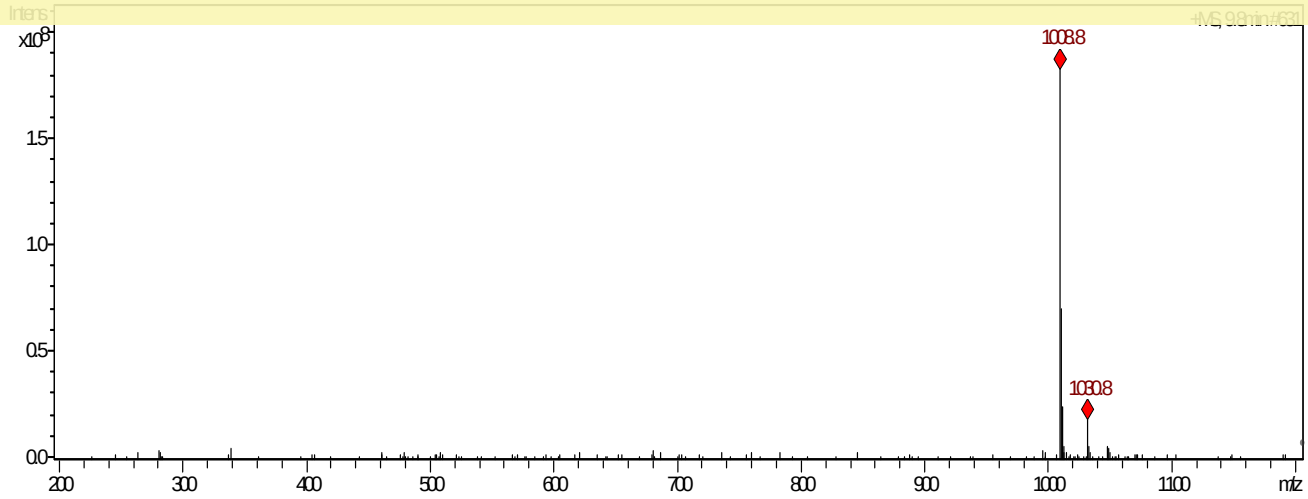
Benefits for registered users:

1.No watermark on the output documents.

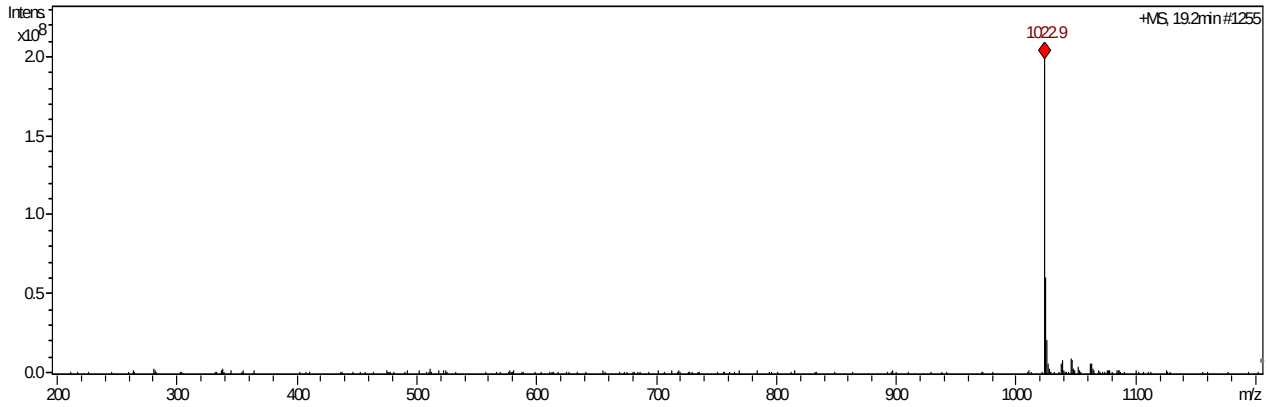
2.Can operate scanned PDF files via OCR.

3.No page quantity limitations for converted PDF files.

Remove Watermark Now



Appendix (25) Mass spectrum obtained from the MS1 analysis of peak retained at 13 min for standard surfactin after HPLC analysis.



This is a watermark for the trial version, register to get the full one!

Benefits for registered users:

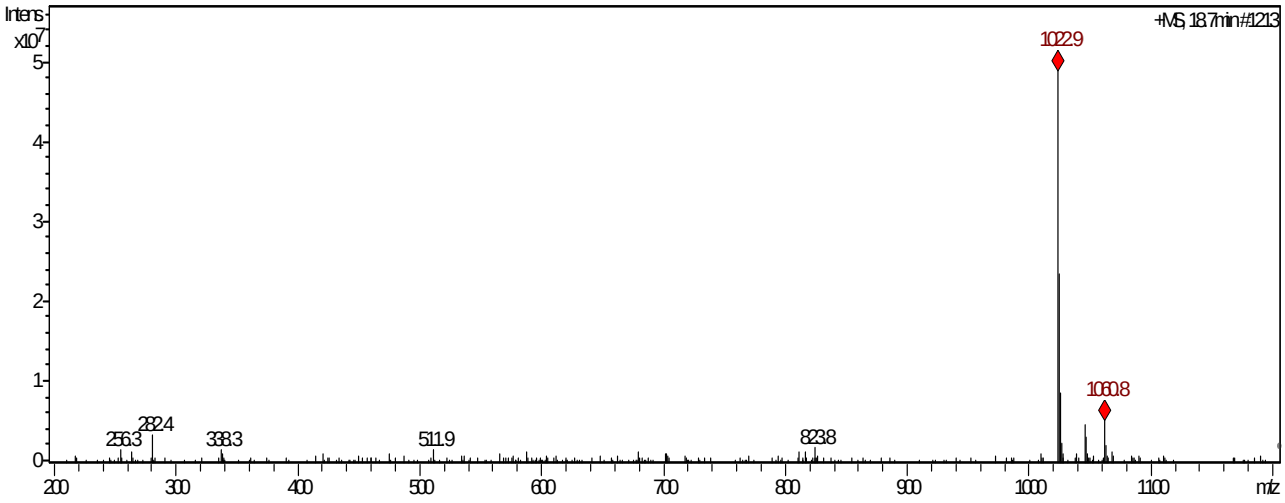
1.No watermark on the output documents.

2.Can operate scanned PDF files via OCR.

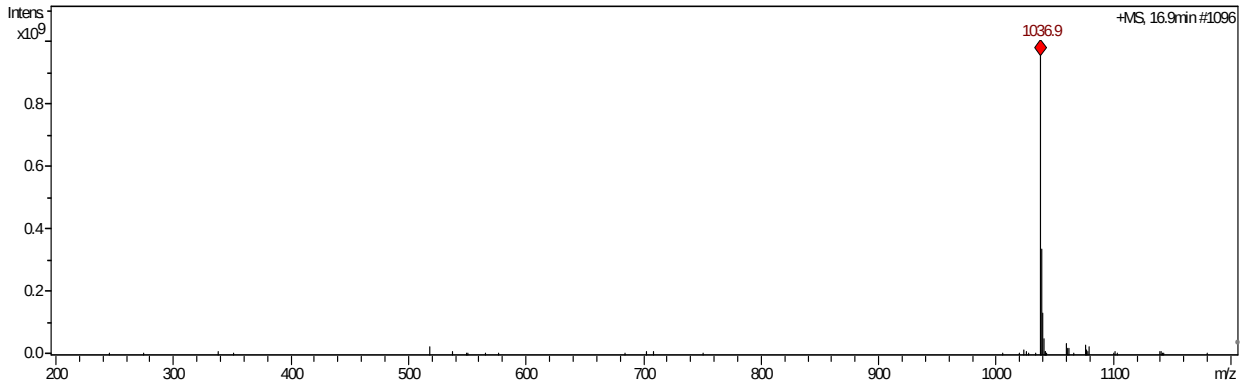
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Wondershare™



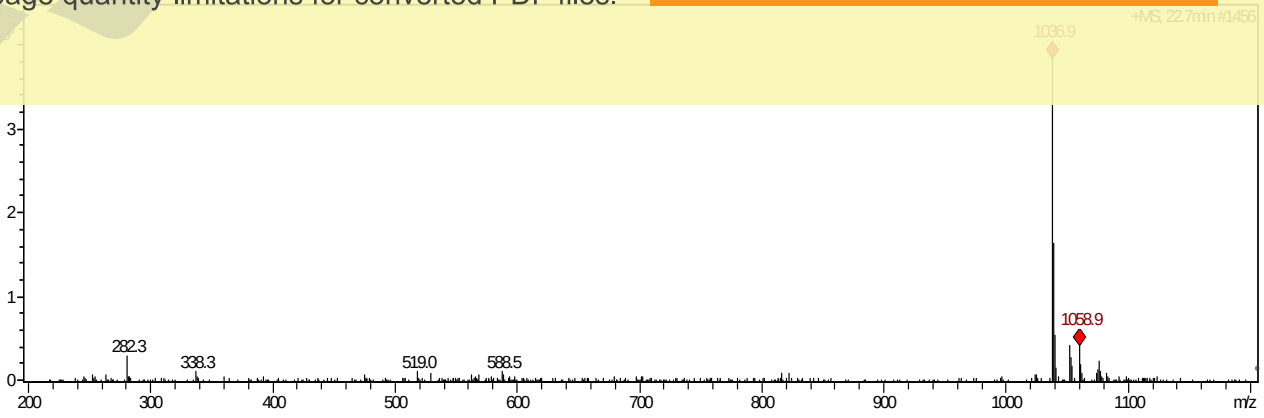
Appendix (27) Mass spectrum obtained from the MS1 analysis of peak retained at 16.9 min for standard surfactin after HPLC analysis.



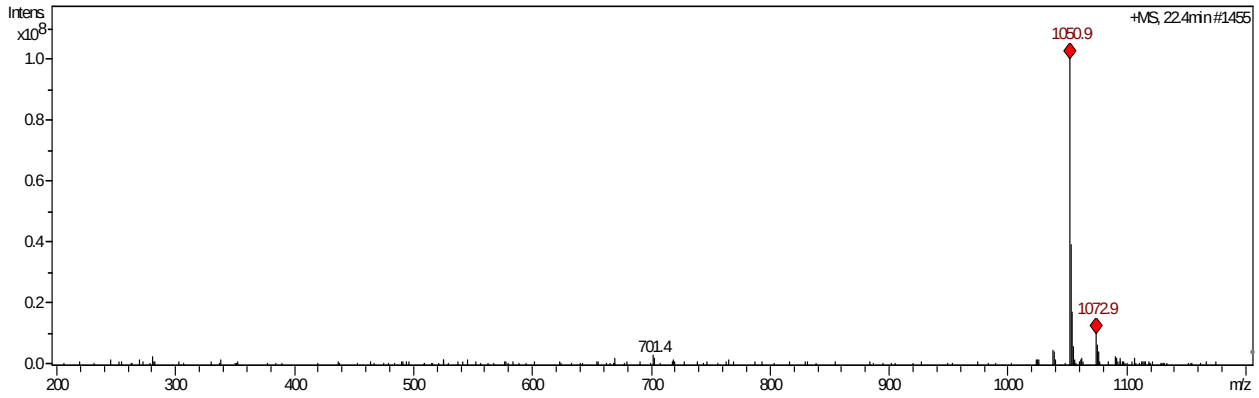
This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now



Appendix (29) Mass spectrum obtained from the MS1 analysis of peak retained at 22.4 min for standard surfactin after HPLC analysis.



This is a watermark for the trial version, register to get the full one!

Benefits for registered users:

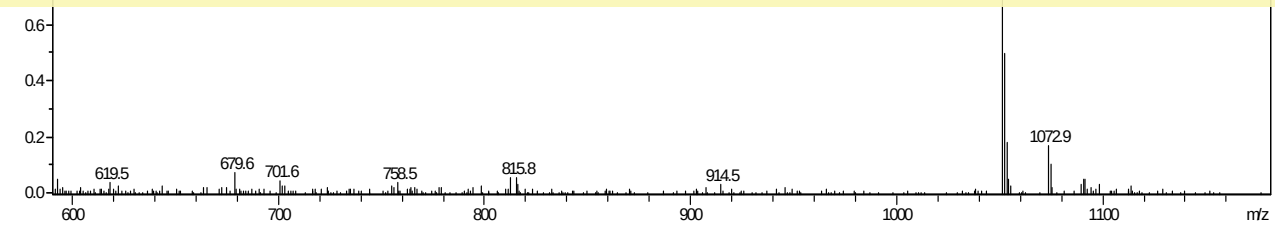
1.No watermark on the output documents.

2.Can operate scanned PDF files via OCR.

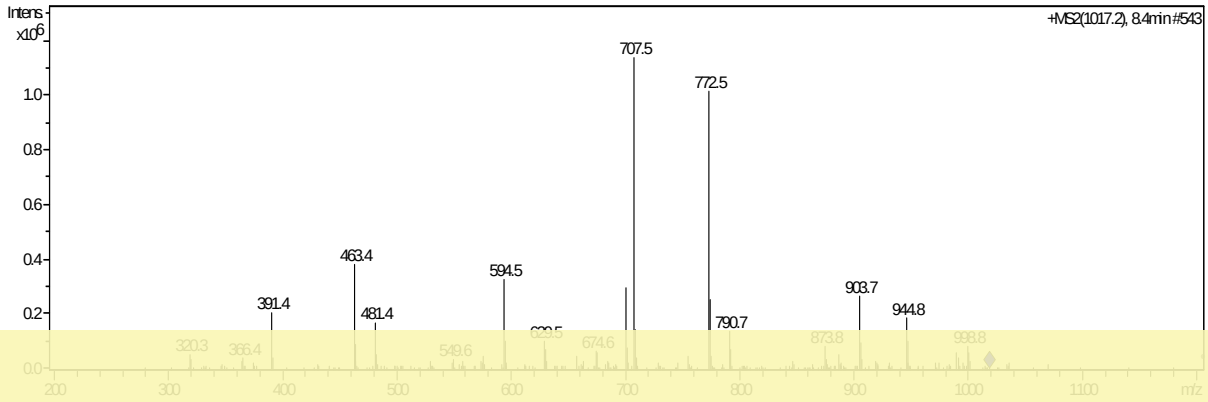
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Appendix (30) Mass spectrum obtained from the MS1 analysis of peak retained at 25.7 min for *B. PC* lipopeptide after HPLC analysis.



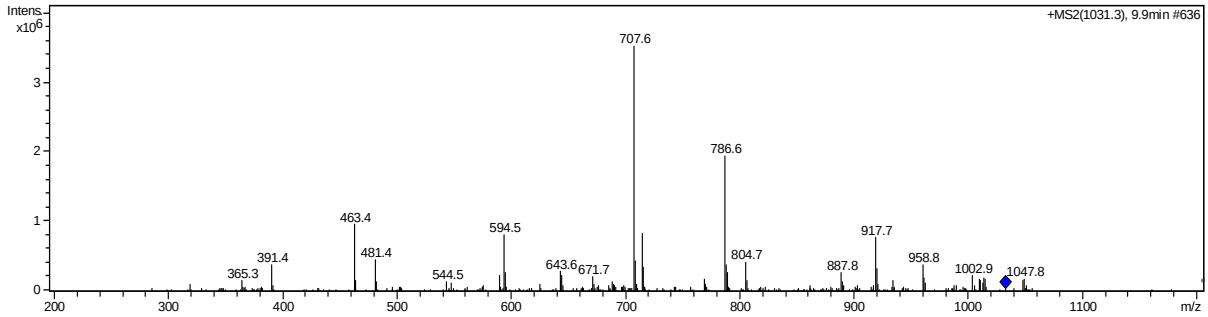
Appendix (31) Mass spectrum of *B.*B6 lipopeptide obtained from the MS2 analysis of the peak of $[M+Na]^+$ at m/z 1017.



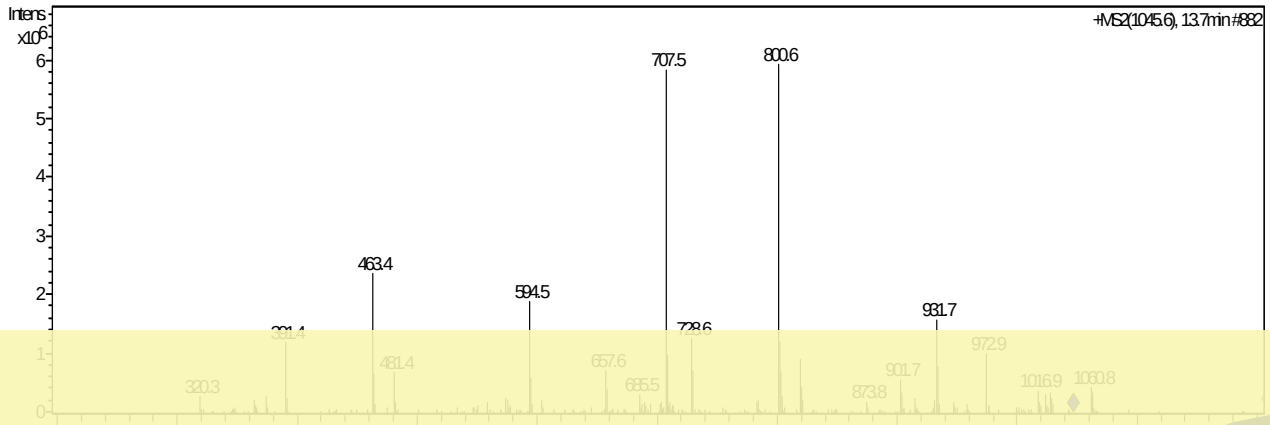
This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now



Appendix (33) Mass spectrum of *B.*B6 lipopeptide obtained from the MS2 analysis of the peak of $[M+Na]^+$ at m/z 1045 .



This is a watermark for the trial version, register to get the full one!

Benefits for registered users:

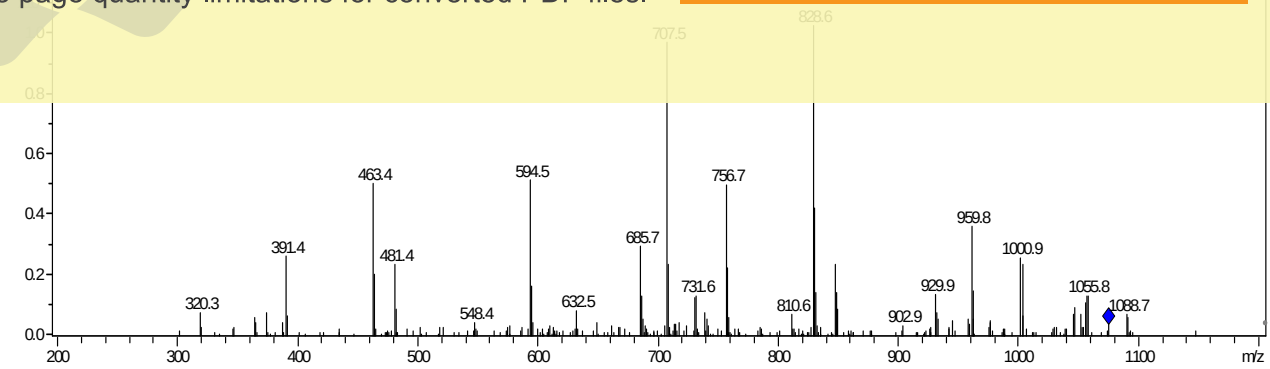
1.No watermark on the output documents.

2.Can operate scanned PDF files via OCR.

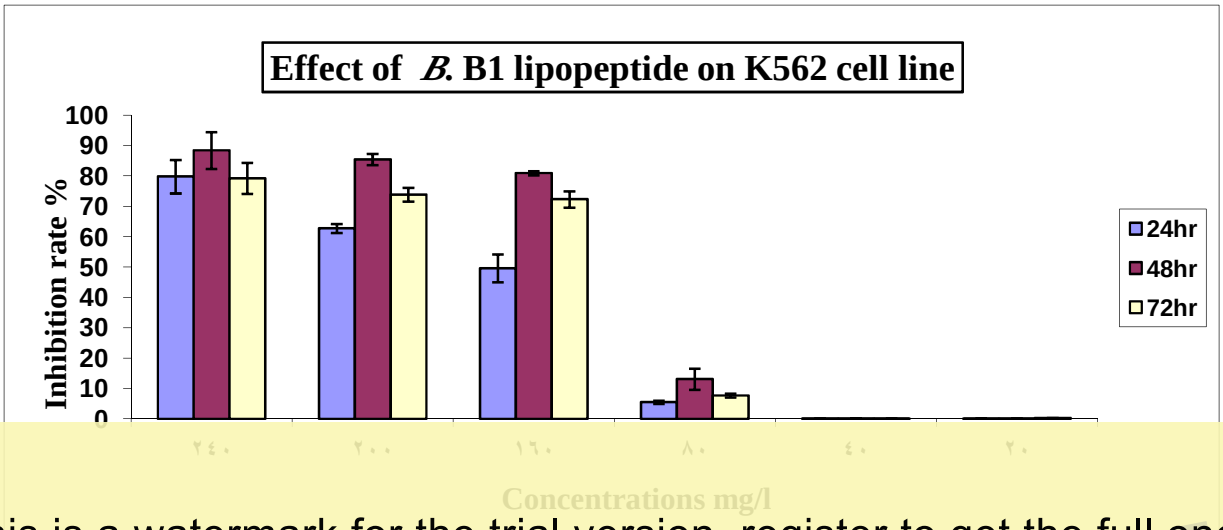
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Appendix (34) Mass spectrum of *B.*B6 lipopeptide obtained from the MS2 analysis



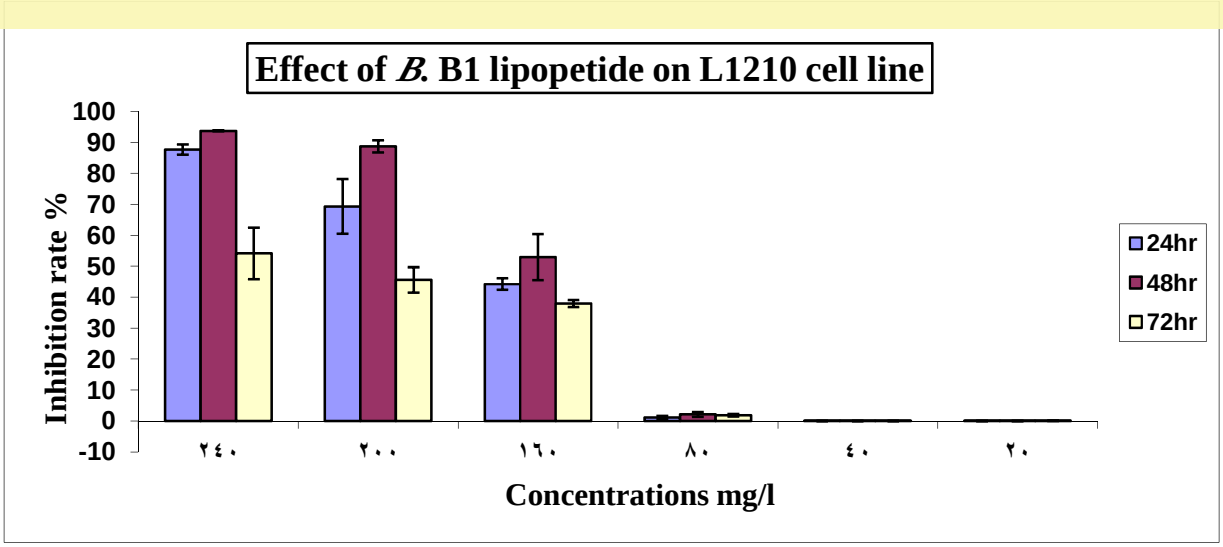
Appendix (35) Effect of *B.* B1 lipopeptide on K562 cell proliferation for different incubation times (24,48,72)hrs.



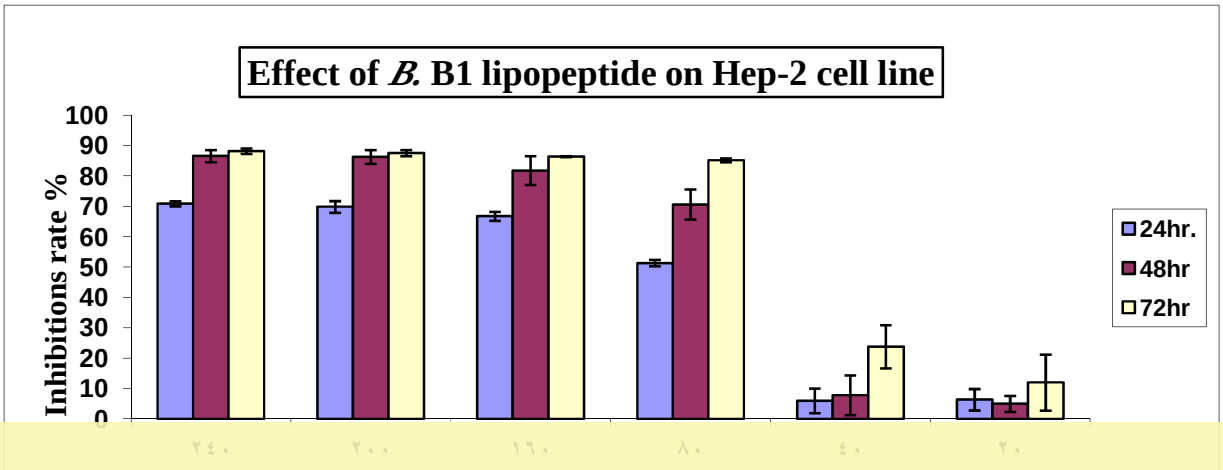
This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now



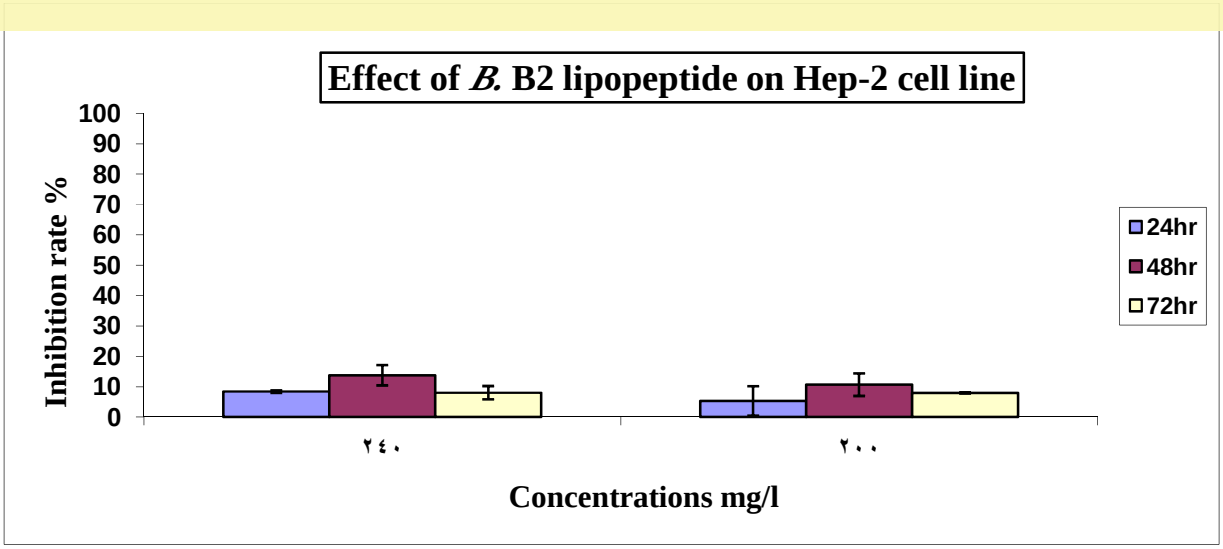
Appendix (37) Effect of *B.* B1 lipopeptide on Hep-2 cell proliferation for different incubation times (24,48,72)hrs.



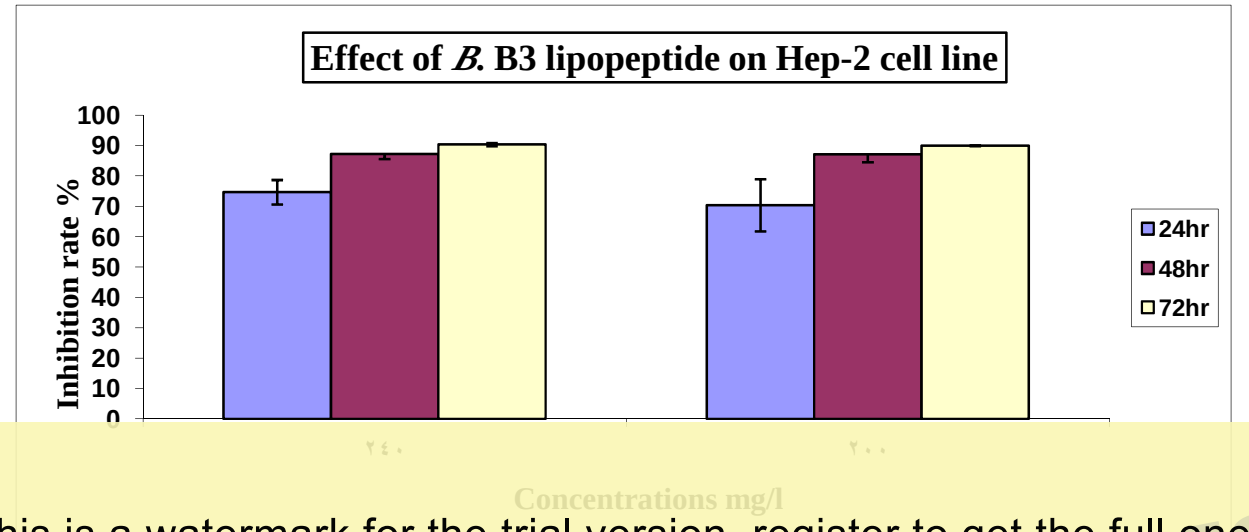
This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now



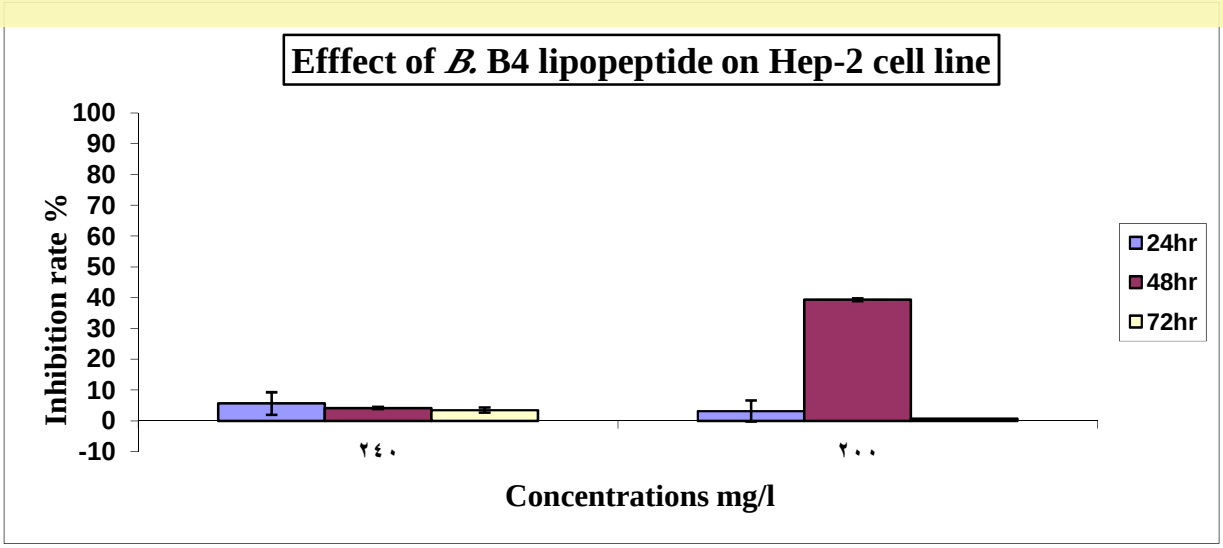
Appendix (39) Effect of *B.* B3 lipopeptide on Hep-2 cell proliferation for different incubation times (24,48,72)hrs.



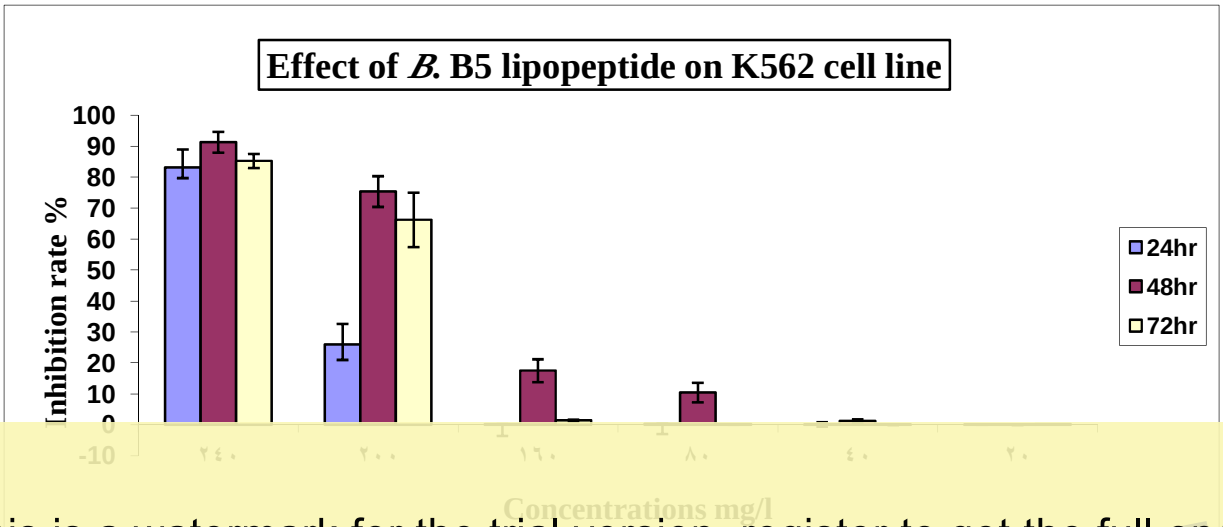
This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now



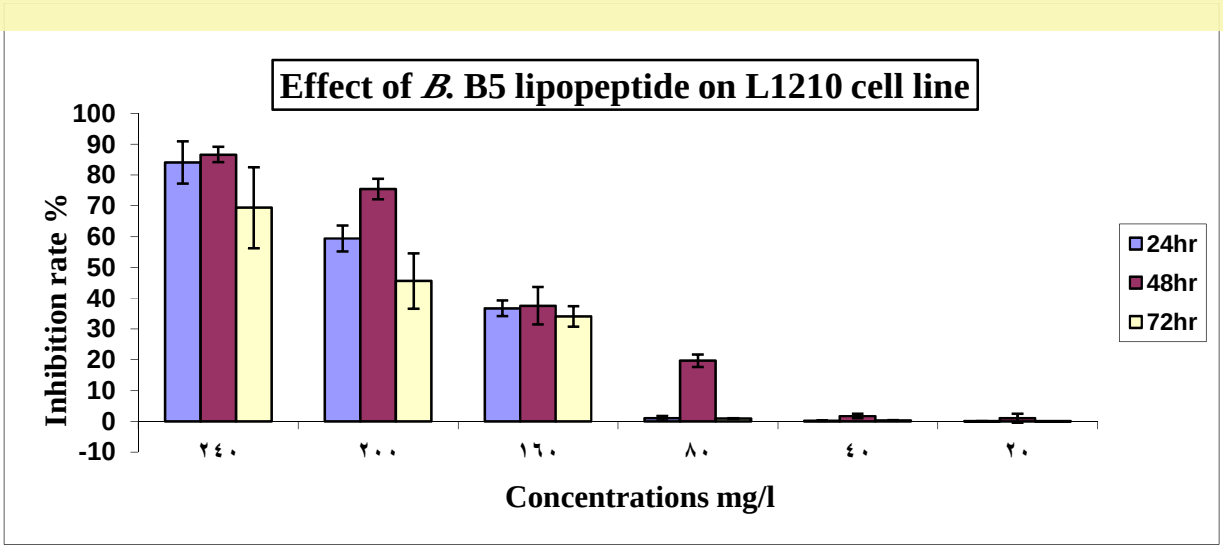
Appendix (41) Effect of *B.* B5 lipopeptide on K562 cell proliferation for different incubation times (24,48,72)hrs.



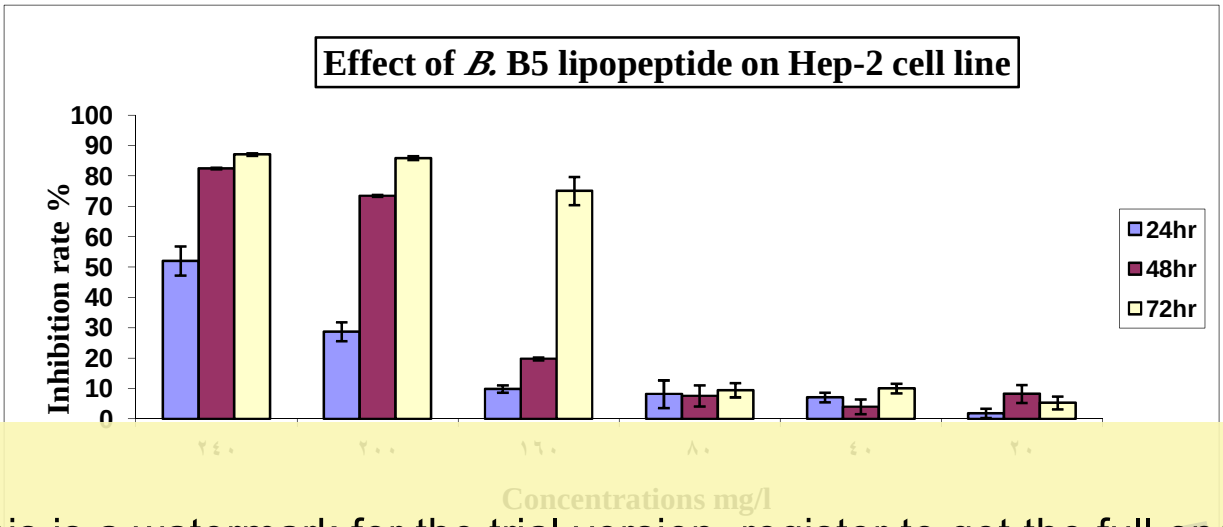
This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now



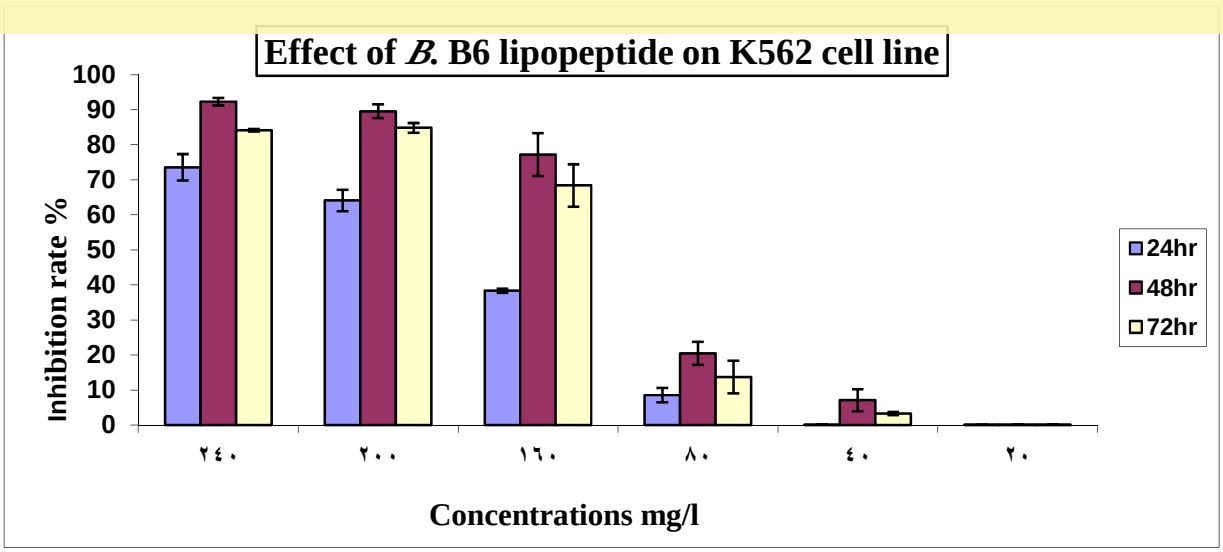
Appendix (43) Effect of *B.* B5 lipopeptide on Hep-2 cell proliferation for different incubation times (24,48,72)hrs.



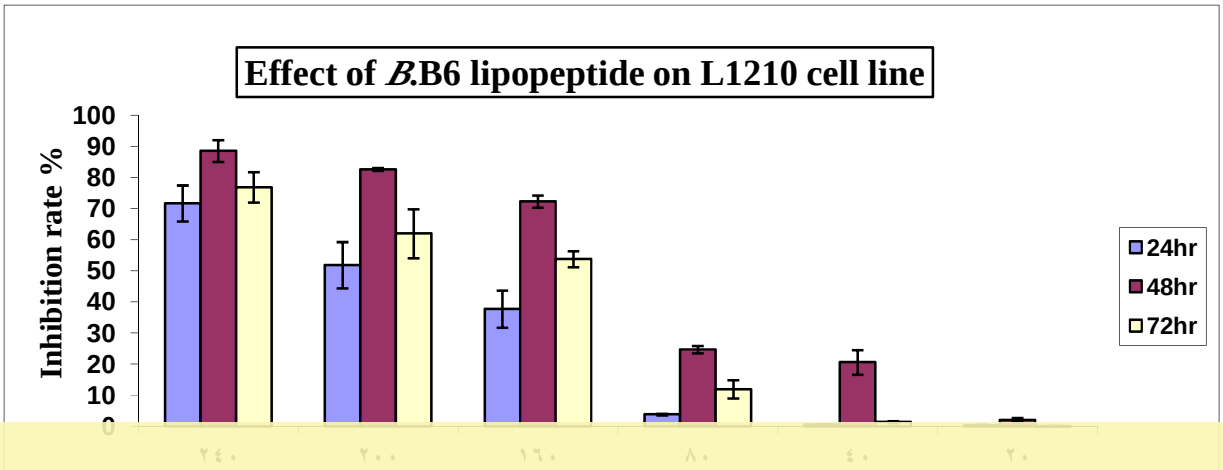
This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now



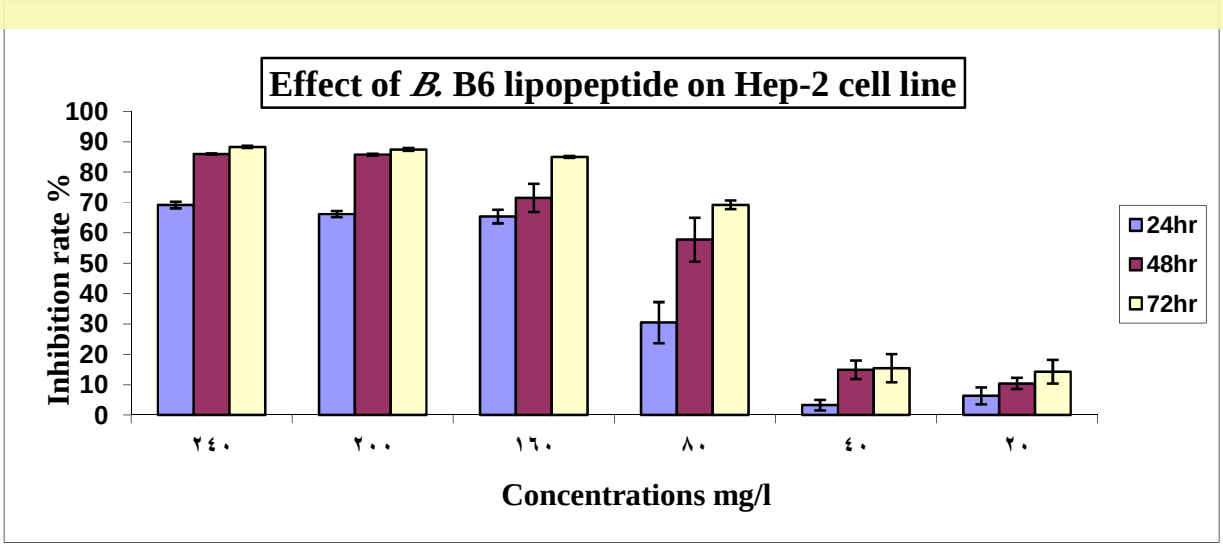
Appendix (45) Effect of *B.* B6 lipopeptide on L1210 cell proliferation for different incubation times (24,48,72)hrs.



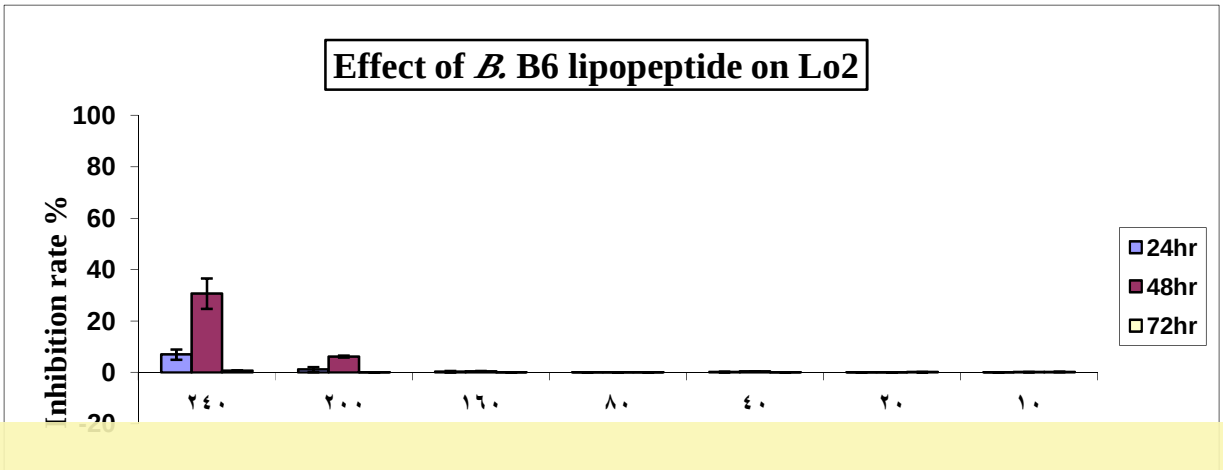
This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now



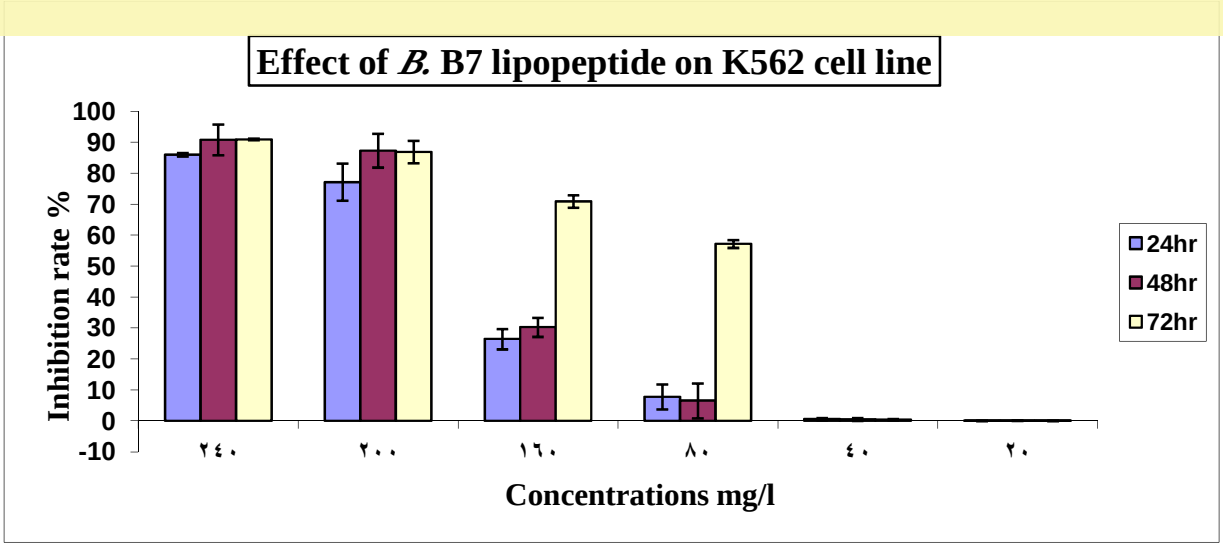
Appendix (47) Effect of *B.* B6 lipopeptide on Lo2 cell proliferation for different incubation times (24,48,72)hrs.



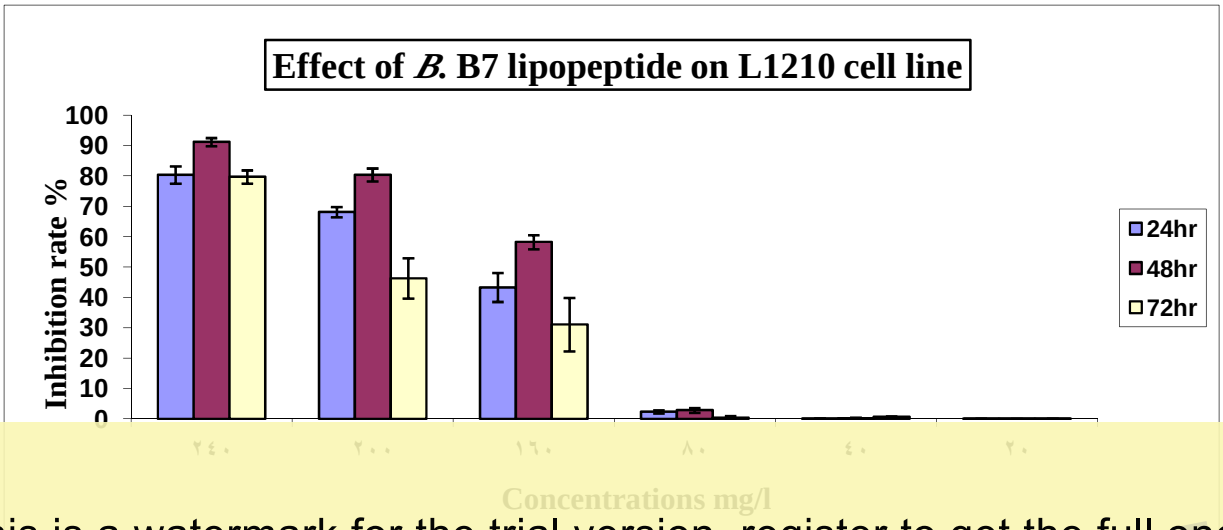
This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now



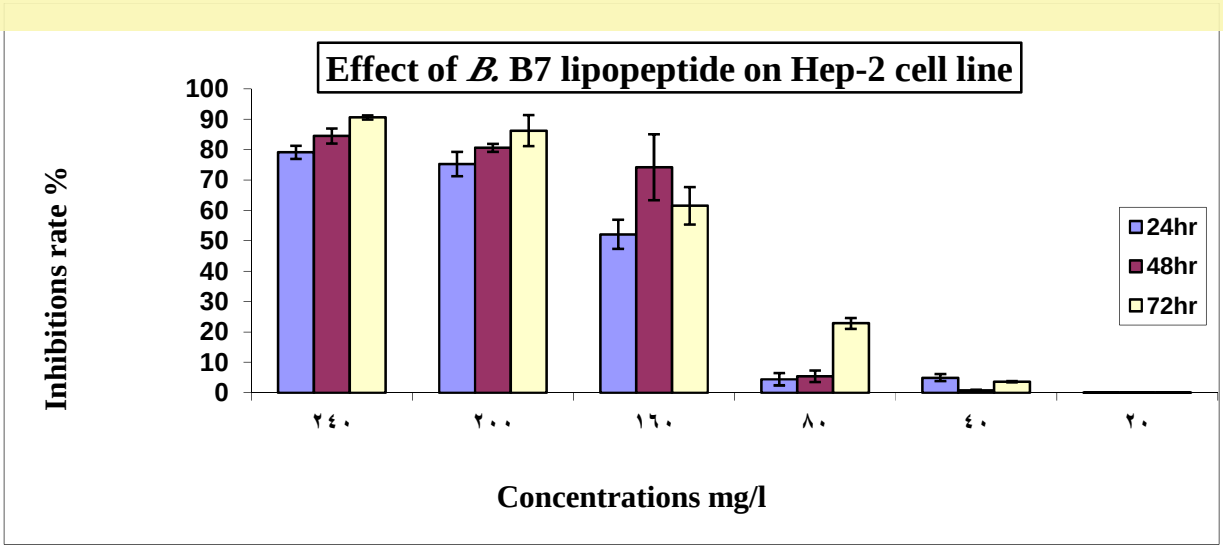
Appendix (49) Effect of *B.* B7 lipopeptide on L1210 cell proliferation for different incubation times (24,48,72)hrs.



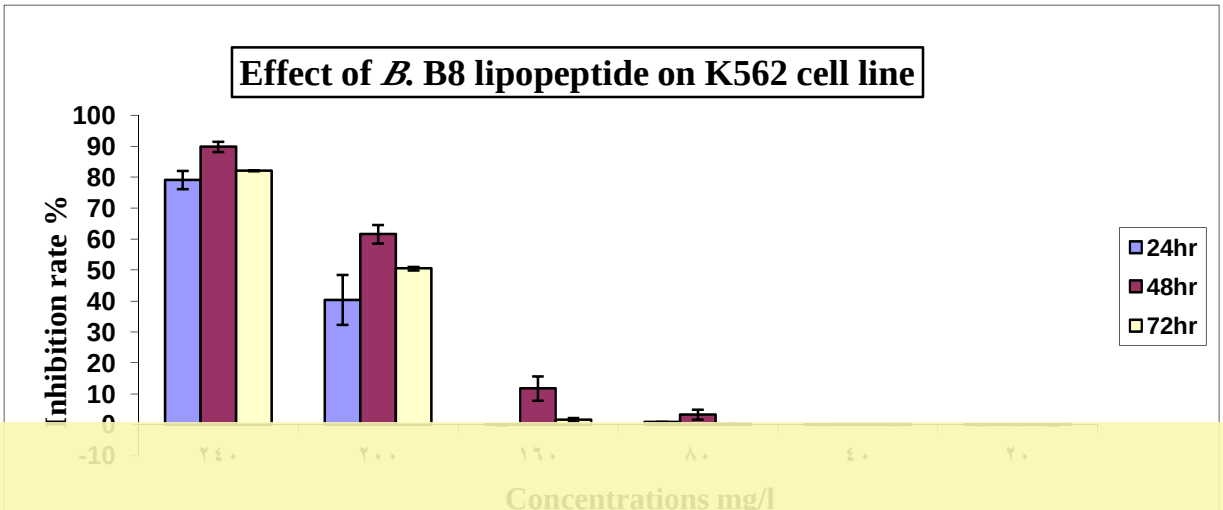
This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now



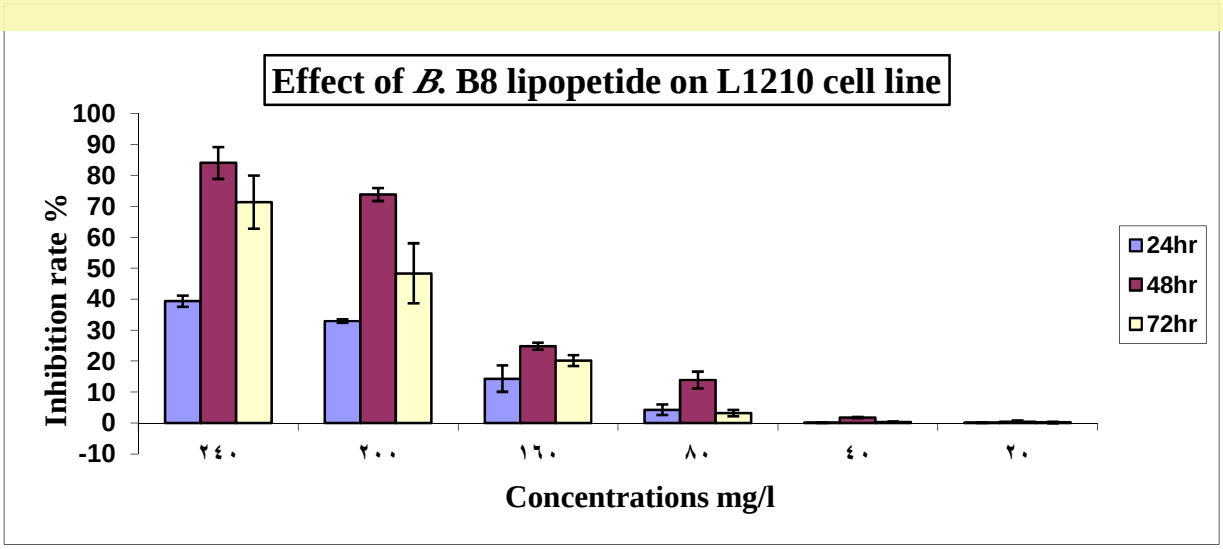
Appendix (51) Effect of *B.* B8 lipopeptide on K562 cell proliferation for different incubation times (24,48,72)hrs.



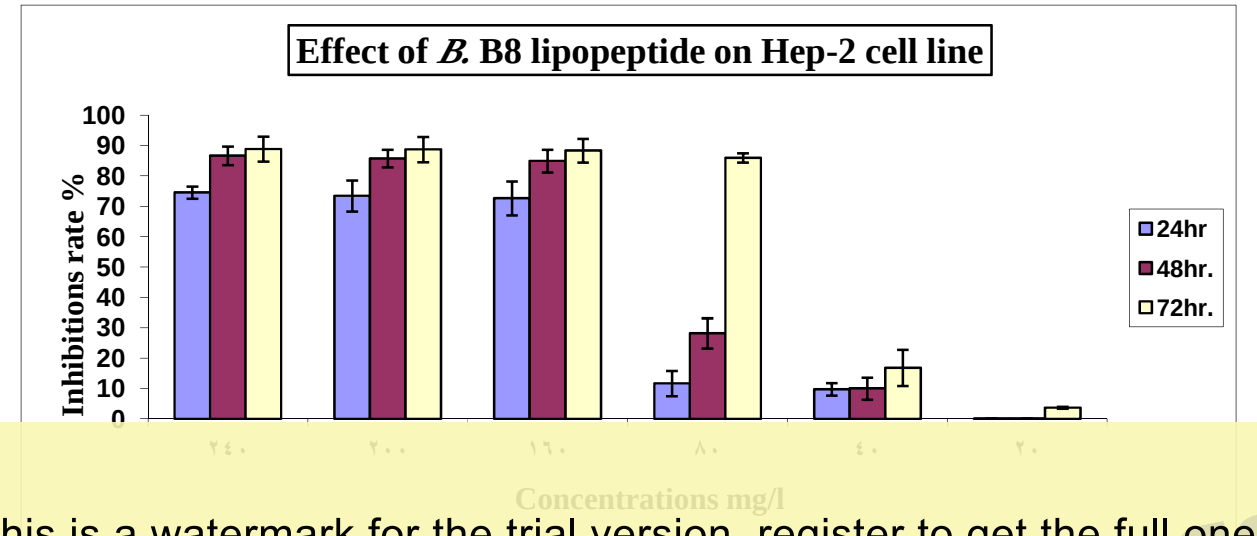
This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now



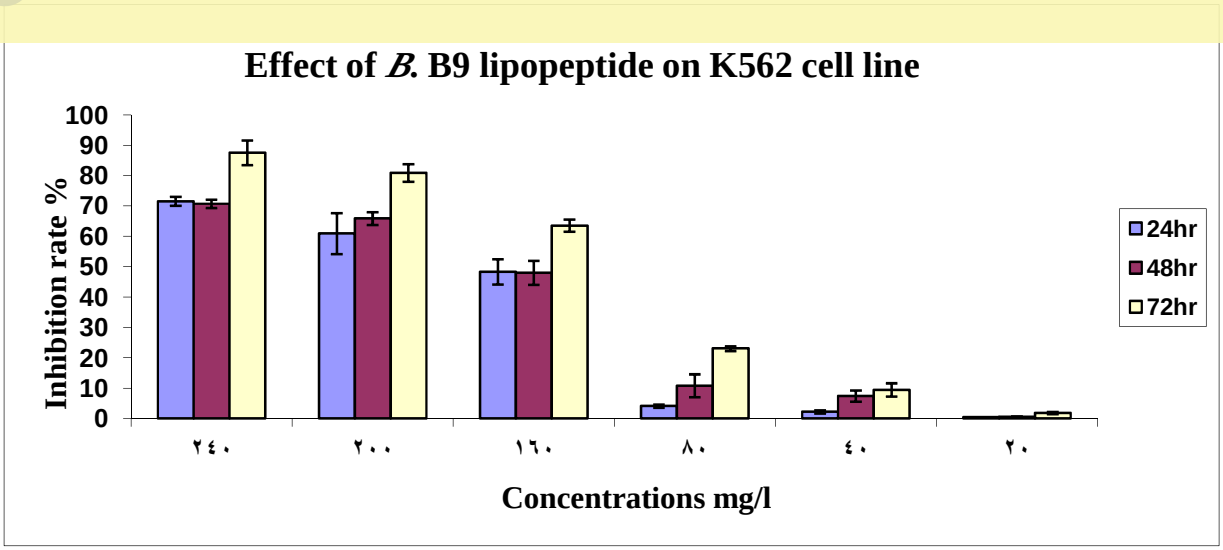
Appendix (53) Effect of *B.* B8 lipopeptide on Hep-2 cell proliferation for different incubation times (24,48,72)hrs.



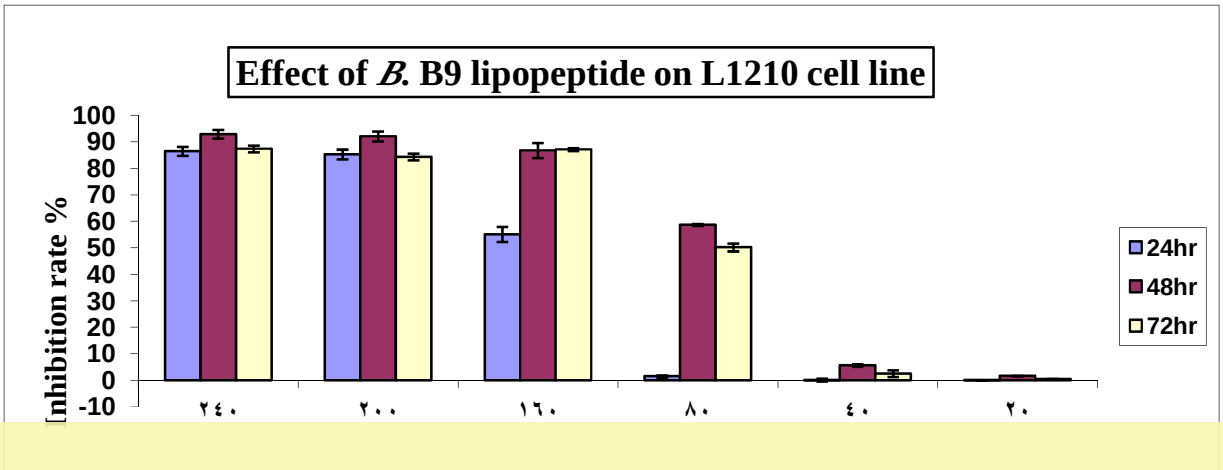
This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now



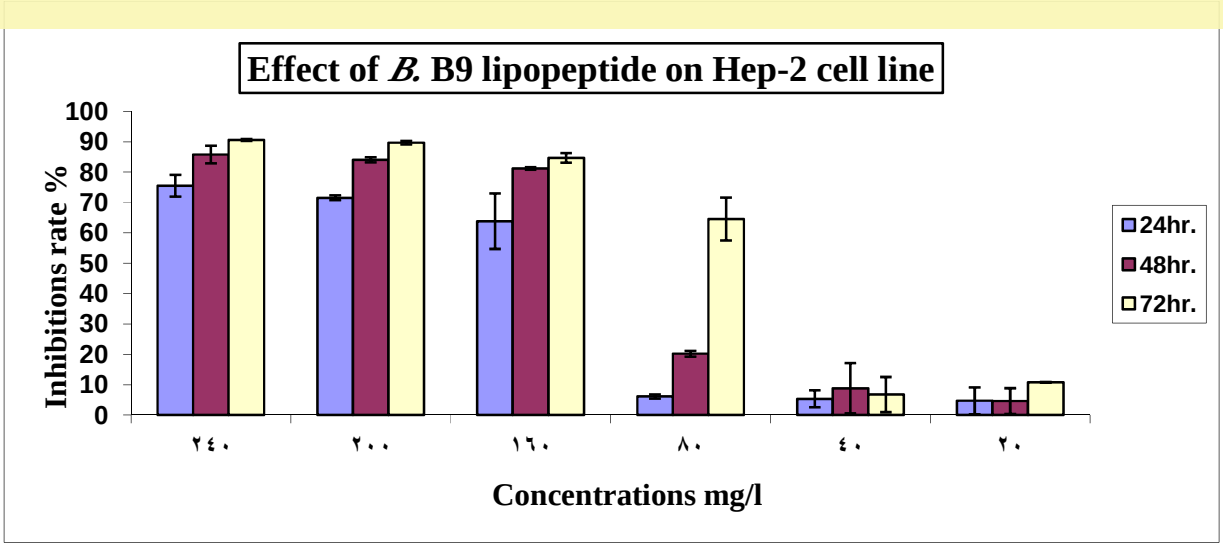
Appendix (55) Effect of *B.* B9 lipopeptide on L1210 cell proliferation for different incubation times (24,48,72)hrs.



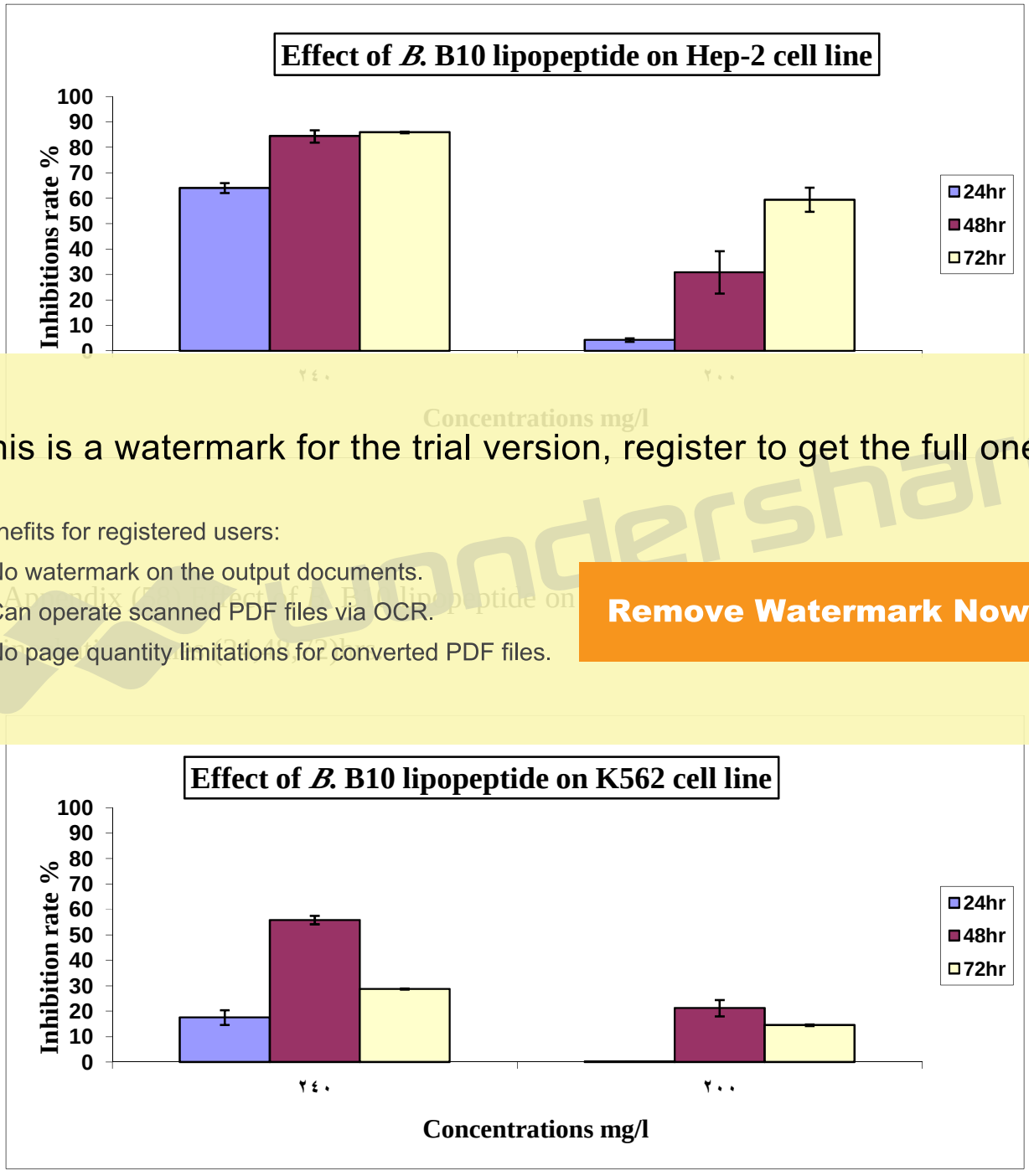
This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now



Appendix (57) Effect of *B.* B10 lipopeptide on Hep-2 cell proliferation for different incubation times (24,48,72)hrs.

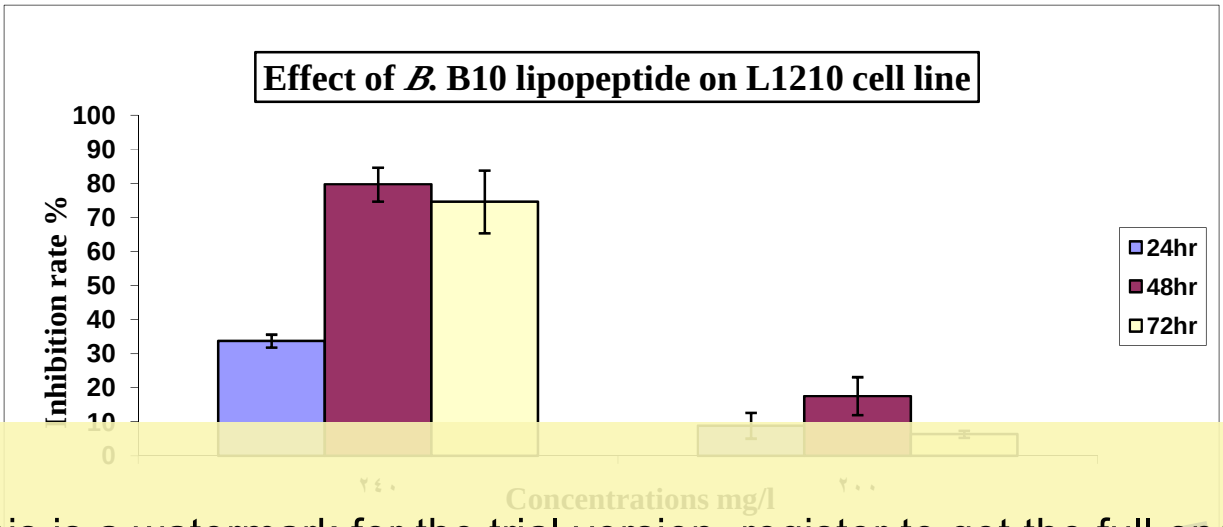


This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Appendix (59) Effect of *B.* B10 lipopeptide on L1210 cell proliferation for different incubation times (24,48,72)hrs.

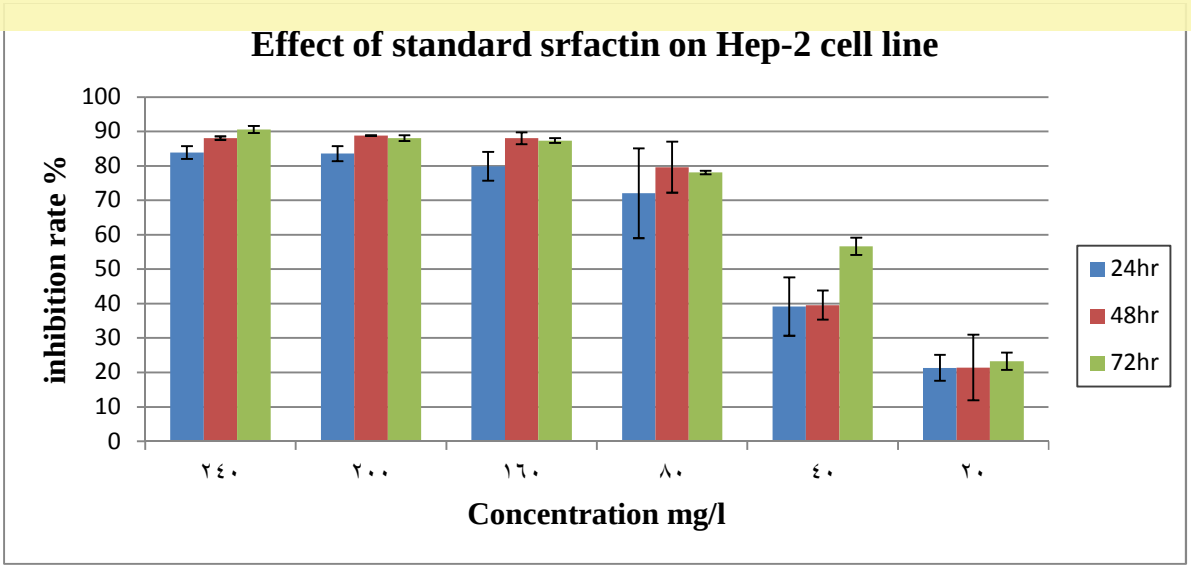


This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Appendix (60) Effect of standard surfactin on Hep-2 cell proliferation for different



This is a watermark for the trial version, register to get the full one!

Benefits for registered users:

1.No watermark on the output documents.

2.Can operate scanned PDF files via OCR.

3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Wondershare™

Biological Activity of Biosurfactants Produced by Locally Isolated *Bacillus* spp.
College of Science/Al –Nahrain University
By
Sura Ali Ibrahim
PhD \2012

Biological Activity of Biosurfactants Produced by Locally Isolated *Bacillus* spp.
College of Science/Al –Nahrain University
By
Sura Ali Ibrahim
PhD \2012

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:

1.No watermark on the output documents.

2.Can operate scanned PDF files via OCR.

3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Wondershare™

Conclusions

1- Ten locally *Bacillus* isolates were capable of producing biosurfactant with high efficiency that can lower (ST) of the cell free supernatant (40-43)%.

2- Molecular identification of these isolates showed that they belong to *Bacillus subtilis* group with similarity (97-100)%.

3- According to TLC and HPLC/MS results, biosurfactant produced by *Bacillus* isolates were belonged to the lipopeptide family, and that produced by *Bacillus* B6 was surfactin with partial sequence of Val-Asp -Leu-Leu-OH_2.

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

4- Purified lipopeptides produced by *Bacillus* isolates (B1, B3, B5, B6, B7, B8, B9, B10) were cytotoxic on L1210 and Hep 2 cell lines in a dose dependent manner.

5- Purified biosurfactant produced by *Bacillus* B7 was found to be the most cytotoxic on L1210 cell line that caused 58.62% inhibition of the cell line growth at the same concentration , however, purified biosurfactans produced by (*B. subtilis* isolates B6, B8, B9) were the most cytotoxic on Hep 2 proliferation with inhibition rate (69.219,86.043,64.6)% at concentration 80mg/l , while purified biosurfactat produced by B.B6 had low effect on the proliferation of normal cell line L02.

٦- Purified surfactin produced by B. subtilis B6 induces apoptosis in Hep-2 cell line through mitochondrial depolarization and caspase -3 activation.

7- Optimum conditions for surfactin production by *Bacillus* B6 isolate were achieved in a medium containing (1%) sucrose, (0,3%) potassium nitrate (1g/l), at pH 8, 30°C, 180rpm of shaking for 72 hrs. The biosurfactant yield was 1.4 g/l of culture medium under the optimum conditions.

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Wondershare™

Dedication

To ...

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Sura

Acknowledgement

First of all Praise to Allah , Lord of the Whole Creation . Mercy and Peace are to the Prophet Mohammed and his Family.

I would like to express my deepest gratitude to my supervisors Dr. Hameed M. Jasim and Dr. Nahi Y. Yaseen for their giddiness and great support throughout my study.

Also I would like to express my great thanks to the Iraqi Ministry of Higher Education and Scientific Research and Al- Nahrain University and everyone who helped me during my research.

My sincere gratitude and deepest thanks must go to Prof. Rui Zhou and all his students in Huazhong Agricultural University (Wuhan / China) especially Yan Wu, Rui Yang, Lei Ju, Cen Wei and Lee Timtin for their support, advice, assistant during my research.

I would like to thank all Iraqis in Wuhan who helped me especially Dr. Hatim Al- Rubaiee , Dr. Rami, Dr. Hussam, Hiam, Kawther, Raid.

I'm grateful to my friend Dr. Ghassan for his continuous advising through my work, and to all my colleagues, Dr. Khalel, Dr. Raghad, Dr. Asmaa , Dr. Rana for their friendship and encouragement .

The greatest thanks and appreciation must go to my mother, husband, brother and my aunt, for their love ,support and understanding, Finally I would like to thank my precious daughter (Sara) who made writing this dissertation the hardest thing to do.

Sura

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Republic of Iraq
Ministry of Higher Education
and scientific Research
Al- Nahrain University,
College of Science,
Department of Biotechnology

Biological Activity of Biosurfactants Produced by Locally Isolated *Bacillus* spp.

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Wondershare™

A Dissertation
Submitted to the College of Science /Al –Nahrain University as a
partial fulfillment of the requirements for the Degree of Doctorate
of Philosophy in Biotechnology

By

Sura Ali Ibrahim
B.Sc. Microbiology / College of Science /Baghdad University /2000
M.Sc. Biotechnology/ College of Science /Baghdad University /2006

Supervised by

Dr. Hameed M. Jasim (Professor)

Dr. Nahi Y. Yaseen (Professor)

January 2012 Rabi alawal 1433

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:

1.No watermark on the output documents.

2.Can operate scanned PDF files via OCR.

3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Wondershare™

1.1 Introduction

Biosurfactants are surface – active compounds produced by a wide variety of microorganisms such as bacteria, yeasts and fungi as membrane components or secondary metabolites (Gautam and Tyagi, 2006). They are a structurally diverse group of amphipathic molecules with both hydrophilic and hydrophobic moieties (Van Hamme *et al*.,2006).

The major classes of biosurfactants include glycolipids, lipopeptides and lipoproteins, phospholipids and fatty acids, polymeric surfactants and particulate surfactants (Cameotra and Makkar, 2004; Salihu *et al*., 2009).

In recent years, the interest in biosurfactants has been remarkably increasing due to many advantages compared with chemical surfactants, including lower toxicity, higher biodegradability, greater compatibility with human skin, and activity at extreme conditions (temperature, pH, salinity) (Muthusamy et al., 2008). They are mainly used in studies on enhanced oil recovery and hydrocarbon biodegradation, solubilization, emulsification and demulsification, and have potential applications in agriculture, cosmetics, pharmaceuticals, detergents, personal care products, food processing, textile manufacturing, laundry supplies, metal treatment and processing, pulp and paper processing and paint industries (Singh *et al*.,2007 ; Rahman and Gakpe, 2008).

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now
Wondershare™

Biosurfactants have various interesting biological activities such as antiviral, antitumor, antibacterial and antifungal activities, also they can be used as immunoregulators, adhesive agents, ligands for binding immunoglibulins, adjuvants for antigen ..etc (Banat *et al*., 2010 ; Fathabad, 2011).

Among the many classes of biosurfactants, lipopeptides produced by *Bacillus* spp. strains are the most powerful ones that possess many biological activities especially the antitumor activity that include the inhibition of the

carcinoma cells proliferation and induction of (differentiation , cytotoxicity ,cell cycle arrest, and apoptosis) (Wang *et al*., 2007 ; Cao *et al*.,2009a).

In Iraq , cancer is a growing problem that increases considerably every year, one of the treatment regiments is the chemotherapy which is hampered by the problem of drug-resistance, so a call for discovery of more effective agents to treat cancer is becoming increasingly urgent , for this purpose, new drugs have being synthesized and tested (WHO, 2006).

New trials for cancer treatment have been performed by many researchers in various countries including Iraq; these trials included using gene therapy, immunotherapy, biological therapy and bacterial byproducts (Mulherkar, 2001;Al-Qadoori, 2004 ; Al-Saffar, 2010; Visagie and Joubert , 2010;). However, using biosurfactants as novel bacterial products has not been tested in

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Wondershare™

- Aims of study

The present study was designed to

Hence this study aimed to achieve the following targets:

- Isolation of *Bacillus spp*. from different localities contaminated with hydrocarbons.
- Selection and identification of efficient isolates of *Bacillus* spp. producing biosurfactant .
- Purification of biosurfactant to homogeneity level.
- Application of the biosurfactants as antitumor agents

1.2 Literature review

1.2.1 Genus *Bacillus*

1.2.1.1 General features

It is a large Genus of Gram-positive rods that were first recognized and named by Ferdinand Cohn in 1872 , they may occur singly or in chain, some are strict aerobes and others are facultative anaerobes. The vegetative bacilli are large (0.5 ×1.2 µm to 2.5 ×10 µm) and straight, they can form highly resistant dormant endospores in response to nutrient deprivation and other environmental stresses.

The majority of vegetative cells are mesophiles with temperature optimum 30 °C or 45 °C, but the genus contains also a number of thermophilic species with optimum at 55 °C or higher. Cells usually are motile by means of peritrichous flagella, some species are capsulated, some produce extracellular media that contain sucrose (Holt *et al*., 1994; Priest, 2003). *Bacillus* strains vary in their requirements that are imposed on the culture such as (medium composition, temperature of incubation, humidity,etc.), Some *Bacillus* species tend to swarm on solid media, especially if plates are not dried to remove the surface moisture before inoculation (Duguid ,1996 ; Rudner *et al*.,1998).

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now

Endospores in pure culture are regular in size, shape and position within the sporangium, in most species they are oval (ellipsoidal) in shape, and central or subterminal in position. In a few species the spores are spherical in shape and in a few they are terminal in position, when they viewed under microscope unstained they appear edged in black and very bright and refractile (Holt *et al*., 1994; Duguid , 1996; Piggot and Hilbert, 2004).

Endospores formation is affected by some factors including the temperature of growth, the pH ,aeration, presence of minerals , presence of certain carbon or nitrogen compounds and the concentration of the carbon or nitrogen source, in some circumstances a starvation for phosphorus source ,population density, cell cycle (Errington,1993 ; Piggot and Hilbert,2004 ;Goesselsberger *et al.*,2009).

Endospores are highly resistant to environmental stress such as high temperature , low temperature ,irradiation, presence of strong acids, disinfectants, nisin, , high sodium chloride concentration etc. Mature spores have no detectable metabolism , a state that is described as cryptobiotic, they retain viability indefinitely such that under appropriate environmental conditions, they germinate into vegetative cells (Nicholson, 2002 ; Faille *et al*., 2002; Cartman *et al*., 2008).

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

1.2.1.2 Classification and phylogeny

The earlier taxonomy of the bacilli was very confused, yielding more than 150 named species which often described on the basis of single strains, the number was reduced by Smith *et al*., (1952) which based on the shape of the endospore and its position in the mother cell or sporangium (Priest *et al*.,1988; Priest, 2008).

This taxonomic was accepted until the introduction of modern taxonomic techniques such as numerical phonetics, DNA base composition determination and DNA reassosiation experiments, 16S rDNA sequence analysis , which found that *Bacillus* comprise more heterogenous species than scientists expected as shown in the GC content variation of known species of *Bacillus* which range from 32%-69% as listed in Bergeys Manual of Systemic Bacteriology (1986) by Claus and Berkeley. This indicates considerable genetic diversity among species and suggests that the genus should perhaps be split into several, more homogeneous taxa , so in the 1990s the genus *Bacillus* comprises in excess of 60 species as listed by

Konemann *et al.*(1997), but this taxonomy is not stable as sequence analysis has led and continues to lead, on the one hand, to the separation of groups of species from the core genus *Bacillus* to form new genera and, on the other hand, to the definition of novel genera to allocate new isolate.

Recently, according to Garrity *et al.* (2004), *Bacillus* is only one out of a whole series of genera of aerobic endospore-forming bacteria(AEFB) in which it can be classified as follows:

Phylum BXIII. *Firmicutes* phy. nov.

Class III. *Bacilli*

Order I. *Bacillales*

Family I. *Bacillaceae*

Genus I. *Bacillus*

Species [1]

Sub species [2]

The descriptions of these genera and species are based to a varying extent upon morphological, biochemical, physiological, and chemotaxonomical traits and on the results of various molecular genetic techniques.

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Wondershare™

1.2.1.3 Natural products of *Bacillus* spp.

Members of the *Bacillus* genus are often considered as microbial factories for the production of a vast array of biologically active molecules with the potential for technical and scientific applications, on the other hand, several strains of *Bacillus* species also produce compounds toxic to mammalian cells (Lugan ,1988; Outtrup and Jörgensen 2002).

For example *Bacillus* genus produce exocellular enzymes (e.g. amylases and proteases) that form over one-half of the total commercial enzyme volume (Crueger and Crueger ,1982; Fogarty and Kelly ,1990), also some *Bacillus* spp produce different kinds of surface active compounds which known as Biosurfactants (e.g. surfactin, iturins, lichenysin A) that have a wide range of applications , also many strains of *Bacillus* produce several bacteriocin-like substances, e.g. some strains of *B. cereus* produce the bacteriocin cerein 7, a pore-forming peptide of 3940 Da (Oscariz *et al*. 1999, Oscariz and Pisabarro 2000).

Bacillus spp. can also produce a vast array of antibiotics that active against important pathogens such as methicillin-resistant *Staphylococcus aureus* (Chatterjee *et al*. 2005).

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now

1.2.2 Surfactants

The word surfactant derives from the contraction of the terms surface-active-agent, several molecules which [illegible] their amphiphlilic composition of two functional moieties: polar with the main hydrophilic group and non-polar with lipophilic trait that tend to partition preferentially at the interface between fluid phases with different degrees of polarity and hydrogen bonding such as oil / water or air / water interfaces (Desai and Banat, 1997; Rahman and gakpe,2008). The formation of such an ordered molecular film at the interface lowers the interfacial energy (interfacial tension) and surface tension which is responsible for the unique properties of surfactant molecules (Lin, 1996).

Surfactants are defined by their capability of reducing the surface tension (ST), critical micelle concentration (CCM) (which is the minimum surfactant

concentration required for reaching the lowest interfacial or surface tension values) , interfacial tension and hydrophilic-lipophilic balance (HLB) , (surfactants with HLB values less than 6 are more soluble in the oil phase ; while those with HLB values between 10 and 18 have the opposite characteristics) (Cooper and Zajic, 1980 ; Parkinson, 1985).

The hydrophobic part of the surfactant is a long – chain of fatty acids , hydroxy fatty acids , hydroxyl fatty acid or α- alkyl –β-hydroxy fatty acids which is usually a C8 to C22 alkyl chain or alkylaryl that may be linear or branched while the water soluble end (hydrophilic) can be a carbohydrate, amino acids, cyclic peptide, phosphate, carboxylic acid or alcohol (Oberbremer *et al*., 1990 ; lin *et al*., 1994a).

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now

There are a broad classes of surfactants, petroleum based chemical surfactants and biologically based surfactants produced from animal or plant materials or of microbial origins which known also as biosurfactants (Horowitz *et al*., 1990 ; Mulligan *et al*., 2002 ; Nitschke *et al*., 2005).

2.2.1 Petroleum – based chemical surfactants

Surfactants under this category are synthesized from petroleum refining by-products that are found in light cuts (gasoline and kerosene) coming from atmospheric distillation and catalytic cracking or by polymerization of short chain olefin, particularly in C3 and C4 (Salager, 2002).

They can be classified into anionic, cationic, amphoteric, and nonionic classes depending on the nature of their polar grouping, regarding the hydrophobic moiety of the molecule, it is a hydrocarbon chain in most common surfactants; however, in some more specialized surfactants, this hydrophobic part can be a nonhydrocarbon chain such as a polydimethylsiloxane or a perfluorocarbon (Van Ginkel, 1989).

Anionic surfactants are negatively charged which is usually due to a sulphonate ,sulphur , carboxylates , or phosphates group. They are the most commonly used surfactants that accounting for about 50 % of the world production. (Int .2)

A general formula may be ascribed to anionic surfactants as follows:

* Carboxylates: $C_nH_{2n+1}COO^-X$
* Sulphates: $C_nH_{2n+1}OSO_3^-X$, (Figure 2-1)
* Sulphonates: $C_nH_{2n+1}SO_3^-X$
* Phosphates: $C_nH_{2n+1}OPO(OH)O^-X$

With n = 8–16 atoms and the counter ion X is usually Na^+.

Several other anionic surfactants are commercially available such as sulphosuccinates, isethionates and taurates and these are sometimes used for [illegible]

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

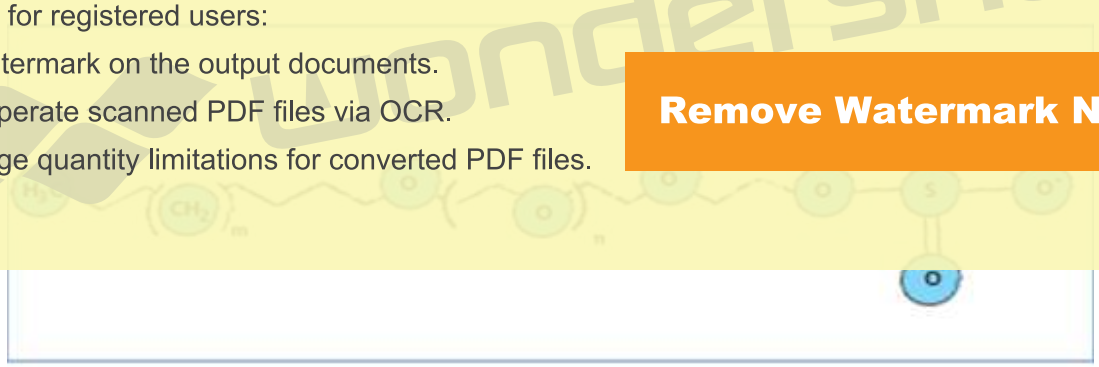


Figure (1-1) Alkyl ether sulphate as an example of anionic surfactant (Tadros, 2005)

Nonionic surfactants that lack ionic constituent, they come as a close second with about 45% of the overall industrial production. They do not ionize in aqueous solution, because their hydrophilic group is of a non dissociable type, such as alcohol, phenol, ether, ester, or amide (Sarney and Vulfson, 1995).

There are two major classes of anionic surfactants:

- Ethoxylated surfactants such as fatty acid ethoxylates with the general formula RCOO-$(CH_2CH_2O)_nH$.
- Multihydroxy products such as glycol esters, glycerol (and polyglycerol) esters(Tadros , 2005).

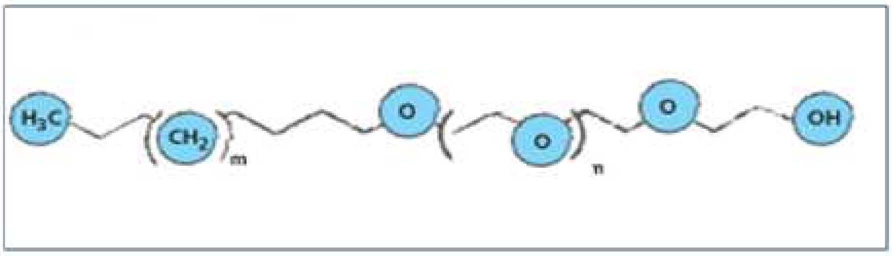


Figure (1-2) Non ionic surfactants (Sarney and Vulfson, 1995)

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now
Wondershare™

Cationic surfactants are dissociated in water into an amphiphilic cation and an anion, most often of the halogen type (Figure 1-3). The most common kinds of these surfactants are the quaternary ammon[illegible] [illegible]lly [illegible] groups. (Tadros , 2005) .

These surfactants are in general more expensive than anionics, because of the high pressure hydrogenation reaction to be carried out during their synthesis. (Salager, 2002)

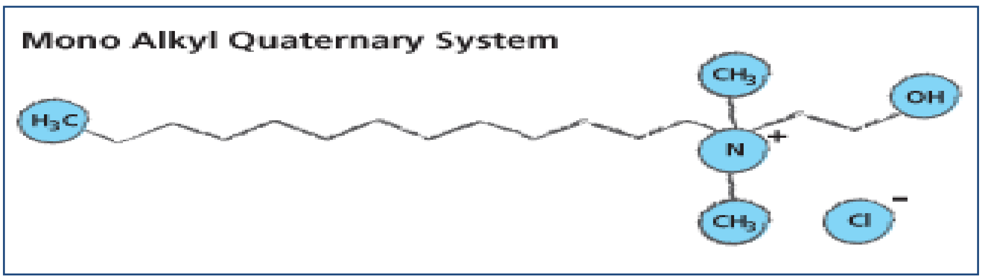


Figure (1-3) Cationic surfactants (Salager, 2002)

- **Amphoteric surfactants** that have both positively and negatively charged moieties in the same molecule such as Alkyl betaines, alkyldimethylamines , imidazonilinum derivatives (Salager, 2002).

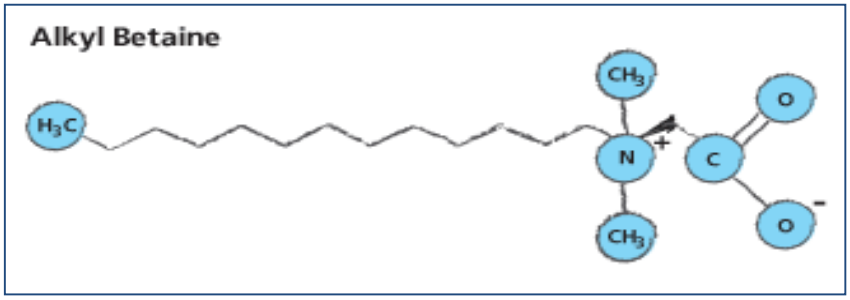


Figure (1-4) Amphoteric surfactants (Salager, 2002)

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now

consists of zwitterionic phosphatidylethanolamine and phosphatidylcholine, its mainly used as a pharmaceutical recipient for drug delivery and intravenous nutrition (Wang and Wang, 2008).

Gelatin is a high – molecular – eight polymer that obtained through the partial hydrolysis of collagen with dilute acid or base, it's a relatively poor protein surfactant, but its emulsifying properties can be improved by enzyme – catalyzed attachment of hydrophobic side chains (Dickinson, 1993). The main sources of commercial gelatin are bovine skin and bones and pigskin. It is mainly used as a stabilizer, thickener and texturizer in food and non – food applications (Karim and Bhat, 2008).

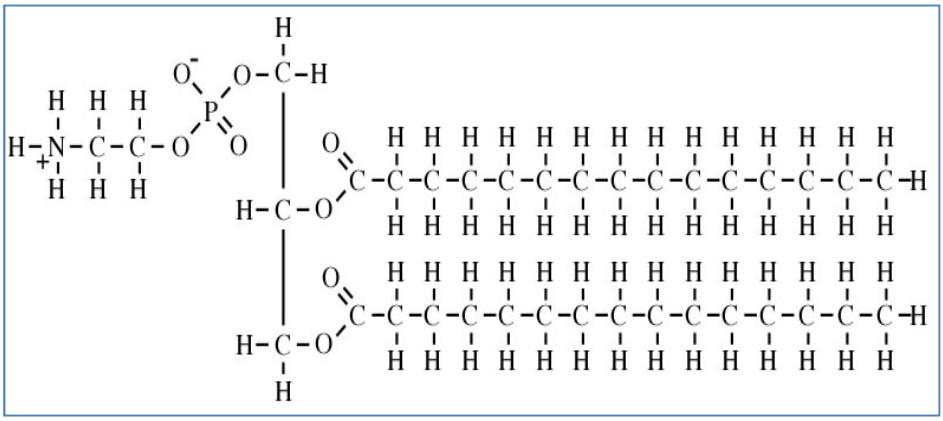


Figure 1-5 Chemical composition of lecithin (Wang and Wang, 2008)

1.2.2.3 Plant – derived surfactants

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

beans, kidney beans and lentils; the saponin content in soybeans is 5–6% (Oakenfull, 1981).

Another example are the plants surface-active proteins. Soy protein is one of the most important plant-derived protein surfactants. Soybeans contain about 40% protein and 20% oil. Soy proteins are mainly globulins and can be classified into 2S, 7S, 11S, and 15S fractions. Soy proteins are available in three major forms that vary in protein content: soy flours, soy protein concentrates and soy protein isolates (Iwabuchi and Yamauchi, 1987).

1.2.2.4 Biosurfactants

Biosurfactants are surface active metabolites produced by microorganisms (Bacteria, yeasts and fungi) when grown on water miscible or oily substrates or both (Arino *et al*., 1996; Kiran *et al*., 2009; Rufino *et al*., 2011). They either remain adherent to microbial cell surfaces or are secreted in the culture broth (Olivera *et al*., 2009; Fathabad,2011). Their molecular masses generally range from 500 to 1500 Da.(Van Hamme *et al*.,2006).

Although , most biosurfactants are considered to be secondary metabolites , some may play essential roles for the survival of biosurfactant – producing microorganisms through facilitating nutrients uptake or microbial – host interactions or by acting as biocide agents or promoting the swarming motility of microorganisms and participating in the physiological processes of growth and differentiation (Ahimou *et al*., 2000; Kearns and Losick, 2003; Das *et al*., 2008).

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now

1.2.2.4.1 Classification of Biosurfactants

Biosurfactants are classified according to their chemical composition and microbial origin. For example Biermann *et al*. (1987) group biosurfactans based on their composition into glycolipids, lipopeptides , phospholipids, fatty acids, neutral lipids , polymeric and particulate compound. While Kosaric (1992) classify them depending on their structure namely; hydroxylated and cross- linked fatty acids, polysaccharide – lipid complexes, glycolipids, lipoproteins – lipopeptides, phospholipids and complete cell surfaces while Healy *et al*., (1996) group biosurfactants into four main categories namely, glycolipids, phospholipids, lipoproteins / lipopeptides and polymeric. Lastly, Rosenberg and Ron (1999) suggested that biosurfactants can be divided into two categories: low – molecular – mass molecules, which efficiently lower surface and interfacial tension, and high – molecular – mass polymers, which are more

effective as emulsion – stabilizing agents. The major classes of low – mass surfactants include glycolipids , lipopeptides and phospholipids, whereas high – mass surfactants include polymeric and particulate surfactants.

1.2.2.4.2 Types of biosurfactants

There are many types of biosurfactants that produced by various microorganisms, the following are some of the various types of biosurfactants.

1.2.2.4.2.1 Glycolipid biosurfactants

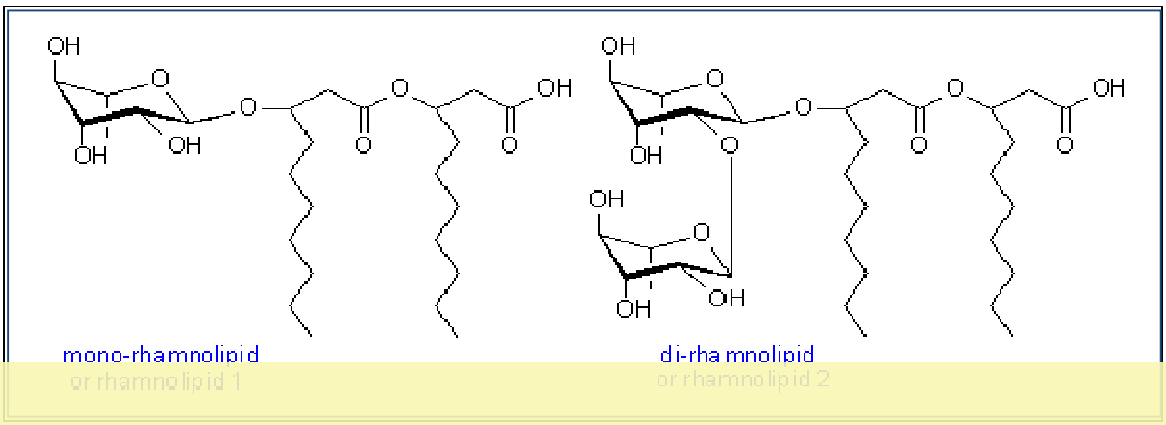
The most known biosurfactants are glycolipids , they are carbohydrates in combination with fatty acids , the linkage is by means of either ether or an ester group. the carbohydrate moiety is one of monosaccharides and disaccharides which include glucose , mannose , galactose, glucuronic acid , rhamnose and galactose sulphate.(Veenanadig *et al*., 2000) while the fatty acid component usually has a comparatively [illegible] phospho[illegible] the [illegible] trehalolipids and sophorolipids (Desai and Banat , 1997; Morita *et al*., 2006).

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now
Wondershare™

- **Rhamnolipids**

They are generally a mixture of homologous species of glycolipids produced by the genus *Pseudomonas* , they composed of one or two molecules of rhamnose linked to one or two molecules of β-hydroxydecanoic acid usually, but other fatty acids may be found depending on the *Pseudomonas* species or growth conditions (Figure 1.6)(Desai and Banat , 1997), they have the ability to lower the interfacial tension against *n* – hexadecane to mN/m and the surface tension to 25 -30 mN/m (Guerra –Santos *et al*., 1986).

Also two unusual rhamnolipids, designated myxotyrosides A and B, have been isolated from a *Myxococcus sp*, they have a rhamnose unit linked to tyrosine and hence to a fatty acid (Ohlendorf *et al*., 2009).



This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now

Figure 1.6 Different types of rhamnolipid biosurfactants (Guerra-Santos *et al*., 1986)

- Trehalolipids

Trehalolipids, trehalose containing

group, such as *Mycobacterium, Rhodococcus ,Corynebacterium, Arthrobacter, Nocardia* and *Gordonia*, different structures have been elucidated particularly in *Rhodococcus* genus(Franzetti *et al*., 2010). They are composed of Trehalose (is a non-reducing disaccharide in which the two glucose units which linked together linked either to Mycolic acids in the *Mycobacterium* and most species of *Crynebacterium* and *Nocardia* (Silva *et al*., 1979 ;Gautam and Tyagi, 2006) or to corynomycolic or nocardomycolic in the case of rest species of *Corynebacterium* and *Nocardia* (Goodfellow *et al*., 1973; Shimakata and Minatogawa ,2000) .

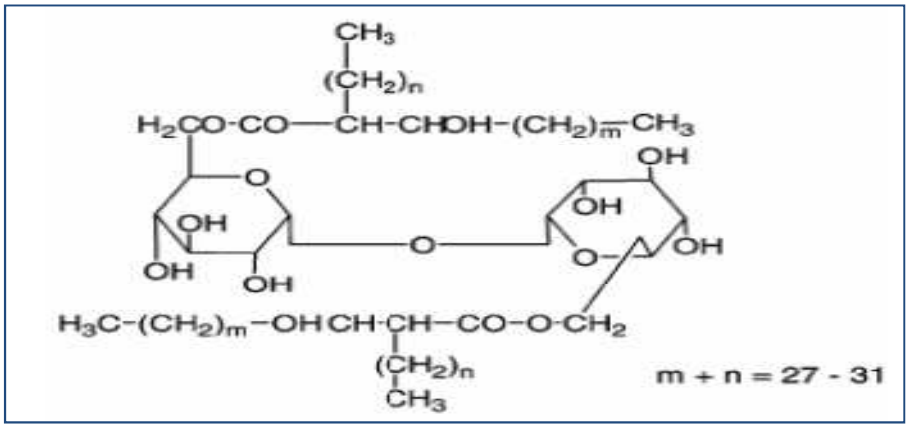


Figure 1.7 Trehalolipid biosurfactant (Ortiz *et al.*, 2008)

- **Sophorolipids**

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now

They are group of biosurfactants produced by some yeast species, and in ... *(sophorose) and a* long chain hydroxyl fatty acid linked by ... (Asmer *et al.*, 1988).

... acidic SLs and the lactone SLs, the ... acidic SLs has a free carboxylic acidic functional group whilst that of the lactonic SLs forms a macrocyclic lactone ring with the 4"- hydroxyl group of the sophorose by intramolecular esterification. (Figure 1.8)

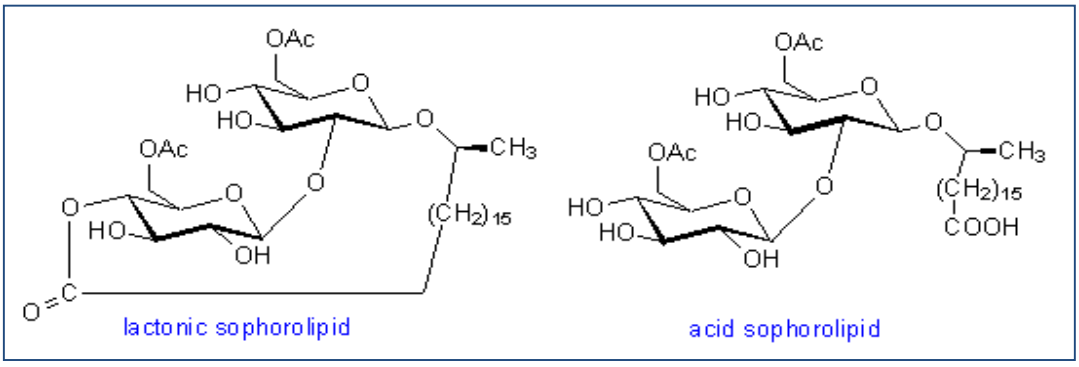


Figure 1.8 Types of Sophorolipid biosurfactants (Hu and Ju ,2001)

1.2.2.4.2.2 Fatty acids biosurfactants

Biosurfactants under this category are produced from alkane as a result of microbial oxidations (Rehn and Reiff, 1981). These fatty acids are either straight chain acids, or complex fatty acids containing OH groups and alkyl branches such as Corynomucolic acids (Kretschner *et al*., 1982) but the most active saturated fatty acids in lowering surface and interfacial tensions are in the range of C12-C14 because the hydrophilic or lipophilic balance of fatty acids is related to the length of the hydrocarbon chain (Rosenberg and Ron, 1999).

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now

polymeric biosurfactant (Rosenberg *et al*., 1979), its polyanionic amphipathic heteropolysaccharide bioemulsifier as mentioned in (Figure 1.9), It's a very effective emulsifying agent for hydrocarbons in water even at a concentration as low as 0.001-0.01% (Zosim *et al*., 1982). Another example of this type of biosurfactants is liposan which was synthesized by using *Candida lipolytica* , its composed of 83% carbohydrate and 17% protein with the carbohydrate portion being a heteropolysaccharides consisting of glucose, galactose, galactosamine and galactoronic acid (Ciriglian and Carman, 1984).

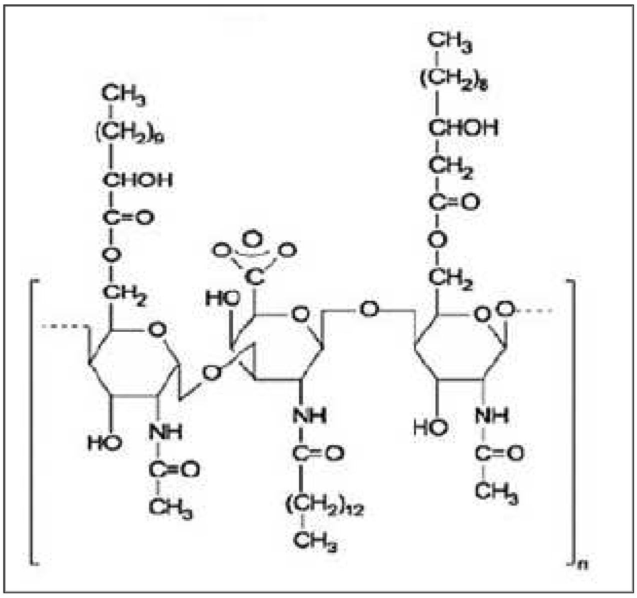


Figure 1-9 Emulsan (Zosim *et al*., 1982)

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now

1.2.2.4.2.5 Particulate biosurfactants

Surfactant activity in most hydrocarbon- degrading and pathogenic bacteria is due to several cell surface componen[illegible] M protein and lipoteichoic acid in grou[illegible] *Staphylococcus aureus*, Layer A in *Aeromonas salmonicida* (Desai and Banat, 1997).

1.2.2.4.2.6 Lipopeptide biosurfactants

Biosurfactants of this type possess remarkable surface- active properties and produced by a wide variety of microorganisms such as *Agrobacterium tumefaciens* that produced lysine- containing lipids (Tahara *et al*., 1976), *Pseudomonas rubescens* produced Ornithine- containing lipids (Yamane,1987), but the majority of these biosurfactants are produced from several species of the genus *Bacillus* that can be classified into three families:(Ongena and Jacques, 2007).

- Lipopeptides of the surfactin family

- Lipopeptides of the iturin family
- Fengycins and various lipopeptides

Bacillus lipopeptides consist of a peptide part containing 7-11 amino acids, either cyclic or linear or a combination of these while the lipid part is composed of β-hydroxy or β-amino fatty acids connected to the peptide backbone. The hydrocarbon length of the fatty acids and amino acid composition may vary, depending on the nutrition of the bacteria, and affect the properties of the lipopeptides. The cyclic structure of the peptide part protects the lipopeptide from enzymatic cleavage and maintains its general stability (Desai and Banat, 1997; Peypoux *et al*., 1999; Muthusamy *et al*., 2008).

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now
Wondershare™

Lipopeptides of the surfactin family (surfactin, lichenysins, Esperin and pumilacidins)(Figure 1.10) contain a cyclic heptapeptide acylated with β-hydroxy fatty acid of variable length (C12 to C16 with n, iso, or anteiso branching (Horowitz *et al*., 1990; Yakimov *et al*., 1996). These peptides are powerful biosurfactants, produced by strains of several *Bacillus* species such as *Bacillus subtilis* (Arima *et al*., 1968), *Bacillus coagulans* (Huszcza and Burczyk, 2006), *Bacillus pumilus* and *Bacillus licheniformis* (Naruse *et al*., 1990; Thaniyavarn *et al*., 2003).

Surfactin produced by *Bacillus subtilis* ATCC 21332 is one of the most powerful biosurfactants was discovered by Arima *et al.* (1968) , it was named surfactin because of its exceptional surfactant activity, its structure was elucidated as that of a macrolide lipopeptide by Kakinuma *et al*., (1969) ,Surfactin contains a heptapeptide (Glu1-Leu2-Leu3-Val4-Asp5-Leu6-Leu7) , it lowers the surface tension from 72 to 27.9 mN/m at concentration as low as 0.005% (Arima *et al*., 1968).

The natural analogue is lichenysin A has Gln in position 1 and Ileu at position 7, It is produced by *B. licheniformis* during anaerobic and aerobic growth, it was first isolated from oil wells and its structure was elucidated by Yakimov *et al*. (1995) and Yakimov *et al*. (1999), another analog is pumilacidin which has Leu at position 4, whereas other variations are located at position 7.

Bacillus coagulans has been found to produce several surfactins, four main components with molecular weights 1007, 1021 and 1035 Da were separated.

Their structures have been confirmed by spectrometric and spectroscopic studies and by acid hydrolysis. The compounds were found to represent two pairs of surfactin isoforms in which beta-hydroxy-iso-C14 or anteiso-C15 fatty acids are linked to the [Leu7] or [Val7] heptapeptide moiety by both an amide group and a lactone bond (Hueso and Peypoux, 1996).

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

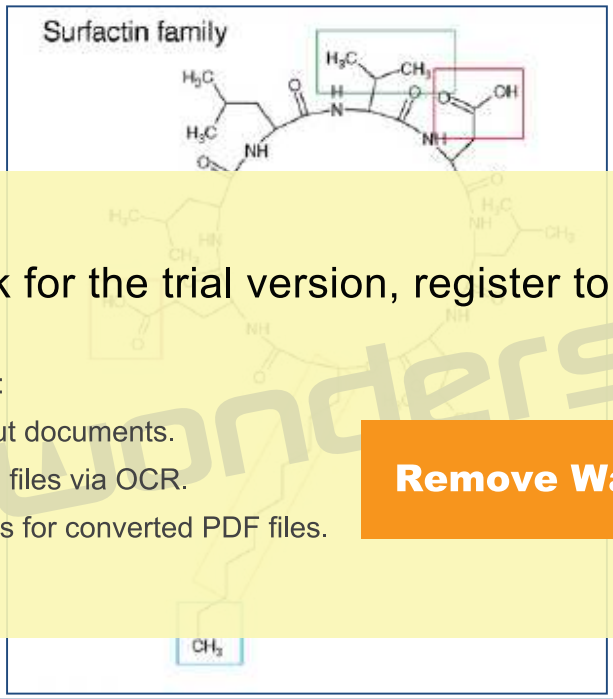
- **Lipopeptides of the iturin family**

Lipopeptides of the iturin family, such as [illegible] (Maget-Dana and Peypoux, 1994; Jacques, 2007), they contain a cyclic heptapeptide acylated with β-amino fatty acids with a chain length of C14 to C16 (Peypoux *et al*. 1978, Hourdou *et al*. 1989). Iturin A contains the heptapeptide Asn1-Tyr2-Asn3-Gln4-Pro5-Asn6-Ser7, whereas in the other members the amino acid residues in the heptapeptides vary slightly; e.g. mycosubtilin has Asn1-Tyr2-Asn3-Gln4-Pro5-Ser6-Asn7 (Yu *et al*. 2002).

- **Fengycins and various lipopeptides**

Fengycins (Figure 1.12) and the structurally similar plipastatins are distinguished from the other lipopeptides by the way in which the β-hydroxy fatty acid is linked with the polar dipeptide which is associated with the cyclic

octapeptide. The cyclic structure of fengycin *cyclo*(*D-allo*-Thr1-*L*-Glu2-*D*-Ala3/*D*-Val3-*L*-Pro4-*L*Gln5-*L*-Tyr6-*L*-Ile7-*D*-Tyr8)*D*-Orn9-*L*-Glu10-β-OH-FA) is formed when *D*-Tyr8 is connected with amide bonds to *D-allo*-Thr1 and *D*-Orn9 and forms a lactone bond with *L*Ile7. The structure of fengycin A contains *D*-Ala3 instead of the *D*-Val3 of fengycin B (Volpon *et al*.,2000).



This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

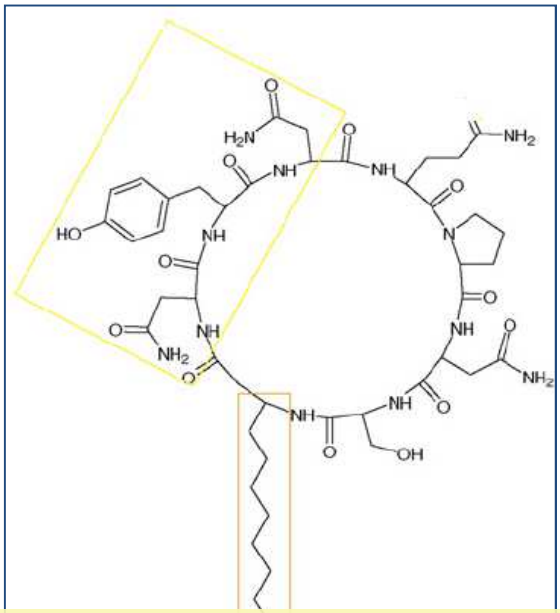
Wondershare™

Variants		Lenght and branching of the acyl chain
Esperin**	**L-Glu-L-Leu**-D-Leu-**L-Val**-L-Asp-D-Leu-**L-Leu-COOH**	
Lichenysin***	**L-XL_1-L-XL_2**-D-Leu-**L-XL_4**-L-Asp-D-Leu-**L-XL_7**	*i*-C_{13}, *ai*-C_{13}, *n*-C_{14}, *i*-C_{15}, *ai*-C_{15}
Pumilacidin	**L-Glu-L-Leu**-D-Leu-**L-Leu**-L-Asp-D-Leu-**L-XP_7**	
Surfactin	**L-Glu-L-XS_2**-D-Leu-**L-XS_4**-L-Asp-D-Leu-**L-XS_7**	*i*-C_{14}, *n*-C_{14}, *i*-C_{15}, *ai*-C_{15}

** the β-carboxyl of Asp_5 is engaged in the lactone
*** or halobacillin
XL_1 = Gln or Glu ; XL_2 = Leu or Ile ; XL_4 and XL_7 = Val or Ile ;
XP_7 = Val or Ile ;
XS_2 = Val, Leu or Ile ; XS_4 = Ala, Val, Leu or Ile ; XS_7 = Val, Leu or Ile

n, linear
i, iso
ai, anteiso

Figure (1.10) Surfactin family (Ongena and Jacques, 2007)



This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Figure (1.11) Iturin Family (Ongena and Jacques, 2007)

Fengycins have stereoisomeric composition different from those of plipastatin. Fengycins contain *D*-Tyr8 instead of the *L*-Tyr8 of plipastatins and *L*-Tyr6 instead of the *D*-Tyr of plipastatins (Volpon *et al*. 2000). While Hathout *et al*. (2000) reported from *Bacillus thuringiensis kurstaki* HD-1 an antifungal compound structurally resembling plipastatin and fengycins.

Analogues of iturin and fengycin were reported with a double bond in the fatty acid part such as in *B. thuringiensis* strain CMB26 that produced an analogue of fengycin with a double bond in the fatty acid. It was fungicidal, bactericidal, and

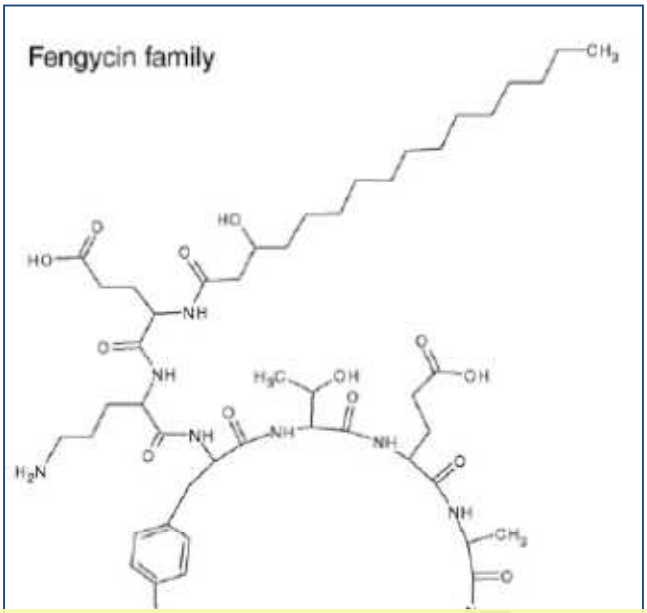
insecticidal, and more effective against fungi than was iturin or surfactin (Kim *et al*. 2004). The fatty acids in iturins were predominantly C16 and C17 (Vater *et al*. 2002, Deleu *et al*. 2005). Recently *B. subtilis* strain GA1 was described as producing three lipopeptides: surfactin, fengycin, and iturins (Toure *et al*. 2004).

Another group of cyclic hexapeptide lipopeptides contains six amino acids acylated with 15-guanidino-n-hydroxypentadecanoic acid (bacillopeptins, fusaridins and LI-F. The fusaricidins (Kajimura and Kaneda 1997, Beatty and Jensen, 2002) and LI-F (Kurusu *et al*. 1987, Kuroda *et al*. 2000) from *Paenibacillus polymyxa* were fungicidal and antibacterial. LI-F compounds containing an azole group were toxic to ddY mice (Kuroda *et al*. 2001). The target of the azole group containing fungicidals target is cytochrome P450 which is also present in mammals (Yoshida 1988) while polymyxins lose their antibiotic activity when administered orally (Mclean *et al*. 2002) and impair mitochondrial functions (Rodriquez and Acosta 1996).

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now
Wondershare™

Several methods for screening and estimating of biosurfactants have been developed.These methods were as follows:

- Drop-collapse method is one of the qualitative methods used to determine the presence of biosurfactant. Tugrul and Cansunar (2005) conducted experiments to confirm the reliability of the method using polystyrene microwell plate; oil-coated wells collapse was observed when the culture broth contained biosurfactant and there was no change in the shape of the droplets in the absence of biosurfactant.



This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

** double bond between carbons 2-3, 3-4 or 13-14 were reported for some acyl chains

Figure (1.12) Fengycins family (Ongena and Jacques, 2007)

- Thin layer chromatography (TLC) is also used in preliminary characterization of the biosurfactant where the cell free extract containing biosurfactant is separated on a silica gel plate using chloroform: methanol: water (70:10:0.5, v/v/v); this is then followed by using color developing reagents. Lipopeptide biosurfactant showed red spots in the presence of ninhydrin reagent, while glycolipid biosurfactant is detected as yellow spots when anthrone is used as the color reagent (Yin *et al.*, 2009).

- Additionally, blood agar hemolysis tests is another method used; where the organisms with biosurfactant ability are streaked on blood agar plates and incubated at 40 °C. The plates are visually monitored for the presence of clearing zone around the colonies which is indicative of surfactant biosynthesis. The diameter of the clear zones depends on the concentration of the biosurfactant produced (Youssef *et al.*, 2004; Ghojavand *et al.*, 2008).
- Surface tension measurement by a du Nöuy ring-type tensiometer is one of the simplest techniques used. The surface tension measurement is carried out at room temperature after dipping the platinum ring in the solution for a while in order to attain equilibrium conditions. A higher biosurfactant concentration in the test sample provides a lower surface tension until the critical micelle concentration (CMC) is reached. The CMC is the point where the surface tension remains stable despite the changes in concentration (Desai and Banat, 1997; Grosman *et al.*, 2002).

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now

(UV, MS, evaporative light scattering detection, ELSD) for identification and quantification of biosurfactants (Heyd *et al.*, 2008).

1.2.2.4.4 Fermentation and production of surfactin

Most nutritional and production studies were done with batch cultures, usually in flasks with shaking , but occasionally in small-scale or large-scale fermentors (Cooper *et al.*1981; Mulligan and Gibbs 1990; Lin *et al*. 1994b).

A laboratory-scale cyclone column fermentor was used for continuous, phased growth with feedback control, based on the concentration of dissolved oxygen (Sheppard and Cooper, 1990). As the earlier studies carried out in nutrient

broth gave a very low yield (0.1 g/ l)(Arima *et al*. 1968), in subsequent studies a minimal mineral salts medium, containing NH_4NO_3 (0.05 M) as the inorganic nitrogen source and glucose (4%) as the carbon source, was defined by Cooper (Cooper's medium).Studies of the mineral requirement clearly established the need for and the stimulatory effect of iron and manganese (Cooper *et al*. 1981).

For continuous operation, a critical nitrogen/iron/manganese molar ratio of 920:7.7:1.0 was determined and was found to sustain surfactin production for at least 36 generations (Sheppard and Cooper 1991). Other work has been done in the semisynthetic medium of Landy *et al*. (1948), which contains L-glutamic acid (5 g/l) as the organic nitrogen source, glucose (2%) as the carbon source and a trace of metal cations (Landy's medium) (Nakano *et al*. 1988; Sandrin *et al*. 1990).

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

differences in the ability of different amino acids to support surfactin production has been found, yields being(0.6±0.8 g /l) (Sandrin *et al*. 1990). When they are used as the sole nitrogen source of a culture medium, some hydrophobic amino acids insert themselves directly into selected positions of the peptide sequence, thus amplifying the original structural microheterogeneity via the production of variants (Peypoux and Michel 1992).

Competition with cellular growth is probably one of the reasons for the rather disappointing product yields. In fact, in most of the studies the levels of increase are marginal and are probably due to differences in the process conditions, i.e.physicochemical and engineering parameters or nutritional factors; conversely, the replacement of Cooper's nitrogen source and the introduction of O2 limitation,

which redirects the energy efflux into product synthesis, have led to a productivity of(7 g /l), about 10 times as high as Cooper's basal yield (Kim *et al.* 1997).

B. subtilis was cultivated under aerobic conditions and at temperatures from 30 °C to 37 °C.

de Roubin *et al.* (1989) study the correlation between the primary metabolism of the cell and the secondary metabolism using UV mutagenesis, it was possible to isolate an overproducing strain of *B. subtilis* ATCC 21332, the mutant ATCC 51338. This mutant lowers the isocitrate dehydrogenase (a Krebs cycle enzyme) activity to 30 times less than that of the parent and produces 4 times more surfactin, i.e. (1.1 g/ l). The decrease of enzyme activity can also be achieved by O2 limitation or by addition of citric acid to the production medium, and they showed that under these conditions surfactin was increased. These observations suggest that the overall rate of surfactin synthesis may be subject to some control mechanisms, such as O2 availability, the rate of utilization of carbon substrates via oxidative catabolism

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now

1.2.2.4.5 Genes for surfactin synthesis

The organization of the biosynthetic gene cluster of surfactin was published in the early 1990s by different researcher groups (Cosmina *et al.*, 1993; Fuma *et al.*, 1993; Nakano *et al.*, 1988).

It composed of a large operon of 25 kb, named srfA ,(Figure 2-13), which is also responsible for sporulation and competence development (Nakano *et al.* 1991; Hamoen *et al.* 1994). (Figure 1.13)

It contains four modular open reading frames, ORF1 (srfA-A), ORF2 (srfA-B), ORF3 (srfA-C) and ORF4 (srfA-D), the first three of them encoding the three

respective enzymes E1A, E1B and E2 needed to make the heptapeptide sequence (Vollenbroich *et al*. 1994).

The two nucleotide regions for the L-leucine epimerases are located at the 3` portion of the srfA-A and srfA-B genes. The two coded polypeptides show sequence similarities to other putative bacterial racemases (Marahiel *et al*. 1997).

At the end of the srfA-C gene, one region (TE, thioesterase) codes for an enzyme homologous to fatty acid thioesterases type I, The terminal gene srfD encodes a protein with high homology to external thioesterases of type II, It has been reported that SrfD is a repair enzyme, which regenerates with acetyl-CoA or with incorrect amino acids mischarged peptidyl carrier protein (PCP) domains during non ribosomal peptide synthetases assembly (Schwarzer *et al*., 2002; Yeh *et al*., 2004). Its action affects the surfactin production as the knockout disruption in 1998 (Schneider and Marahiel, 1998) and its NMR structure and reaction mechanism were elucidated by Koglin *et al*. (2008).

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now

The gene encoding the downstream [illegible] its nucleotide sequence was established by Nakano *et al*. (1992). The encoded enzyme, termed sfp, belongs to the superfamily of 4`PPTases that function as primers of the non-ribosomal peptide and siderophore synthesis via a post-translational phosphopantetheinylation of thiotemplates (Walsh *et al*. 1997).

1.2.2.4.6 Synthesis of Surfactin

Surfactin as one of the lipopeptide is synthesized by non ribosomal peptide synthetases (NRPSs), they are megaenzymes organized in iterative functional units called modules that catalyze the different reactions leading to peptide transformation (Stein, 2005).

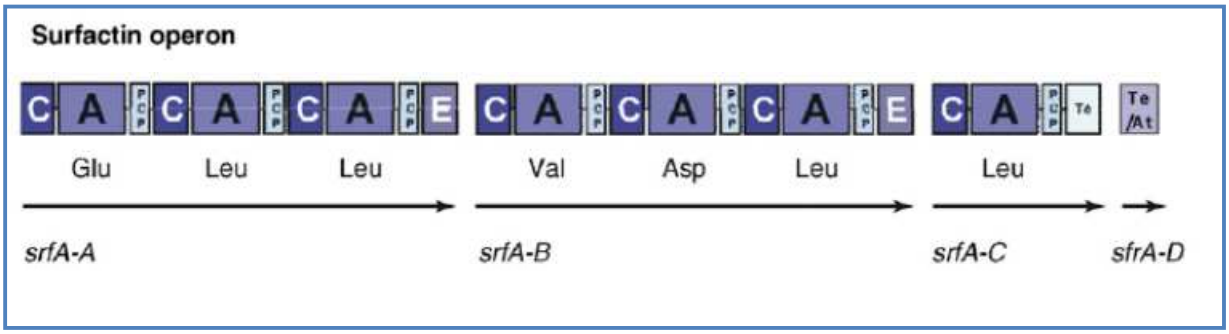


Figure (1-13) Surfactin operon (Ongena and Jacques, 2007)

Each module is subdivided into several catalytic domains responsible for each biochemical reaction (Sieber *and* Marahiel , 2003; Walsh, 2004).

The NRPS is responsible for one reaction cycle of selective substrate recognition and activation as an adenylate (A-domain), tethering of a covalent intermediate as an enzyme-bound thioester (Peptidyl-Carrier-protein, or T-domain), and peptide bond formation (Condensation, or C-domain) (Figure 1.14) (Finking and Marahiel, 2004).

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now

A typical cycle adds ~350 amino acids to the growing peptide and generates an enzymatically stabilized aminoacyl adenylate. This mechanism resembles the amino-acylation of tRNA synthetases during ribosomal peptide biosynthesis (Walsh, 2004).

(ii) The thiolation or peptidyl carrier domain (80 aa) is equipped with a 4`-phosphopantetheine (PPan) prosthetic group to which the adenylated amino acid substrate is transferred and thioesterified under release of AMP. Thus, the PPan cofactor acts as thiotemplate and as a swinging arm to transport intermediates between the various catalytic centers. The peptidyl carrier proteins are post-translationally converted from inactive apoforms to their active holoforms by dedicated PPan transferases (Lambalot *et al*., 1996).

(iii) The formation of a new peptide bond is catalysed by condensation domains (450 aa) located between each pair of adenylation and peptidyl carrier domains (Walsh , 2004).

The linear assembly line-like arrangement of multiple of such core units (i–iii) ensure the co-ordinated elongation of the peptide product.

Mechanism of peptide biosynthesis has been outlined in the concept of the 'Multiple Carrier Model of Nonribosomal Peptide Biosynthesis at Modular Multienzymatic Templates' (Stein *et al*., 1996).

So the surfactin biosynthesis starts with the acylation of the first amino acid glutamate, which is activated by the three-modular enzyme SrfAA (Menkhaus *et al*., 1993; Steller *et al*., 2004). These ten domains comprising synthetase exhibits typical N-terminal condensation domain that is often used for substitution module. However, NRP-synthetases producing lipopeptides usually feature such N-terminal C domains and that is why they are believed to be involved in the acylation step [illegible] and the third module of the synthetase SrfAA as well. The third module converts L-Leu into the D-configurated isomer by its C-terminal epimerization domain (Figure 1.15). SrfAB incorporates the following three amino acids (Val-Asp-D-Leu) while SrfAC is responsible for the activation and incorporation of the last leucine residue and catalysis of product release by cyclization (Cosmina *et al*., 1993; Tseng *et al*., 2002).

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now
Wondershare™

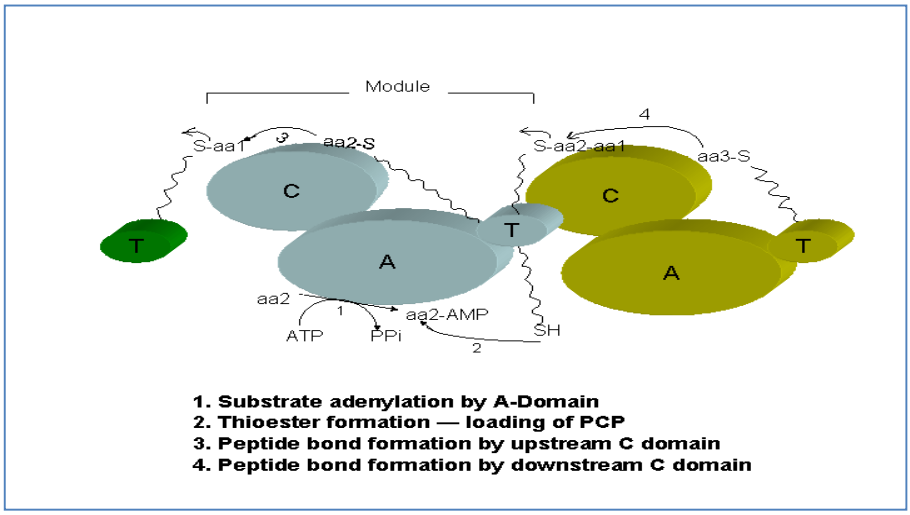


Figure (1.14) Non ribosomal peptide synthetases model(Int.3)

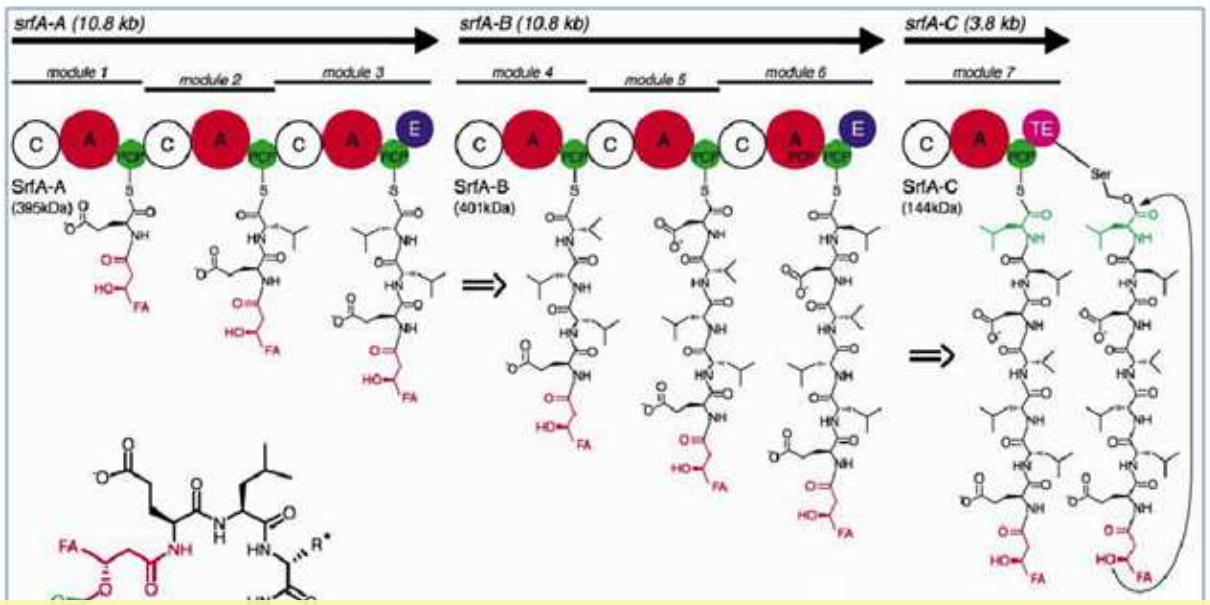
This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

biosurfactants, include solvent extraction, adsorption followed by solvent extraction, precipitation, crystallization, centrifugation and foam-fractionation (Kowall *et al*., 1998 ; Davis *et al*., 2001 ; Hsieh *et al*., 2004 ; Chen and Juang , 2008) .

The optimal approaches for biosurfactant recovery depend on the type and nature of the substrates and fermentation technique and on the type and physicochemical properties of the desired biosurfactants (Lin, 1996).



This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Most of the microbial surfactants are amphiphilic molecules with a higher ratio of hydrophobic to hydrophilic character and hence, can be easily isolated by the traditional methods of solvent extraction, precipitation and crystallization (Desai and Banat, 1997; Mukherjee *et al*., 2006).

Membrane separation processes are also well suited to those downstream processing steps that involve the recovery, concentration and purification of the biomolecules (Muthusamy *et al*., 2008).

Ultrafiltration is a pressure-driven membrane separation technique for dissolved and suspended materials based on size and molecular scale; It is a very simple procedure and requires no phase change, no chemical addition and little

energy. For molecules from 10 A ° to 500–1000 A ° diameter, ultrafiltration is useful both for product concentration by solvent removal and purification by removal of low molecular weight impurities under hydrostatic pressure (Chtioui *et al*.,2005).

The major filtration characteristics of a solute on passing through a membrane are usually judged by such parameters as permeate flux, rejection coefficient, concentration factor, solute yield or purification factor and hence, the recovery efficiency (Sen and Swaminathan ,2005).

The recovery process including concentration and purification of the product constitutes a major part in the economics of the whole process of biosurfactant production; this often determines the viability of the process itself, often the presence in low concentrations in the fermentation broth, the amphiphilic nature and foam-forming ability of biosurfactants pose serious impediments to efficient separation by classical methods (Mukherjee *et al*., 2006).

Separation of biological molecules usin [illegible] [illegible] properties. Biosurfactant molecu [illegible] ability to form micelles or miceller aggregates at concentrations higher than the critical micelle concentration (CMC) and hence can easily retained by high molecular weight cut-off ultrafiltration membranes (Chtioui *et al*.,2005).

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now

The lipopeptide biosurfactant, surfactin, produced by various strains of *Bacillus subtilis* has been reported to be concentrated and purified from the cell-free fermentation broth using ultrafiltration method (Mulligan and Gibbs , 1990 ; Lin and Jiang , 1997).

A relatively higher molecular weight cut-off membrane was found to be quite effective in the recovery of surfactin, since it forms micelle aggregates of about 50–100 molecules at the CMC (Sen and Swaminathan ,2005).

1.2.2.4.8 Applications of biosurfactants

Surfactants are one of the most frequently used chemicals in our daily lives due to their unique properties, they had applications in an extremely wide variety of industrial processes (Table 1-1), in spite of that there are many advantages of the biosurfactants as compared to their chemically synthesized counterpart as they characterized by: (Lin, 1996 ; Vollenbroich *et al*., 1997; Kitamoto *et al*., 2002; Singh and Cameotra, 2004; Ongena and Jacques, 2007; ; Rahman and Gakpe , 2008 ; Xu *et al*.,2011).

- **Biodegradability**

  Biosurfactants are easily biodegradable and thus particularly suited for environmental applications such as bioremediation and dispersion of oil spills (Banat, 1995).

- **Chemical diversity**

  The chemical diversity of naturally produced amphiphiles offer a wider selection of molecules and biocatalysts with [illegible] applications (Cameotra and Makkar, 2004; Fakruddin, 2011).

- **Bio- compatibility and digestibility**

  Which allow their application in cosmetics, pharmaceuticals and as functional food additives (Gautam and Tyagi, 2006; Williams, 2011).

- **Availability of raw materials**

  Biosurfactants can be produced from microorganisms grown on raw materials which are available in large quantities (Mukherjee *et al*., 2006; Singh *et al*., 2007).

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now
Wondershare™

- **Acceptable production economics**

  Biosurfactants can be produced from industrial wastes and by – products and this is of particular interest for bulk production (Mukherjee *et al*., 2006; Salihu *et al*., 2009).

- **Use in environmental control**

  Biosurfactants can be used in handling industrial emulsions , control of oil spills , biodegradation and detoxification of industrial effluents and in bioremediation of contaminated soil (Banat, 1995).

1.2.2.4.9 Biological activity of biosurfactants produced by *Bacillus spp.*

Many lipopeptides that produced by *Bacillus spp.* have attained increasing [illegible] antiviral and antitumor agents, immunomodulators or specific toxins and enzyme inhibitors. The mode of action of most of these compounds has not been clarified in detail, although it is likely that their surface [illegible] phospholipids of the biomembranes and the lipopeptides have amphiliphilic structures. Thus, lipopeptides are capable of penetrating into cells, with the lipophilic hydrocarbon chain interacting with the plasma membrane lipid moiety while the polar amino acids in the peptide part interact with the polar phosphatidyl moieties. Whether lipopeptides are able to damage the integrity of the plasma membrane or create ion selective pores depends on the nature of the lipopeptides and on the phospholipids of the membranes.(Cameotra and Makkar , 2004 ; Singh and Cameotra, 2004 ;Ongena and Jacques, 2007 ; Fathabad, 2011).

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now

Table (1-1) Industrial applications of chemical surfactants and biosurfactants (Muthusamy *et al.* , 2008).

Industry	Application	Role of surfactants
Petroleum	Enhanced oil recovery	Improving oil drainage into well bore; stimulating release of oil entrapped by capillaries; wetting of solid surfaces; reduction of oil viscosity and oil pour point; lowering of interfacial tension; dissolving of oil
	De-emulsification	De-emulsification of oil emulsions; oil solubilization; viscosity reduction, wetting agent
Environmental	Bioremediation	Emulsification of hydrocarbons; lowering of interfacial tension; metal sequestration
	Soil remediation and flushing	Emulsification through adherence to hydrocarbons; dispersion; foaming agent; detergent; soil flushing
	Emulsification and de-emulsification	Emulsifier; solubilizer; demulsifier; suspension, wetting, foaming, defoaming, thickener, lubricating agent
	Functional ingredient	Interaction with lipids, proteins and carbohydrates; protecting agent
	Microbiological	Physiological behaviour such as cell mobility, differentiation, competition
	Biomedical and therapeutics	Flexibility; adhesive agents; immunomodulatory molecules; vaccines; gene therapy; microbubble preparation
Agricultural	Biocontrol	Facilitation of biocontrol mechanisms of microbes such as parasitism, antibiosis, competition, induced systemic resistance and hypovirulence
Bioprocessing	Downstream processing	Biocatalysis in aqueous two-phase systems and microemulsions; biotransformations; recovery of intracellular products; enhanced production of extracellular enzymes and fermentation products
Cosmetic	Health and beauty products	Emulsifiers, foaming agents, solubilizers, wetting agents, cleansers, antimicrobial agent, mediators of enzyme action

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Wondershare™

1.2.2.4.9.1 The biological activity of surfactin family of lipopeptides

Surfactin and its derivatives is one of the most effective biosurfactants that have many interesting biological activitites . They can inhibit fibrin clot formation, induces formation of ion channels in lipid bilayer membranes, inhibits cyclic adenosine monophosphate (cAMP),inhibits platelet and spleen cytosolic phospholipase A2 ,exhibits antiviral and antitumor activities and show antimycoplasma properties (Singh and Cameotra, 2004).

It has been found that Surfactin in concentrations of 30-64 µM was cytotoxic to several human and animal cell lines (Vollenbroich *et al*. 1997) and provoked hemolysis (Dufour *et al*. 2005).

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Inactivation of enveloped viruses such as vesicular stomatitis virus (VSV), simian foamy virus (SFV), and suid herpesvirus 1 (SHV 1) by surfactin depended on its hydrophobicity: the C14 and C15 isoforms were more antiviral than C13 (Kracht *et al*. 1999).

The biological effects of surfactin and lichenysin A are probably due to their strong surfactant properties. However, surfactin also forms cation-selective K+ > Na+ channels in black-lipid membrane (BLM)(Sheppard *et al*. 1991). Surfactin-producing *B. subtilis* strains have high swarming motility and biofilm formation, whereas surfactin-nonproducing strains did not swarm or form biofilm (Connelly

et al. 2004). Surfactin promotes bacterial cell motion by lowering the surface tension (Kinsinger *et al*. 2003, Hofemeister *et al*. 2004, Mukherjee and Das; 2005).

The amphiphilic structures of the lipopeptides surfactin and lichenysin A explain their ability to form micelles and to penetrate the plasma membrane but in spite of all these properties surfactin has no remarkable fungitoxicity which may be due to the different in the sterol contents of the biological membranes to which surfactins can react with (Carrillo *et al*., 2003).

- **The antitumor activity of the surfactin family**

Over the past years, only a few studies have examined the actual effect of lipopeptide on tumour cells. Studies by Kameda's group (1974) proved that [illegible] the first workers to provide evidence that lipopeptide extracted from *Bacillus natto* KMD 2311 has antitumour activity [illegible] while Sudo's group (2000) has reported the ability of succinoyl trehalose lipids to inhibit [illegible] promyelocytic leukaemia cells.

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now

In addition, Wakamatsu *et al*. (2001) discovered that lipopeptide induces neuronal differentiation in rat adrenal phaeochromocytoma PC12 cells and provided the groundwork for the use of microbial extracellular lipopeptide as a novel reagent for the treatment of cancer cells.

Another studies were conducted by kim *et al*., (2007) to study the effect of surfactin on the proliferation of LoVo cells, a human colon carcinoma cell line, they found that Surfactin strongly blocked the proliferation of LoVo cells by inducing pro-apoptotic activity and arresting the cell cycle, according to several lines of evidence on DNA fragmentation, Annexin V staining, and altered levels of poly (ADP-ribose) polymerase, caspase-3, p21WAF1/Cip1, p53, CDK2 and cyclin E. The anti proliferative activity of surfactin was mediated by inhibiting

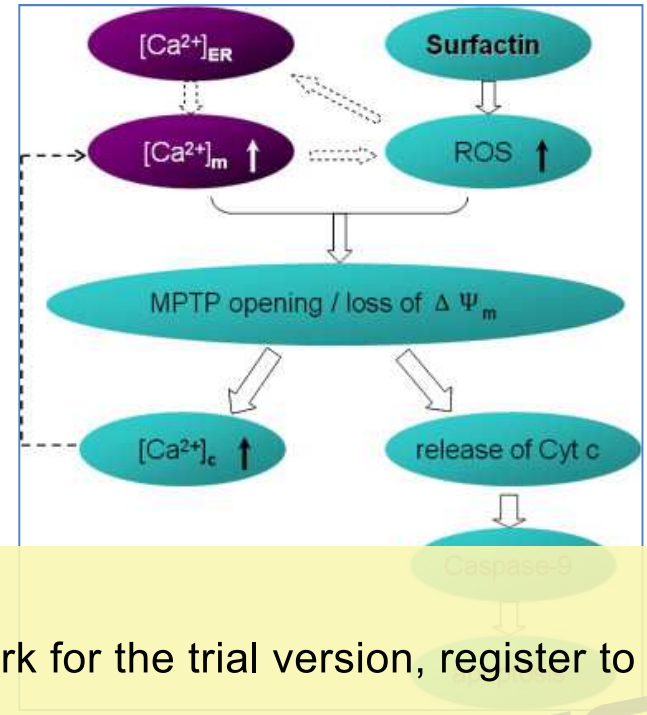
extracellular- related protein kinase and phosphoinositide 3-kinase/ Akt activation, as assessed by phosphorylation levels. Therefore, surfactin may have anti-cancer properties as a result of its ability to down regulate the cell cycle and suppress its survival, this study was followed by the work of Cao *et a*l., (2009a) who isolate surfactin from *Bacillus natto* TK-1 and test its antitumor activity on MCF-7 human breast-cancer proliferation, they found that the inhibition effect of surfactin was a dose- and time-dependent and the antitumour activity was associated with cell apoptosis determined by typical morphological changes.

In 2009 another group of researchers (Wang *et al*., 2009) published their work twhich studied the molecular mechanisms involved in surfactin(isolated from *B. natto* T-2) -induction of apoptosis in human leukemic K562 cells, they found that the effect through Induction of Ca^{2+} concentration and this process stimulated protein kinase ERK activation which evoked ERK phosphorylation. Thus ERK phosphorylation subsequently activated Pro-Cytochrom C and Caspase-3 leading to apoptosis (Ca[Cl

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now

2.2.4.9.2 The Biological activity of iturin family of lipopeptides

Biological effects of the iturin family peptides are due to their capability of forming ion-conducting pores (Maget-Dana and Peypoux, 1994). Iturin A and bacillomycin L provoked hemolysis and released potassium from erythrocytes (Latoud *et al*. 1986, Aranda *et al*. 2005). Iturin A induced morphological changes in human erythrocytes (Thimon *et al*. 1994). Iturin A, bacillomycins, and mycosubtilin formed channels in BLM (Maget-Dana *et al*. 1985a,b, Maget-Dana and Ptak 1990). Mycosubtilin altered the permeability of the plasma membrane, releasing nucleotides, proteins, and lipids from yeast cells (Besson and Michel 1989) and lysing erythrocytes (Besson *et al*. 1989). The lipopeptides of the iturin family are more active in membranes containing cholesterol, such as mammalian



This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Fig. 1.16) Mechanism of apoptosis induced by surfactin (Wang *et al*., 2009)

Surfactin from the ergosterol-containing fungal c
actively from the cells of *Saccharomyces cerevisiae* mutants containing cholesterol in the membrane instead of ergosterol (Latoud *et al*.1990). Nucleotides, proteins, polysaccharides, and lipids leaked from *S. cerevisiae* cells exposed to iturin A (Latoud *et al*. 1987) while the lethal concentration in *S. cerevisiae* was 10-60 µg/ml (Besson *et al*. 1984). Iturin and bacillomycin L form ion pores by aggregation in the membranes and interacting with sterols (Quentin *et al*. 1982, Maget-Dana and Peypox 1994, Volpon *et al*. 1999). Mycosubtilin formed pores in dimyristoylphosphatidylcholine (DMPC) membranes by interacting with the phospholipids, forming a (1:2) complex with cholesterol, thus stabilizing the ion pore (Maget-Dana and Ptak 1990).

1.2.2.4.9.3 The Biological activity of fengicin family of lipopeptides

Fengycins and plipastatins inhibit phospholipase A2, an enzyme affecting inflammation, acute hypertensions, and blood platelet aggregation (Volpon *et al.* 2000). *Bacillus thuringiensis* strain CMB26 produced an analogue of fengycin with a double bond in the fatty acid. It was fungicidal, bactericidal, and insecticidal, and more effective against fungi than was iturin or surfactin (Kim *et al.* 2004). In low molar ratios from 0.1 to 0.5 of fengycin/dipalmitoylphosphatidylcholine (DPPC) membrane fengycin forms pores and at a ratio of > 0.66 it acts as a detergent that solubilize membrane (Deleu *et al.* 2005).

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Wondershare™

List of Abbreviations

Abbreviations	Meaning
A_{260}	Absorbance at 260nm
A_{280}	Absorbance at 280nm
AMP	Adenosine mono phosphate
A-domain	Adenylation - domain
AEFB	Aerobic endospore-forming bacteria
Ala	Alanine
α	Alpha
ATCC	American Type Culture Collection
aa	Amino acids
A °	Angstrom
Asn	Asparagine
Asp	Aspartic acid
bp	Base pair
β	Beta
BLM	Black-lipid membrane
C-domain	Condensation - domain
CMC	Critical micelle concentration
cAMP	cyclic adenosine monophosphate
Caspase	cysteine-dependent aspartate-specific proteases
DNA	Deoxyribonucleic acid
D.W	Distilled water
ELSD	Evaporative Light Scattering Detection
ERK	Extracellular-related protein kinase
Glu	Glutamic acid
Gln	Glutamine
g/ l	Gram per liter
GC ratio	Guanine- Cytosine ratio
HPLC	High performance liquid chromatography
HLB	Hydrophilic-lipophilic balance
Ileu	isoleucine
kb	Kilo base

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Wondershare™

KDa	Kilo dalton
Leu	Leucine
µM	Micromolar
µl	Micro litter
µg	Microgram
µm	micrometer
mg/ml	Milligram per milliliter
mm	millimeter
mN/m	Millinuton per meter
M	Molar
nm	nanometer
NCBI	National centre for biotechnology
NRPS	Non ribosomal peptide synthetases
ORF	Open reading frame
Orn	Ornithin
O2	Oxygen gas
PCP	Peptidyl carrier protein
Phe	Phenyl
pH	Sign of potential
16S rDNA	Sixteen s ribosomal deoxy ribonuclease acid
SLs	Sophorolipids
SHV 1	Suid herpesvirus 1
ST	Surface tension
TLC	Thin layer chromatography
TE	Thioesterase
Thr	Threonine
Tyr	Tyrosine
UV	Ultra violet
U/ml	Unit per milliter
Val	Valine
VSV	Vesicular stomatitis virus
v/v/v	Volume by volume by volume

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now
Wondershare™

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Wondershare™

List of Contents

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:

1.No watermark on the output documents.

2.Can operate scanned PDF files via OCR.

3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Wondershare™

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:

1.No watermark on the output documents.

2.Can operate scanned PDF files via OCR.

3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Wondershare™

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:

1.No watermark on the output documents.

2.Can operate scanned PDF files via OCR.

3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Wondershare™

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:

1.No watermark on the output documents.

2.Can operate scanned PDF files via OCR.

3.No page quantity limitations for converted PDF files.

Remove Watermark Now

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:

1.No watermark on the output documents.

2.Can operate scanned PDF files via OCR.

3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Wondershare™

List of Figures

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:

1.No watermark on the output documents.

2.Can operate scanned PDF files via OCR.

3.No page quantity limitations for converted PDF files.

Remove Watermark Now

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:

1.No watermark on the output documents.

2.Can operate scanned PDF files via OCR.

3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Wondershare™

List of Tables

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:

1.No watermark on the output documents.

2.Can operate scanned PDF files via OCR.

3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Wondershare™

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:

1.No watermark on the output documents.

2.Can operate scanned PDF files via OCR.

3.No page quantity limitations for converted PDF files.

Remove Watermark Now

2. Materials and methods

2.1 Materials

2.1.1 Equipments and apparatus

The following equipments and apparatus were used in this study:-

Equipment	Company /Origin
Agarose gel tank with power supply	Beijing Six One Instrument Company /China
Autoclave	Shcnan /China
Biophotometer	Eppendrof /Germany
Centricon centrifuge filter (cut off 30 KDa)	Millipore Corp. /USA
Cooling centrifuge	Beckman /USA
[illegible]	[illegible]
Elisa reader	Multiscan EX; Labsystems /China
Eppendrof centrifuge	Eppendrof
Fluorescence microscope	Olympus
Gel casting tray	Bio Rad/Germany
HPLC-ESI/MS (Agilent 1100 series) with Zorbax Eclips XDB-C18 Analytical (4.6x150) mm	Agilent Technologies /USA
Ice maker	Shcnan
Incubator	Memmert /Germany
Incubator with CO_2 supply	Memmert
Laminar air flow	Jia Jie /china

Followed →

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now
Wondershare™

Luminometer	Promega / USA
Lyophylizer	Sartorius /Germany
Magnetic stirrer	Fuhe 78-1
96 well microtiter plate , 6 well plate	Hangzhou Yanhui /China
Microfuge	Heraeus sepatech /Germany
Variable micropipettes(10, 100, 1000)µl	Eppendorf /Germany
Microwave oven	Media /china
Milipore filter unit	Millipore Company
Minicentrifuge	Crystal MLX -206 /Germany
Flasks of tissue culture: plastic disposable of T-25 cm^2, T-75 cm^2,	Iwaki /Japan
pH- meter	Sartorius
Oven	Fuhe 78-1
Sensitve balance	Sartorius
Shaker incubator	Memmert
Tensiometer	Laryee/China
Thermal cycler	Bio Rad /USA
TLC tank	Sigma/ USA
UV transilluminator	Beckman Instruments/USA
Vortex mixer	Qilinbeirer
Water bath	Atom/ Eryland

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:

1.No watermark on the output documents.

2.Can operate scanned PDF files via OCR.

3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Wondershare™

2.1.2 Chemicals and biological materials

The following chemicals and biological materials were used in this study:-

Materials	Company / Origin
Agarose	Sigma /USA
Ammonium chloride(NH_4Cl)	Sigma
Ammonium nitrate (NH_4NO_3)	Sigma
Ammonium sulphate ($(NH_4)_2SO_4$)	Sigma
Boric acid	Sigma
Calcium chloride dihydrate ($CaCl_2.2H_2O$)	Sigma
Chloroform	Sigma
Corn oil	Wuhan oil Food /China
Copper sulphate pentahydrate ($CuSO_4.5H_2O$)	Sigma
Dipotassium hydrogen phosphate (K_2HPO_4)	
Disodium hydrogen phosphate (Na_2HPO_4)	
DNA mark Trans 2K and 5K	Trans/China
Ethylene diamine tetra acetic acid (EDTA)	Sigma
Ferrous sulphate heptahydrate ($FeSO_4.7H_2O$)	Sigma
Glucose	Sigma
Glycerol	Sigma
Machine oil	Atago Company /China
Hydrochloric acid (HCl)	Sigma
Isopropanol	Sigma
Magnesium sulphate ($MgSO_4$)	Sigma

Followed ⟶

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Materials	Company / Origin
Manganese sulphate ($MnSO_4$)	Sigma
Manganese sulphate monohydrate ($MnSO_4.H_2O$)	Sigma
Manganese sulphate tetrahydrate ($MnSO_4.4H_2O$)	Sigma
Methanol	Sigma
Mineral oil	Al Dorah oil refineries
Lithium chloride hexa hydrate $NiCl_2.6H_2O$	Sigma
Olive oil	Wuhan for food
Penicillin	Sigma
Peptone	Sigma
Phosphate buffer saline (PBS)	Biochrom /Germany
Potassium dihydrogen phosphate (KH_2PO_4)	Sigma
Potassium nitrate (KNO_3)	Sigma
Sodium acetate	Sigma
Sodium hydroxide (NaOH)	Sigma
Sodium nitrate ($NaNO_3$)	Sigma
Streptomycin powder	Sigma
Surfactin standard	Sigma
Sodium molbdate dihydrate ($Na_2MoO_4.2H_2O$)	Sigma
Disodium selenoxide (Na_2SeO_4)	Sigma
Sucrose	Sigma
Urea	Sigma
Yeast extract	Sigma
Zinc sulphate heptahydrate ($ZnSO_4.7H_2O$)	Sigma
Aluminium potassium sulfate($AlK(SO_4)_2$	Sigma

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Wondershare™

2.1.3 Reagents and solutions

2.1.3.1 Solution EA (Youssef *et al.*, 2004)

This solution was prepared by dissolving 25 g of $MgSO_4$ in 950 ml of D.W., then volume was completed to 1000 ml in a volumetric flask, and sterilized by autoclaving.

2.1.3.2 Solution EB (Youssef *et al.*, 2004)

To prepare this solution 100g of $(NH_4)_2SO_4$ was dissolved in 950 ml of D.W., then volume was completed to 1000 ml in a volumetric flask, and sterilized by autoclaving.

2.1.3.3 Solution EC (Youssef *et al.*, 2004)

It was prepared to be consisted of the following components:

Component	g/l
$FeCl_3$	3
$MnSO_4.H_2O$	3
NaCl	1
$CaCl_2.2H_2O$	0.1
$ZnSO_4.7H_2O$	0.1
$FeSO_4.7H_2O$	0.1
$CuSO_4.5H_2O$	0.01
$AlK(SO_4)_2$	0.01
$Na_2MoO_4.2H_2O$	0.01
Boric acid	0.01
Na_2SeO_4	0.005
$NiCl_2.6H_2O$	0.003

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now

All components were dissolved in 950 ml of D.W., then volume was completed to 1000 ml in a volumetric flask, pH was adjusted to 7 and sterilized by filtration.

2.1.3.4 Manganese and magnesium sulfate solution (Jacques *et al.*, 1999)

This solution was prepared by dissolving 0.4 g of $MnSO_4.H_2O$ and 5 g of $MgSO_4$ in 950 ml of D.W., then volume was completed to 1000 ml in a volumetric flask.

2.1.3.5 Trace elements solution (Jacques *et al.*, 1999)

It was prepared to be consisting of the following components:

Component	Weight (g)
$ZnSO_4.7H_2O$	2.32
$MnSO_4.H_2O$	
$CuSO_4.5H_2O$	
Boric acid	0.56
EDTA	1
$NiCl_2.6H_2O$	0.004
KI	0.66

All components were dissolved in 950 ml of D.W., then volume was completed to 1000 ml in a volumetric flask, pH was adjusted to 7 and sterilized by filtration.

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now
Wondershare™

2.1.3.6 Heat inactivated fetal bovine serum (Wang *et al.*,2007)

It was prepared by incubating fetal bovine serum in the water bath at 56 °C for 30 min.

2.1.3.7 PCR mixture

PCR master mixture was supplied by Takara company. It was composed of the followings:

Component	Volume(µl)
Template DNA	<0.5
(10×) Easy Taq Buffer (Mg^{++})	5
$MgSO_4$ (100mM)	1
dNTPs (25 mM)	5
Easy Taq DNA polymerase	1
Forward and reverse primers	1

Then volume was completed to 50 µl with D.W.

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Wondershare™

2.1.3.8 TLC solvent mixture (Symmank *et al* ., 2002)

It was prepared by mixing chloroform , methanol and deionized D.W. at ratios of 65:25:4 (v/v/v).

2.1.3.9 Biosurfactant standard solution

It was prepared according to Symmank *et al*. (2002) by dissolving 50 mg of biosurfactant in 1 ml of methanol.

2.1.3.10 Rhodamine B reagent (0.25%)

This reagent was prepared by dissolving 0.25 g of rhodamine B in 100 ml of absolute ethanol.

2.1.3.11 Trypsin solution

It was prepared according to Seo –young *et al.*, (2007) by dissolving 0.25 g of trypsin and 0.02 g EDTA in 100 ml of phosphate buffer solution (pH 7.4), mixed gently and sterilized by filtration.

2.1.3.12 Phosphate buffer saline (PBS 1%)

It was prepared by dissolving 10 g of Instamed PBS Dulbecco (Biochrom AG, Germany) in 1 liter of sterilized D.W.

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

1 staining buffer (5x).

2.1.3.14 TBE buffer solution (Lema *et al.*, 1994)

To prepare stock solution of TBE buffer (5×), 54 g of Tris base, 27.5 g of boric acid were dissolved in 900 ml D.W., then 20 ml of 0.5 M EDTA was added, pH was adjusted to 8.3 and the volume was completed to 1000 ml with D.W.

2.1.3.15 Ethidium bromide (10mg/ml)

Stock solution of ethidium bromide was prepared according to Maniatis *et al*., (1982) in a concentration of 10 mg/ml by dissolving 100 mg of ethidium bromide in 10 ml of D.W.

2.1.3.16. Hanks balanced salt solution (HBSS):

It was prepared to be consisting of the following components:

Component	Weight (g/l)
$CaCl_2$	0.14
KCl	0.40
K_2HPO_4	0.06
$MgCl_2.6H_2O$	0.10
$MgSO_4.7H_2O$	
$NaHCO_3$	0.35
Na_2PO_4	0.09
D-glucose	1.00
Hepes	2.08

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Wondershare™

Each of the above constituents was dissolved separately. $CaCl_2$ was added last, and made up in 1L. pH adjusted to 7,the solution was sterilized by filtration (Freshney,2000).

2.1.3.17 Trypan Blue Stain:

Trypan blue powder (1g) was dissolved in (100ml) Hank´s solution (2.1.3.17). The solution was filtered by Watman filter paper stored at 4ºC until use then diluted 1:10 in Hank´s solution for using (Freshney, 2000).

2.1.4 Culture media (Fluka/ Germany)

The following media were used in this study

2.1.4.1 Ready made culture media

- Nutrient agar
- Nutrient broth

These media were prepared as recommended by the manufacturing company and sterilized by autoclaving.

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now

2.1.4.2.1 medium (Youssef *et al.*, 2004)

This medium was consisted of the following components

Component	Weight (g)
KH_2PO_4	2.1
K_2HPO_4	13.9
Sucrose	10
NaCl	0.5
Yeast extract	50
$NaNO_3$	1

All components were dissolved in 950 ml D.W., pH was adjusted to 6.9, then volume was completed to 1000 ml and sterilized by autoclaving. After cooling, 10 ml of solution EA (item 2.1.3.1), solution EB (item 2.1.3.2) , and solution EC (item 2.1.3.3) were added to 1 liter of the above medium.

2.1.4.2. 2 Jacques medium (Jacques *et al*.,1999)

This medium was consisted of the following components

Component	Weight (g)
KH_2PO_4	1.9
Sucrose	20
Peptone	30

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

After all components were dissolved in 950 ml D.W., 9 ml of manganese – magnesium solution (item 2.1.3.4) ,and [illegible] added, respectively, pH was adjusted to 7, then volume was completed to 1000 ml and sterilized by autoclaving.

2.1.4.2.3 Bushnell –Hass medium (Patel and Desai,1997)

This medium was consisted of the following components

Component	Weight (g)
KH_2PO_4	1
K_2HPO_4	1
NH_4NO_3	1
$MgSO_4.7H_2O$	0.2
$CaCl_2.2H_2O$	0.02
$FeSO_4.7H_2O$	0.05
Yeast extract	0.5

All components were dissolved in 950 ml D.W., before 1 ml of trace element solution (item 2.1.3.5) was added, pH was adjusted to 7, then volume was completed to 1000 ml and sterilized by autoclaving.

2.1.4.2.4 Blood agar medium

This medium was prepared according to manufacturing company (Fluka / Germany) by dissolving 31 g of blood agar base in 900ml D.W., pH was adjusted to 7.3, then volume was completed to 950 ml with D.W., and sterilized by autoclaving . After cooling, 50 ml of defibrinated sheep blood was added and mixed thoroughly then poured into petridishes.

2.1.4.2.5 RPMI 1640 medium

This medium was enriched with 10% heat inactivated fetal bovine serum (item 2.1.3.6), 100U/ml penicillin and 100µg Streptomycin.

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

2.1.4.2.6 Dulbeccos modified Eagls medium

This medium was enriched with 10% heat inactivated fetal bovine serum (item 2.1.3.6), 100U/ml penicillin and 100µg Streptomycin.

2.1.5 Biological kits

2.1.5.1 Genomic DNA extraction kit ver. 2

Takara Minibest bacterial genomic DNA extraction kit ver. 2 (Takara Biotechnology ,Japan) was consist of the following solutions and buffers:

- SP buffer , RNAase AI , Lysozyme , EDTA buffer , Solution A , Solution B , Solution C, DB buffer , Rinse A , Rinse B , Elution Buffer

2.1.5.2 Cell titer 96 non- radioactive cell proliferation assay (MTT assay) (Promega, USA)

This kit was consist of two solutions:

- Dye solution (colorimetric 3-(4, 5- dimethylthiazol-2-yl)-2, 5diphenyl tetrazolium bromide)
- Solubilization solution / stop mix

2.1.5.3 Caspase –G10 3/7 assay kit: (Promega, USA)

This kit was composed from the following components:-

- Caspase –G10 substrate (lyophilized)

This kit was consist of the following:-

Jc-1 (2000×), JC-1staining buffer, positive control

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now

2.2 Methods

2.2.1 Samples collection

After removing the soil surface layer, 45 soil samples heavily contaminated with engine oils were collected from different fuel stations in addition to Al- Dorah oil refinery in Baghdad Province from Feb. to Aug., 2008 .

2.2.2 Isolation of *Bacillus* spp.

Isolation of *Bacillus* spp. was carried out according to Claus and Barkeley (1986) by adding 4 g from each soil sample to 20 ml of sterile D.W. , mixed thoroughly then heated to 80 °C with gentle agitation for 10 min. and left to cool at room temperature , then 0.1 ml aliquot was taken from each sample and spreaded on a nutrient agar plates and incubated aerobically for 24 hrs. at 30 °C.

After incubation, colonies appeared with different shapes and sizes were selected for further identification.

2.2.3 Identification of bacterial isolates

2.2.3.1 Morphological and cultural characteristics

Colony size, shape and color of bacterial colonies were studied on nutrient agar plates according to Fritz (2002).

Microscopical examinations

Single colony of each bacterial isolate was fixed on a clean slide and stained with Gram stain to study its Gram reaction and spore formation under the light compound microscope (Atlas *et al*., 1995).

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Wondershare™

2.2.3.3 Molecular identification

Molecular identification of bacterial isolates were achieved according to the analysis of DNA sequences and degree of similarity of 16S rDNA.

Bacterial isolates suspected to be *Bacillus* spp. were selected and propagated individually in nutrient broth for 18 hrs. at 30°C to obtain fresh cultures for extraction of genomic DNA.

2.2.3.3.1 Extraction of Genomic DNA

Genomic DNA was extracted from local isolates of *Bacillus* spp. using Takara Minibest Bacterial Genomic DNA Extraction Kit ver. 2 (2.1.5.1) as follows:

- Aliquots of 4 ml from fresh culture of each bacterial isolates was centrifuged, at 10000 rpm (4 °C) for 2 min., then 150µl aliquots of sp buffer and RNAase AI solutions were added respectively to cells precipitate followed by the addition of 20 µl of lysozyme solution and incubated at 25°C for 10 min.
- After incubation , 30 µl of EDTA buffer solution was added, and left at 25 °C for 5 min., then 200 µl of solution A was added and [illegible] for 10 min.

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

- Aliquot of 400 µl of solution B was added [illegible] by inverting for 15 [illegible] C was adde[illegible] rpm for 1 min.
- Supernatant was discarded; recentrifuged at 12000 rpm for another 1 min to obtain pellet.
- Aliquot of 400 µl of solution DB was added, shacked, then contents were transferred to spin column in filter cup and centrifuged at 12000 rpm for 1 minute.
- The spin column was transferred to another filter cup, then 500 µl of Rinse A solution was added to the column and centrifuged at 4°C, 12000 rpm for 1minute.
- Aliquot of 700 µl of Rinse B solution was added and centrifuged at 12000 rpm for 1 minute.

- Spin column was placed in a new filter cup, then 60µl of elution buffer was added and incubated at 25 °C for 10 min., centrifuged at 12000 rpm for 1 minute, then filtrate which containing genomic DNA was collected.

2.2.3.3.2 Quantization of DNA concentration (Maniatis *et al*., 1982)

Purity and concentration of DNA solution was measured by using Biophotometer device, by adding 8 µl of DNA sample to 72 µl of D.W. in a quartz cuvete and the absorbency at 260 nm and 280 nm was measured after calibration with D.W. at 260 nm and 280 nm respectively, Pure DNA has an A_{260} /A_{280} ratio of 1.7-1.9.The concentration of double strand DNA (µg/ml)was measured directly by the device.

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

2.2.3.3.3 Gel electrophoresis (Maniatis *et al*., 1982)

Gel tray was first prepared by sealing [illegible] ends of the gel tray. The agarose solution w[illegible] agarose in [illegible]0 ml of TBE (1×) prepared in (2.1.3.14) until agarose was dissolved by heating and solution is clear, after cooling to about 55°C, Ethidium bromide solution (2.1.3.15) was added at a concentration of 0.5 µg/ml. Finally gel solution was poured into tray to a depth of about 5mm and allowed to a solidify for about 20 min at room temperature followed by removing the comb from the tray and placed in the electrophoresis chamber , submerged with TBE buffer (1×) , DNA samples were prepared by mixing 1µl of the (6×) gel loading dye with 5 µl of the DNA then loaded in the wells, electrophoresis was done at 72 v until dye markers have migrated in appropriate distance, then gel was removed and visualized under UV light in the transiluminator.

2.2.3.3.4 Amplification of 16S rDNA

Two sets of primers were used as listed in table (2-1) to amplify 16S rDNA sequence for identification of bacterial isolates (Goto *et al*., 2000; Thomas, 2004).

Table (2-1) Types of primers used to amplify the 16S rDNA sequence of bacterial isolates.

Primer	type	Sequence	Size (bp)	Tm (°C)
27F	Forward primer	5'-AGAGTTTGATCCTGGCTCAG-3'	20	61
1492R	Reverse primer	5′-ACGGTTACCTTGTTACGACTT -3′	21	59
B16SF	Forward primer	5′-TGTAAAACGACGGCCAGTGCC TA ATACATGCAAG TCGAACG-3′		
B16SR	Reverse primer	5′-CAGGAAACAGCTATGACCATG CCCGTAG	39	60

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now

PCR reaction was performed by adding 3µl of the genomic DNA isolated from locally isolated *Bacillus* spp. as DNA template to 27 µl of a PCR mixture (2.1.3.7). The initial denaturation step was done at 94°C for 1 min. followed by 30 cycles at 94 °C for 30 sec. at 47 °C for 30 sec. and at 72 °C for 1.5min , then a final extension reaction was achieved at 72 °C for 10 min. PCR products were sequenced by Huazhong agricultural company using ABI310 DNA sequencer and the ABI PRISM Big Dye Terminator Cycle Sequencing kit version 3.1 (Perkin-Elmer Applied Biosystems,CA,U.S.A.). DNA sequences were analyzed to determine the degree of similarity using the National Centre for

Biotechnology Information (NCBI ; Bethesda , MD,USA) Blast system (http://www.ncbi.nlm.nih.gov/BLAST).

2.2.4 Sterilization methods (Atlas *et al.*, 1995)

2.2.4.1 Dry heat sterilization

Glassware were sterilized in an oven at 160°C for 3 hrs.

2.2.4.2 Moist heat sterilization

Media and solutions were sterilized by autoclaving at 121°C (15 pounds/ inch^2) for 30 min.

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now

2.2.4.3 Membrane sterilization

Milipore filters (0.22µm) were used to sterilize heat sensitive solution.

2.2.5 Maintenance of bacterial isolates(Atlas *et al.*, 1995)

2.2.5.1 Short term storage

Bacterial isolates were incubated on nutrient agar plates for 24 hrs. at 30 °C then wrapped with a parafilm , these plates can be store at 4 °C for a week as stock culture.

2.2.5.2 Medium term storage

Bacterial isolates were maintained by streaking on nutrient agar slants in screw- capped tubes containing 5 ml of the medium and incubated at 30 °C for 24 hrs. , these slants can be stored for a few months at 4 °C.

2.2.5.3 Long term storage

Aliquetes of 8.5 ml of the exponential phase of the bacterial growth was added to 1.5 ml of sterilized glycerol in a screw – capped tubes and stored at - 20 °C.

2.2.6 Screening of biosurfactant production by local isolates of *Bacillus* spp.

2.2.6.1 Preparation of inoculums

Single colony of each bacterial isolates was selected and used to inoculate 10 ml of nutrient broth medium, and incubated in shaker incubator (180 rpm) for 18 hrs. at 30 °C, then10 µl of fresh culture was used to inoculate 10 ml of the nutrient broth medium supplemented with solid waste oil in shaker with the same conditions.

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now

E medium and Jacques medium, separately, in Erlenmyer flasks (250 ml) in triplicate, and incubated aerobically (without shaking) for E medium and with shaking at 180 rpm for Jacques medium at 30 °C for three periods (24 , 48 and72 hrs.) .Each culture was then centrifuged at 4 °C, 10000 rpm. for 15 min. Production of biosurfactant was investigated in cell- free supernatant.

2.2.6.3 Qualitative screening

Qualitative screening for the ability of local isolates of *Bacillus* spp.of biosurfactants production were achieved by two methods:

2.2.6.3.1 Detection of hemolytic activity (Banat, 1993)

Haemolytic activity was detected on blood agar medium by streaking each local isolate on blood agar plate and incubated for 48 hrs. at 30 °C, then plates were visually inspected for the formation of clear zones around the growing colonies.

2.2.6.3.2 Oil spreading method (Morikawa *et al*., 2000)

This method was performed by adding 20 µl of crude oil to the surface of water layer in large petridishes (25 cm diameter) (50ml /petridish). 10 µl of cell – free supernatant were then added to the surface of oil layer. The diameter of clear zone on the oil surface was measured in centimeters which represent the biosurfactant activity. The means of diameters of the triplicates samples of each isolate were determined.

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now

Quantitative screening for biosurfactant production by local isolates of *Bacillus* spp. was achieved by measuring surface tension of cell – free supernatant according to McInerney *et al*. (1990) using Du Nouy ring tensiometer and by estimation of biosurfactant dry weight .

2.2.6.4.1 Surface tension measurement

Surface tension for each sample was measured by equilibrating 15 ml of cell- free supernatants for 15 min in a small weighing dish prior to measuring the surface tension in which the reading was recorded at the moment when the instrument's ring was detached from the surface of supernatant that immersed in it.

After calibration with D.W., glycerol and isopropanol, respectively, the percentage of lowering the surface tension was calculated according to the following equation.

$$\text{Percentage (\%) of lowering the surface tension} = \frac{ST1-ST2}{ST1} * 100$$

ST1= Surface tension of the medium or water without any addition.

ST2= Surface tension of the medium after cell growth or addition of biosurfactant.

2.2.6.4.2 Estimation of biosurfactant dry weight

Cell –free supernatants were subjected to acid precipitation by adding drops of 6N HCl to a final pH of 2 and allowing the precipitate to form at 4°C for overnight.

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Precipitate was collected by centrifugation with15000 rpm at 4°C for 15 min, then pellets were resuspended by 5ml of D.W. then adjusted to pH 7 with sodium hydroxide solution and finally lyophilized.

2.2.7 Optimization of biosurfactant production

Optimum conditions for biosurfactant production by the selected isolate of *Bacillus* spp. was determined according to Makkar and Cameotra (1998).

Optimum conditions included the optima of : carbon source, carbon source concentration , nitrogen source, nitrogen source concentration, dipotassium hydrogen phosphate concentration , pH , temperature, time of incubation and shaking speed respectively.

Each experiment was done in an Erlenmyer flask containing 50 ml of the production medium (Bushnell – Hass medium at pH 7) by inoculating with 0.5 ml of fresh culture of the selected isolate , then flask was incubated in shaker

incubator (180 rpm) at 30 °C for 72 hrs. After incubation , medium pH, surface tension , OD of bacterial growth and biosurfactant dry weight were measured.

2.2.7.1 Effect of carbon source

Five carbon sources (Sucrose, Glucose ,Olive oil, Corn Oil, Machine oil) were used to determine the optimum for biosurfactant production by the selected isolate of *Bacillus* spp. Each of these carbon sources was added to the production medium in a concentration of 2% (w/v) or (v/v).

2.2.7.2 Effect of carbon source concentration

Different concentrations (0.5,1,2,3,4 and 5%) of the optimum carbon source were added ,separately, to the production medium to determine the optimum for biosurfactant production by the selected isolate of *Bacillus* spp.

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now

2.2.7.3 Effect of nitrogen source

Eight nitrogen sources (NH_4NO_3 ,NH_4Cl, KNO_3, $NaNO_3$, $(NH_4)_2SO_4$, Urea, Peptone and Tryptone) were used to determine the optimum for biosurfactant production by the selected isolate of *Bacillus* spp. These nitrogen sources were added to the production medium in a concentration of 0.1% (w/v) or (v/v).

2.2.7.4 Effect of nitrogen source concentration

Different Concentrations (0.15, 0.3, 0.6, 1, 1.5 and 2%) of the optimum nitrogen source were added to the production medium to determine the optimum for biosurfactant production by the selected isolate of *Bacillus* spp.

2.2.7.5 Effect of K_2HPO_4 concentration

Different concentrations (0.03, 0.05, 0.1, 0.15, 0.2, 0.5, 1, 1.5, and 2 g/L) of K_2HPO_4 were examined to determine the optimum for biosurfactant production by the selected isolate of *Bacillus* spp.

2.2.7.6 Effect of medium pH

The production medium was adjusted to different pH values (5,6,7,8,9) to determine the optimum for biosurfactant production by the selected isolate of *Bacillus* spp.

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now

2.2.7.7 Effect of temperature

To study the effect of temperature on the ability of the selected isolate of *Bacillus* spp., the production medium was incubated at different temperatures (40, 37, 30 and 25 °C) to determine the optimum for biosurfactant production.

2.2.7.8 Effect of incubation period

To study the effect of incubation period on the ability of the selected isolate of *Bacillus* spp in biosurfactant production , production medium was incubated for 12, 24, 36, 48, 60, 72 and 96 hrs.

2.2.8 Purification of biosurfactant

Biosurfactant, produced by the selected isolate of *Bacillus* spp., was purified according to Moran *et al*. (2002) and Das *et al*. (2008). This was achieved by centrifugation of the culture medium (10000 rpm) for 10 min at 4°C. Cell –free supernatant was subjected to acid precipitation by adding drops of 6N HCl to a final pH of 2 to allow precipitation at 4°C overnight. Acid precipitate was collected

by centrifugation (15000 rpm) at 4°C for 15 min., then pellet was resuspended 2 ml of D.W., pH was adjusted to 7 with agitation for complete dissolving, then biosurfactant precipitate was lyophilized and weighed . From the lyophilized biosurfactant precipitate ,200 µg was dissolved in 4 ml of D.W. and filtered through a Centricon Centrifuge Filter (Mw Cut.off = 30 KDa) at 5000g .

Retentates were diluted in 50% methanol and filtered again. Filtrates were dried at 40 °C for 24 hrs. to obtain the biosurfactant.

2.2.9 Characterization of the biosurfactant

2.2.9.1 Thin layer chromatography (Symmank *et al.*, 2002)

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

In this method, drops of the purified biosurfactant (2 mg/ml methanol) and surfactin standard solution prepared in (2.1.3.9) were spotted quantitatively using capillary tubes in a distance of 1 cm from the bottom of silica gel plates (20*20 cm). The distance between each spot was 2 cm. The silica gel plates were immersed from the sp[illegible] containing 0.5 ml of the solvent mixture prepared in (2.1.3.8) and covered by the lid to allow the migration of solvent from the bottom to the top of the plates until reaches the end of the upper side (1.5 cm from the top), then plates were removed from the tank , air dried and sprayed with D.W. for detection of hydrophilic compounds or sprayed with rhodamine B prepared in (2.1.3.10) for detection of the presence of lipids under ultraviolet light. The relative mobility (R*m*) of each spot was calculated according to the following equation.

$$\text{Relative mobility } (Rm) = \frac{\text{Distance travelled by the compound}}{\text{Distance travelled by the solvent}}$$

2.2.9.2 High Performance Liquid Chromatography

The molecular mass of pure biosurfactant produced by the selected isolate was determined by HPLC-ES-MS (electrospray-ionization MS, Agilent Technology, USA) (figure 2-1)equipped with an electrospray ion source. The electrospray needle and capillary voltage were operated at 4.5 and 10kV respectively.

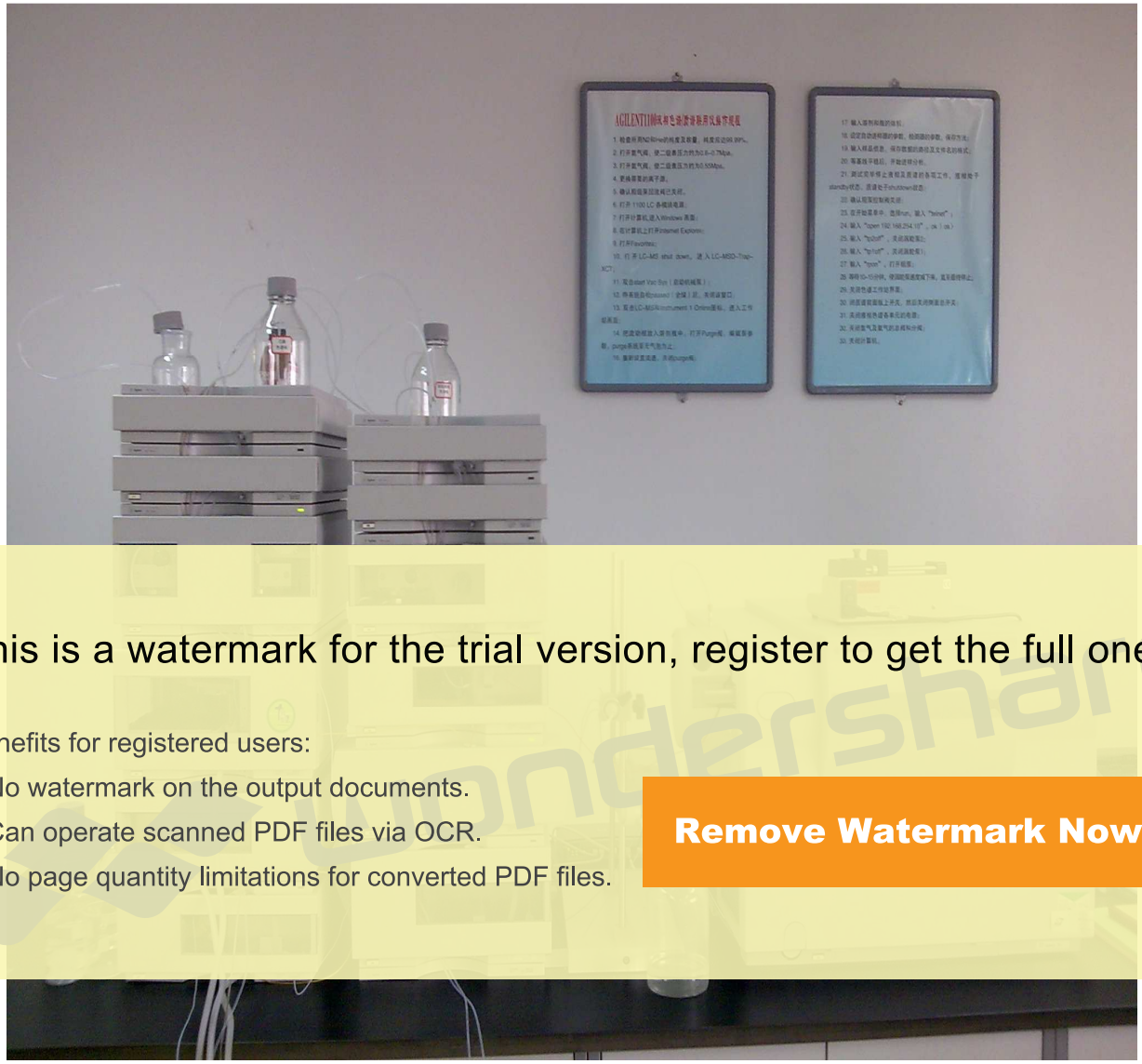
Other conditions were in accordance with the manufacturer`s recommendations. HPLC was equipped with a Zorbax Eclipse XDB-C_{18} analytical column (4.6×150 mm; 5µm particle size). The mobile phase consisted of two parts ,A and B. A was acetonitrile , and B was 0.1% formic acid, 5mmol/l ammonium acetate and D.W.. The parts were combined at an A/B ratio of 90:10(v/v). The injection volume of the standard was 10 µl while of the sample was 20 µl and flow rate was 0.5 ml/min. After HPLC analysis, amino acids sequence of the lipopeptide was determined by MS/MS , in which the [illegible] ionized biosurfactant molecules were selected in the mass spectrometer (MS1). Sodium ionized fragments produced were analyzed and recorded in the second stage mass spectrometer (MS2). The spectra were recorded using a Macromass, Wythenshaw, Manchester, U.K.).The capillary voltage was 3.50 kV and the collision gas was argon.

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now

2.2.10 Studying the antitumor activity

2.2.10.1 Cell lines

Four cell lines [Human leukemia (K562) ,Human epidermal larynx carcinoma cell line (Hep-2) , Mice leukemia (L1210)] were purchased from Cell Bank of Shanghai Institute for Cell Biology (Shanghai , China) ,while the normal liver cell line (Lo2) was purchased from cell bank of Chung Shan hospital (Shanghai , China).



This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Figure (2-1) HPLC-ESI/MS (Agilent 1100 series) with Zorbax Eclips XDB-C18 Analytical (4.6x150) mm.

- **Human Leukemia (K562)**

The human K562 cell line was established by Lozzio and Lozzio (1975) from a 53 year old patient with chronic myelogenous leukemia in blast crisis. The cells are non-adherent and rounded, in culture they exhibit much less clumping

than many other suspension lines (Lozzio and Lozzio ,1979). This cell line was used at a passage number 15.

- **Human epidermal Larynx carcinoma cell line (Hep-2)**

The Hep-2 cell line was established in 1952 by Moore *et al*. from tumors that had been produced in irradiated-cortisonized weanling rats after injection with epidermoid carcinoma tissue from the larynx of a 56-year-old male, they form adherent cells. A hardy cell line, Hep-2 resists temperature, nutritional, and environmental changes without a loss of viability (Toolan, 1954).

It has high proliferation rate and a 23 hours cell cycle (Grem and Fisher, 1989).

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now

- **Mice Leukemia (L1210)**

The murine lymphoid leukemia cell line (L1210) cell line was established in 1960 [illegible] of 8 month [illegible] are grow in suspension with doubling time 10-1[illegible]

Its used in routine screening programs of chemical agents and natural products for cytotoxic activity; preliminary testing for antitumor activity by the NCI in cancer chemotherapy screening studies. This cell line was used at a passage number 15.

- **Normal human liver cell line (Lo2)**

The lo2 cell line was established from an adult Chines mail by Cell Biology of Shanghai institute of Cell bank of China (Liu *et al*., 2007), this cell line was used at a passage number 7.

Cells were recovered by rapid thawing at 37°C in a water bath, centrifuged at 800 rpm for 10 min. at room temperature, then resuspended in 5ml culture medium [(2.1.4.2.5) for K562 and L1210, (2.1.4.2.6) for Hep-2 and Lo2] transferred to a T-25 tissue culture flask, which was incubated in a humidified atmosphere with 5% CO_2 and 95% air at 37°C for 24 hrs. After incubation, the medium was replaced with a fresh one.

Counting of viable cells was carried out using trypan – blue dye (0.4%).Dead cells take up the dye and appear blue under microscope while living cells exclude the dye and appear white.

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

the cell line from the flask surface by adding 2 ml trypsin solution (2.1.3.11) for each flask and incubated in a humidified atmosphere with 5% CO_2 and 95% air at 37°C for few minutes until the cells were round up, then dispersed gently , trypsin was inactivated by adding 15 ml of the culture media since its supplemented with fetal bovine serum (2.1.4.2.6) and subdivided into two flasks which incubated under the same conditions.

2.2.10.3 Cytotoxicity tests

2.2.10.3.1 Preparing the cell lines (Cao *et al.*,2009b)

Cell lines were seeded in 96 well microtiter plate for the cell viability study (MTT) and incubated for (48-72) hrs. to a final concentration of 100000 cell/ ml ,then treated with different concentrations [2,4,8,16,32,64 µg/ml] of biosurfactant in methanol produced and purified from the selected isolates and standard surfactin for 24,48 and 72 hrs. before testing with kit(2.1.5.2).

In order to study caspase activity, Hep-2 cell line was seeded in 96well microtiter plate and incubated for 72 hrs. to the final concentration of 20000 cell/ ml then treated with 8µg/ml of the biosurfactant preparation from the selected isolate for 6,12 and 18 hrs. before testing with kit(2.1.5.3), while the studying of mitochondrial membrane potential required the seeding of Hep-2 cell line in well plate and incubated for 72 hrs. to the final concentration of 100000 cell/ ml then treated with 8µg/ml of biosurfactant preparation from the selected isolate for 6,12 and 18 hrs. before testing with kit(2.1.5.4) , all the assays were done in triplicate.

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now

2.2.10.3.2 Cell Titer 96 Non- Radioactive cell Proliferation Assay (MTT assay)

- **Method:**

A volume of 15 µl of the dye solution was added to each well of the 96 well microtiter plate that contain 100 µl of the treated cells or blank then incubated at 37°C for 4 hrs. in a humidified (5%) CO_2 atmosphere , after incubation 100 µl of the solubilization solution / stop mix was added to each well and left for an hour, the contents of each well was mixed and finally the absorbance at 570 nm wavelength of the plate was recorded .

The cell inhibition rate (%) was calculated according to the following equation (Wang *et al*., 2007):

Cell inhibition rate (%) = [(Average absorbance of control cells− Average absorbance of treated cells)/ Average absorbance of control cells]×100

Data were analyzed by 2-way analysis of variance with ANOVA- test followed by Duncan test. Data were presented as means of three replicates ± SD. The level of significance $P < 0.05$ was used for analysis of variance test (ANOVA) (Al-Mohammed *et al*., 1986).

2.2.10.3.3 Caspase – G10 3/7 assay kit

- **Reagent preparation**
  - Caspase – G10 3/7 buffer and lyophilized Caspase – G10 3/7 substrate were first equilibrated to room temperature before used.
  - Contents of the Caspase – G10 3/7 buffer was transferred into the vial containing Caspase – G10 3/7 substrate and mixed until the substrate was thoroughly dissolved to form the Caspase –G10 3/7 reagent.

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now

- **Reaction preparation**

The following reactions were prepared to detect caspase -3 and -7 activities in cell Culture.

* Blank reaction :compose of the following Caspase – G10 3/7 reagent, vehicle solvent of protein ,cell culture medium without cells.
* Negative control : Caspase –G10 3/7 reagent and vehicle –treated cells in medium.

Blank reaction was used to measure background luminescence associated with the cell culture system and Caspase –G10 3/7 reagent ,so the value for the blank reaction was subtracted from experimental values.

- **Method**

* For each well of cells treated with biosurfactant (2.2.10) equal volume of the reagent was added and mixed well, then incubated for 3 hrs.
* luminescence from the plate was measured by luminometer

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

2.2.10.3.4 Mitochondrial membrane potential assay kit with JC-1

- **Method:**

For the six –well plates in a hole , culture medium was sucked then cells were washed with PBS buffer(2.1.3.13) followed by the addition of 1ml cell medium. A certain dol of JC-1 dye working s[illegible] supernatant sucked and washed twice with JC-1 staining buffer (1x). Finally 2 ml of cell culture fluid was added then observed with fluorescence microscope (fluorescence wave length (529 nm and 590 nm).

Recommendations

1- Genetic study on the biosurfactant producer locally isolated *Bacillus* isolates (B1,B2,B3,B4,B5,B6,B7,B8,B9,B10) to determine the genetic elements responsible for biosurfactant production.

2- Mutagenesis of *Bacillus* B6 to develop its ability in biosurfactant production using physical and chemical mutagens.

3- Studying the biological activity of the selected isolates as antimicrobial, antiviral, antifungal agents.

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

A

- **Abushady**, H.M.; Bashandy, A.S.; Aziz, N.H. and Ibrahim, H.M.M. (2005). Molecular Characterization of *Bacillus subtilis* Surfactin Producing Strain and the Factors Affecting its Production. Int. J. Agri. Biol., 7(3):337-344.

- **Ahimou**, F.; Jacques, P. and Deleu, M. (2000). Surfactin and iturin A effects on *Bacillus subtilis* surface hydrophobicity. Enzyme Microbiol. technol., 27: 749–754.

- **Akhmaloka**, A. Suharto, A.; Nurbaiti, S.; Tika, I. N. and Warganegara, F.M. (2006). Ribotyping Identification of Thermophilic Bacterium from Papandayan Crater. PROC. ITB Eng. Science., 38 (1): 1-10.

- **Al- Ajlani**, M.M.; Sheikh, M.A.; Ahmad, Z. and Hasnain, S. (2007). Production of surfactin from *Bacillus subtilis* MZ-7 grown on pharmamedia commercial medium. *Microbial Cell Factories* , 6:17-25.

This is a watermark for the trial version, register to get the full one!

- **Al-Araji**, L.; Abd Rahman, R.N.Z.R.; Mahiran, B. and Sa[illegible] (2007). Microbial Surfactant- minireview. Asia P[illegible] 15(5): 99-103.

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now

- [illegible] (2006). Studying S[illegible] Cells from Acute Myeloid [illegible] Thesis, College of Science, Al-Mustansiriya University.

- **Ali** ,A.; Ikramullah, M.; Asif, S.; Ahmad, A.; Jamal, A. and Jaffri, S.A. (2010). Evaluation of lipopeptide (surfactin) production by *Bacillus subtilis*. Biomedica. 26:34-38.

- **Al-Mohammed**, N.T.; Al-Rawi, K.M.; Younis, M.A. and Al-Morani, W.K. (1986). Principle of Statistics. J. Al-Mousl University. Iraq .

- **Al- Qadoori**, J.F.A. (2004). Effect of some local extracts on normal and cancer cells (in vitro). Ph.D. Thesis, College of Science , University of Al-Nahrain.

- **Al-Saffar**, A.Z. (2010). Antitumor , hepatotoxic and cytogenetic effect of the partially purified endotoxin extracted from locally isolated Salmonella typhimurium. Ph.D. Thesis, College of Science, University of Al- Nahrain.

- **Aranda**, F.J.; Teruel, J.A and Ortiz, A. (2005). Further aspects on the hemolytic activity of the antibiotic lipopeptide iturin A. Biochim. Biophys. Acta., 1713:51-56.

- **Arima**, K.; Kakinuma, A. and Tamura, G. (1968). Surfactin, a crystalline peptidelipid surfactant produced by *Bacillus subtilis*: isolation, characterization and its inhibition of fibrin clot formation. Biochem. Biophys. Res. Commun., 31: 488-494 . (Cited by Peypoux *et al*., 1999).

- **Arino**, S.; Marchal, R. and Vandecasteele, J. (1996). Identification and production of rhamnolipidic biosurfactant by *Pseudomonas* sp. Appl. Microbiol. Biotechnol., 45:162-168.

- **Asmer**, H.J.; Slegmund, L.; Fritz, W. and Wrey, V. (1988). Mcrobial production, structure elucidation and bioconversion of sophorose lipid. JAOCS., 65:1460-1466.

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:

1.No watermark on the output documents.

2.Can operate scanned PDF files via OCR.

3.No page quantity limitations for converted PDF files.

Remove Watermark Now

- **Assie**, L.K.; Deleu, M.; Arnaud, L.; Pa[illegible]ot, M.; [illegible]; G[illegible], C.H. and [illegible]uge, E. (2002). Insecticide [illegible] of su[illegible]actins and iturins from a [illegible] Cohn (S[illegible]

- A[illegible] M., Park, G. and Brown, A[illegible] Experimental Microbiology. Mosby – year –Book, Inc.,USA.

B

- **Banat**, I.M. (1993). The isolation of a thermophilic biosurfactant – producing *Bacillus* species. Biotechnol. Lett., 15:591-594.

- **Banat**, I.M. (1995). Characterization of biosurfactantsand their use in pollution removal state of the art (review). Acte Biotechnol., 15(3):251-267.

- **Banat**, I.M.; Franzetti, A.; Gandolfi, I.; Bestetti, G.; Martinotti, M.G.; Fracchia, L.; Smyth, T.J. and Marchant, R. (2010). Microbial biosurfactants production, applications and future potential. Appl. Microbiol. Biotechnol., 87:427–444.

- **Beatty**, P.H. and **Jensen**, S.E. (2002). *Paenibacillus polymyxa* produces

fusaricidin-type antifungal antibiotics active against *Leptosphaeria maculans*, the causative agent of blackleg disease of canola. Can. J. Microbiol., 48:159-169.

- **Belijanski**, M. (2000). The anticancer agent PB-100 selectivity active malignant cell inhibits multiplication of sixteen malignant cell lines, even multidrug resistant. Genet. Mol. Biol., 23:224-235.

- **Besson**, F.; Peypoux, F.; Quentin, M.J. and Michel, G. (1984). Action of antifungal peptidolipids from *Bacillus subtilis* on the cell membrane of *Saccharomyces cerevisiae*. J. Antibiot.,37:172-177.

- **Besson**, F.; Quentin, M.J. and Michel, G. (1989). Action of mycosubtilin on erythrocytes and artificial membranes. Microbios., 59:137-143.

- **Besson**, F. and **Michel**, G. (1989). Action of mycosubtilin, an antifungal antibiotic of *Bacillus subtilis*, on the cell membrane of *Saccharomyces cerevisiae*. Microbios., 59:113-121.

- **Biermann**, M.; Lange, F.; Piorr, R.; Proske, U.; Rutten, H.; Schindler, J. and Sinnwell, V. (1987). Surfactant [illegible] albe, J. (Ed. [illegible] Biochemistry and Genetics, 200[illegible]

- **Bodour**, A.A and **Miller – Maier**, R.M. (1998). Application of a modified drop – collapse techniques for surfactant quantitation and screening of biosurfactant – producing microorganisms. J. Microbiol. Methods, 32:273-280.

- **Bonmatin**, J.M.; Laprévote, O. and Peypoux, F. (2003). Diversity among microbial cyclic lipopeptides: iturins and surfactins. Activity-structure relationship to design new bioactive agents.Comb. Chem. High Throughput Screen., 6: 541-556. Cited by (Seydlová and Svobodová, 2008)

C

- **Cao**, X.-H.; Liao, Z.-Y.; Wang, C.-L.; Yang , W.-Y. and Lu, M.-F.(2009a). Evaluation of a lipopeptide biosurfactant from *Bacillus natto* Tk-1 as a

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

potential source of anti-adhesive, antimicrobial and antitumor activities. Brazilian J. Microbiol., 40:373-379.

- **Cao**, X.H.; Liao, Z.Y.; Wang, C.L.; Cai, P.; Yang, W.Y.; Lu, M.F. and Huang, G.W.(2009b). Purification and antitumor activity of a lipopeptide biosurfactant produced by *Bacillus natto* TK-1. Biotechnol. Appl. Biochem., 52:97-106.

- **Cao**, X.H.; Wang, A.H.; Wang, C.L,.; Mao, D.Z.; Lu, M.F.; Cui, Y.Q. and Jiao, R.Z. (2010). Surfactin induces apoptosis in human breast cancer MCF-7 cells through a ROS/JNK-mediated mitochondrial/caspase pathway. Chem. Biol. Interact., 183(3):357-362.

- **Carrillo**,C.; Teruel, J.A.; Aranda, F.J. and Ortiz, A .(2003). Molecular mechanism of membrane permeabilization by the peptide antibiotic surfactin. Biochim Biophys Acta, 1611:91-97.

- **Cameotra**, S.S. and **Makkar**, R.S. (2004). Recent applications of biosurfactants as biological and immunological molecules. Cur. Opin. Microbiol., 7:262-266.

- **Cartman**, S.T.; Ragione, R.M. and Woodward, M.J. (2008). *Bacillus subtilis* Spores germinate in the chicken gastrointestinal tract. Appl. Environ. Microbiol., 74: 5254-5258.

- **Cerritos**, R.; Vinuesa, P.; Eguiarte, L.E.; Herrera-Estrella, L.; Alcaraz-Peraza, L.D.; Arvizu-Go´mez, J.L; Olmedo,G.; Ramirez, E.; Siefert, J.L. and Souza, V. (2008). *Bacillus coahuilensis* sp. *nov*., a moderately halophilic species from a desiccation lagoon in the Cuatro Cie´negas Valley in Coahuila, Mexico. Int. J. Sys. Evol. Microbiol., 58: 919–923.

- **Chatterjee**, C.; Paul, M.; Xie, L. and van der Donk, W.A. (2005). Biosynthesis and mode of action of lantibiotics. Chem. Rev., 105:633-684.

- **Chen**, S.Y.; Wei, Y.H. and Chang, J.S. (2007a). Repeated pH-stat fed – batch fermentation for rhamnolipid production with indigenous *Pseudomonas* aeruginosa S2. Appl. Microbiol. Biotechnol., 76(1): 67-74.

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:

1.No watermark on the output documents.

2.Can operate scanned PDF files via OCR.

3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Wondershare™

- **Chen**, H.L.; Chen,Y.S. and Juang, R.S.(2007b). Separation of surfactin from fermentation broths by acid precipitation and two-stage dead-end ultrafiltration processes. J. Membr. Sci., 299: 114–121.

- **Chen**, H.L. and **Juang**, R.-S. (2008). Recovery and separation of surfactin from pretreated fermentation broths by physical and chemical extraction. Biochem. Engin., 38: 39–46.

- **Chtioui**, O.; Dimitrov, K.; Gancel, F. and Nikov, I. (2005). Biosurfactants production by immobilized cells of *Bacillus subtilis* ATCC 21332 and their recovery by pertraction. Process. Biochem., 45(11):1795-1799.

- **Ciriglian**, M.C. and **Carman**, G.M. (1984). Isolation of bioemulsifier from *Candida lipolytica*. Appl. Environ. Microbiol., 48(4):747-750.

- **Claus**, D. and **Berkeley**, R.C.W. (1986). Genus *Bacillus*. P.1105-1139 in: Bergey's Manual of Systematic Bacteriology. William and Wilkins, Baltimore.

- **Connelly**, M.B.; Young, G.M. and Sloma, A. (2004). Extracellular proteolytic activity plays a central role in swarming motility in *Bacillus subtilis*. J. Bacteriol., 186:4159-4167.

- **Cooper**, D.G. and **Zajic**, J.I. (1980).Surface active compounds from microorganisms. Adv. Appl. Microbiol., 26: 47-64.

- **Cooper**, D.G.; MacDonald, C.R.; Duff, S.F.B. and Kosaric, N .(1981). Enhanced production of surfactin from *Bacillus subtilis* by continuous product removal and metal cation additions. Appl. Environ. Microbiol., 42: 408-412.

- **Cosmina**, P.; Rodriguez, F.; de Ferra, F.; Grandi, G.; Perego, M.; Venema, G. and van Sinderen, D. (1993). Sequence and analysis of the genetic locus

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:

1.No watermark on the output documents.

2.Can operate scanned PDF files via OCR.

3.No page quantity limitations for converted PDF files.

Remove Watermark Now

responsible for surfactin synthesis in *Bacillus subtilis*. Mol. Microbiol., 8: 821–831.

- **Cossarizza**, A.; Baccarani-Contri, M.; Kalashnikova, G. and Franceschi, C. A. (1993). New method for the cytofluorimetric analysis of mitochondrial membrane potential using the J-aggregate forming lipophilic cation 5,5',6,6'-tetrachloro-1,1',3,3' tetraethylbenzimidazolylcarbocyanine iodide (JC-1). Biochem. Biophys. Res. Commun., 197 (1): 40-45.

- **Crosman**, J.T.; Pinchuk, R.J. and Cooper, D.G. (2002). Enhanced Biosurfactant Production by *Corynebacterium alkanolyticum* ATCC 21511 using Self-Cycling Fermentation. JAOCS, 79(5): 467-472.

- **Crueger**, W. and **Crueger**, A. (1982). Enzymes. In Biotechnology: a textbook of industrial microbiology (eds.) Crueger W, Crueger A., Science Tech. Inc., Madison, Wis. USA pp. 161-174.

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:

1.No watermark on the output documents.

2.Can operate scanned PDF files via OCR.

3.No page quantity limitations for converted PDF files.

Remove Watermark Now

- **Darwish**, M.A.(2007). A study on the [illegible] capsule of locally isolated *Klebsiella* [illegible] cancer cells *in vivo* and *in vitro*. Ph.D. Thesis, College of Science, University of Baghdad.

- **Das**, P. ; Mukherjee, S. and Sen, R.(2008). Antimicrobial potential of a lipopeptide biosurfactant derived from a marine *Bacillus circulans*. J. Appl. Microbiol.,104: 1675–1684.

- **Davis**, D.A. ; Lynch, H.C. and Varley, J. (1999).The production of surfactin in batch culture by *Bacillus subtilis* ATCC 21332 is strongly influenced by the conditions of nitrogen metabolism, Enzyme Microbial. Tech., 25 :322–329.

- **Davis**, D.A.; Lynch, H.C. and Varley, J. (2001). The application of foaming for the recovery of Surfactin from *B. subtilis* ATCC 21332 cultures. Enzyme Microbial Tech., 28: 346–354.

- **Deleu**, M.; Paquot, M. and Nylander, T. (2005). Fengycin interaction with lipid monolayers at the air-aqueous interface-implications for the effect of fengycin on biological membranes. J. Colloid Interface Sci., 283:358-365.

- **de Roubin**, M.R.; Mulligan, C.N. and Gibbs, B.F. (1989) .Correlation of enhanced surfactin production with decreased isocitrate dehydrogenase. Can. J. Microbiol., 35: 854-859.

- **Desai**, J.D. and **Banat**, I.M. (1997). Microbial production of surfactants and their commercial potential. Microbiol. Mol. Biol. Rev., 61(1): 47-64.

- **Dickinson**, E. (1993). Towards more natural emulsifires. Trends Food Sci. Tech., 4:330-334.

- **Drouin**, C.M. and **Cooper**, D.G. (1992). Biosurfactants and aqueous two-phase fermentation. Biotechnol. Bioeng., 40: 86–90.

This is a watermark for the trial version, register to get the full one!

- **Dufour**, S.; Deleu, M.; Nott, K.; Wathelet, B.; Thonart, P. and Paquot, M. (2005). Hemolytic activity of new linear surfactin analogs in relation to their physico-chemical properties. Biochim. Biophys. Acta., 1726:87-95.

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now

- [illegible] Genus *Bacillus*. [illegible] P.P. and Simmons, A. [illegible] Microbiology. Vol. 1. Mackie and McCartney. p.p. 131 – 149.

E

- **Earl**, A.M.; Losick, R. and Kolter, R. (2008).Ecology and genomics of *Bacillus subtilis.* Trends in Microbiol., 16 (6):269-275.

- **Errington**, J. (1993). *Bacillus subtilis* Sporulation: Regulation of Gene Expression and Control of Morphogenesis. Microbiol. Rev., 57: 1-33.

F

- **Faille**, C.; Membre, J. ; Kubaczka, M. and Gavini, F. (2002). Altered ability of *Bacillus cereus* spores to grow under unfavorable conditions (presence of nisin, low temperature, acidic pH, presence of NaCl) following heat treatment during sporulation. J. Food Prot. 65:1930-1936.

- **Fogarty**, W.M. and **Kelly**, C.T. (1990). Recent advances in microbial amylases, p. 71-132. In: Microbial enzymes and biotechnology (2nd ed.). Fogarty, W.M. and Kelly, C.T. (eds). Elsevier Applied Science, London.

- **Fathabad**, E.G. (2011).Biosurfactants in Pharmaceutical Industry: A mini-review. Am.J. Drug Discov. Dev. , 1(1):58-69.

- **Finking**, R. and **Marahiel**, M.A. (2004). Biosynthesis of nonribosomal peptides. Annu. Rev. Microbiol., 58:453-88.

- **Folkman**, J. (2000).Tumor angiogenesis In: Bast, R.C; Kufe, D.W.; Pollock, R.F.; Weichselbom, R.R.; Holland, J.F.; Ferri, E. and Ganster, T.E. (eds.).Cancer Medicine (5 th ed).BC.Decher Inc.Canada.

- **Franzetti**, A.; Gandolfi, I.; Bestetti, G.; Smyth, T.J.P. and Banat, I.M. (2010). Production and applications of trehalose lipids biosurfactants. Euro.J. lipid Sci. Technol., 112:617-627.

- **Fritz**, D. (2004). Taxonomy of the genus *Bacillus* and related genera: The aerobic endospore –forming bacteria . Phytopathology. 94:1245-1248.

- **Fuma**, S.; Fujishima, Y.; Corbell, N.; D'Souza, C.; Nakano, M.M.; Zuber, P. and Yamane, K. (1993). Nucleotide sequence of 50 portion of srfA that contains the region required for competence establishment in Bacillus *subtilis*. Nucleic Acids Res., 21: 93–97.

G

- **Garrity**, G.M.; Bell, J.A. and Lilburn, T.G. (2004). Genus: Bacillus . P 172-180 in: Taxonomic Outline of Bergey's Manual of Systematic Bacteriology (2nd ed.). Springer New York, Inc., USA.

- **Gautam**, K.K. and **Tyagi**, V.K. (2006). Microbial surfactants: A review.J. Oleo. Sci., 55(4): 155-166.

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Wondershare™

- **Ghojavand**, H.; Vahabzadeh, F.; Mehranian, M.; Radmehr, M.; Shahraki, K.A.; Zolfagharian, F.; Emadi, M.A. and Roayae, E. (2008). Isolation of thermotolerant, halotolerant, facultative biosurfactant-producing bacteria. Appl. Microbiol. Biotechnol., 80: 1073-1085.

- **Ghribi**, D. and **Ellouze-Chaabouni**, S. (In press) Enhancement of *Bacillus subtilis* lipopeptide biosurfactants production through optimization of medium composition and adequate control of aeration. Biotech. Research Int., In press.

- **Goesselsberger**, C.G.; Hekele ,O.; Brandstetter, M.; Aumayr, M.; Sommer, R. and Gebeshuber, I.C. (2009). Topography and stiffness characteristics of *Bacillus subtilis* UV-resistant and UV- sensitive. International Conference on Bioengineering & Biomaterials: IC2B'2009.

- **Goodfellow**, M.; Minnikin, D.E.; Patel, P.V. and Mordarska, H. (1973). Free nocardomycolic acids in the classification of nocardiae and strains of the 'rhodochrous' complex. J. Gen. Microbiol., 74(1):1-18.

- **Gould**, A.R.; May, B.K. and Elliott, W.H. (1971). Studies on the protoplast-bursting factor from *Bacillus amyloliquefaciens*. FEBS lett.,14:320-322.

- **Grangemard**, I.; Peypoux, F.; Wallach, J.; Das, B.C.; Labbe, H.C.; Caille, A.; Genest, M.; Maget-Dana, R.; Ptak, M. and Bonmatin, J.M. (1997). Lipopeptides with improved properties: structure by NMR, purification by HPLC and structure activity relationships of new isoleucyl-rich surfactins. J. Peptide Sci., 3:145-154.

- **Grangemard**, I.; Wallach, J.; Maget-Dana, R. and Peypoux, F. (2001). Lichenysin: a more efficient cation chelator than surfactin. Appl. Biochem. Biotechnol., 90:199-210.

- **Grem** , I.L. and **Fisher**, P.H. (1989). Enhancement of 5- flurouracil′s anticancer activity by dipyridamole. Pharmacol. Ther.,40:349-371.

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:

1.No watermark on the output documents.

2.Can operate scanned PDF files via OCR.

3.No page quantity limitations for converted PDF files.

Remove Watermark Now

- **Guerra–Santos**, L.H.; Kappeli, O.and Fiechter, A.(1984). *P. aeruginosa* biosurfactant production in continuous culture with glucose as carbon source. Appl. Environ. Microbiol., 48: 301–305.

- **Guerra-Santos**, L.H.; Kappeli, O. and Flechter, A. (1986). Dependence of *Pseudomonas aeruginosa* continous culture biosurfactant production on nutritional and environmental factors. Appl. Microbiol. Biotechnol., 24:443-448.

H

- **Haddad,** N. I.A.; Wang, J. and Mu, B. (2009). Identification of a Biosurfactant Producing Strain: *Bacillus subtilis* HOB2. Prot. Pep. Lett., 16: 7-13.

- **Hamoen**, L.W.; Eshuis, H.; Venema, G. and van Sinderen, D. (1994). A small gene, designated comS, located within the coding region of the fourth amino acid activation domain of srfA, is required for competence development in *Bacillus subtilis*. Mol. Microbiol., 13:55-63.

- **Hartmann**, A.; Rothballer, M.; Hai, B.; Schloter, M. and Reinhold-Hurek, B. (2004). Identification of N-acyl-homoserine lactone producing rhizosphere bacteria from various cereals and maize using a novel monomeric biosensor. FEMS Microbiol. Ecol., 51:123-135.

- **Hathout**, Y.; Ho, Y.P.; Ryzhov, V.; Demirev, P.and Fenselau, C. (2000). Kurstakins: a new class of lipopeptides isolated from *Bacillus thuringiensis*. J. Nat. Prod., 63:1492-1496.

- **Healy** , M.G.; Devine, C.M. and Murphy, R. (1996). Microbial production of biosurfactants. Resour. Conserv. Recy., 18(1):41-57.

- **Heyd**, M.; Kohnert, A.;. Tan, T.H.; Nusser, M.; Kirschhöfer, F.; Brenner-Weiss,G.; Franzreb, M. and Berensmeier, S. (2008). Development and trends of biosurfactant analysis and purification using rhamnolipids as an example, Anal. Bioanal. Chem., 391: 1579-1590.

- **Hofemeister**, J.; Conrad, B.; Adler, B.; Hofemeister, B.; Feesche, J.; Kucheryava, N.; Steinborn, G.; Franke, P.; Grammel, N.; Zwintscher, A.;

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:

1.No watermark on the output documents.

2.Can operate scanned PDF files via OCR.

3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Wondershare™

Leenders, F.; Hitzeroth, G. and Vater, J. (2004). Genetic analysis of the biosynthesis of non-ribosomal peptide- and polyketide-like antibiotics, iron uptake and biofilm formation by *Bacillus subtilis* A1/3. Mol. Genet. Genomics, 272:363-378.

- **Holt**, J.G.; Krieg, N.R.; Sneath, P.H., Staley, J.T. and Williams, S.T. (1994). Bergey's Manual of determinative Bacteriology. 9th ed., Williams and Wilkins.

- **Hoornstra**, D.; Andersson, M.A.; Mikkola, R. and Salkinoja-Salonen, M.S. (2003). A new method for *in vitro* detection of microbially produced mitochondrial toxins. Tox. in Vit., 17: 745-751.

- **Horowitz**, S.; Gilbert, J.N. and W. Michael Griffin, W.M. (1990). Isolation and characterization of a surfactant produced by *Bacillus licheniformis* 86. J. Indust. Microbiol., 6:243-248.

- **Hourdou**, M.L.; Besson, F.; Tenoux, T. and Michel, G. (1990). Fatty acids and amino acid syntheses in strains of *Bacillus subtilis* producing surfactic and related lipids. 24:940-944.

- **Hsieh**, F.-C.; Li, M.-H.; Lin, T.-C. and [illegible] Characterization of surfactin-producing [illegible] related species based on PCR. Cur. Microbiol., 49: 186–191.

- **Hu**, Y. and **Ju**, L.K. (2001). Purification of lactonic sophorolipids by crystallization. J. Biotechnol., 87(3):263-272.

- **Hue**, N.; Serani, L. and Laprévote, O. (2001).Structural investigation of cyclic peptidolipids from *Bacillus subtilis* by high energy tandem mass spectrometry. Rapid Commun. Mass Spectrom., 2001, 15, 203-209.

- **Huszcza**, E. and **Burczyk**, B. (2003). Biosurfactant Production by *Bacillus coagulans*. J.Surfactants Detergents, 6(1):61-64.

- **Huszcza**, E. and **Burczyk**, B. (2006). Surfactin isoforms from *Bacillus coagulans*. Znaturforsch [C]., 61(9-10):727-733.

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:

1.No watermark on the output documents.

2.Can operate scanned PDF files via OCR.

3.No page quantity limitations for converted PDF files.

Remove Watermark Now

- **Hutchison**, D.J.; Ittensohn, O.L. and Bjerregaard, M.R. (1966). Growth of L1210 mouse leukemia cells in vitro. Experimental Cell Research, 42(1):157-170.

I

- **Iqbal**, S.; Khalid, Z.M. and Malik, K.A. (1995). Enhanced biodegradation and emulsification of crude oil and hyperproduction of biosurfactants by a gamma ray-induced mutant of *Pseudomonas aeruginosa*. Lett. Appl. Microbiol., 21 : 176-179.

- **Isa**, M.H.M. ; Coraglial, D.E.; Frazier, R.A. and Jauregi, P. (2007). Recovery and purification of surfactin from fermentation broth by a two-step ultrafiltration process. J. Membr. Sci., 296 :51–57.

- **Iwabuchi**, S. and **Yamauchi**, F. (1987). Determination of glycinin and beta-conglycinin in soybean proteins by immunological methods. J. Agr. Food Chem., 35: 200–205.

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

J

- **Jacques**, P.; Hbid, C.; Destain, J.; [illegible] E. and Thonart, Ph. (199[illegible]) [illegible] production [illegible] *s*[illegible] design. Appl. Biochem. Biotechnol., 77:223-233.

- **Javaheri**, M.; Jenneman, G.E.; McInerney, M.J. and Knapp, R.M. (1985). Anaerobic Production of a Biosurfactant by *Bacillus licheniformis* JF-2. Appl. Environ. Microbiol., 50: 698-700.

- **Joshi**, S.; Bharucha, C. and Desai, A.J. (2008). Production of biosurfactant and antifungal compound by fermented food isolate *Bacillus subtilis* 20B. Bioresource Technol., 99: 4603–4608.

K

- **Kadhim**, H. A.-D. M. (2006). Biosurfactant production from locally isolated *Bacillus cereus*. M.Sc. Thesis, College of Science, University of Al-Nahrain.

- **Kappeli**, O. and **Finnerty**, W.R. (1979). Partition of alkane by an extracellular vesicle derived from hexadecane – grown *Acinetobacter*. J. Bacteriol., 140(2):707-712.

- **Kajimura**, Y. and **Kaneda**, M. (1997). Fusaricidins B, C and D, new depsipeptide antibiotics produced by *Bacillus polymyxa* KT-8: isolation, structure elucidation and biological activity. J. Antibiot., 50:220-228.

- **Kakinuma**, A.; Hori, M.; Isono, M.; Tamura, G. and Arima, K. (1969) . Determination of amino acid sequence in surfactin, a crystalline peptidolipid surfactant produced by *Bacillus subtilis*. Agric. Biol. Chem., 33: 971-97.

- **Kameda**, Y.; Oira, S.; Matsui, K.; Kanatomo, S. and Hase, T. (1974) Antitumor activity of Bacillus natto. V. Isolation and characterization of surfactin in the culture medium of *Bacillus natto* KMD 2311. Chem. Pharm. Bull. (Tokyo) 22, 938–944. (Cited by Kim *et al*., 2007).

- **Karim**, A.A. and **Bhat**, R. (2008). Gelatin alternatives for the food industry: Recent developments, challenges and prospects. Trends Food Sci. Tech.19: 644-656.

- **Kim**, H.S.; Yoon, B.D.; Lee, C.H.; Oh, H.M.; Katsuragi, T. and Tani, Y. (1997). Production and properties of a lipopeptide biosurfactant from *Bacillus subtilis* C9. J. Ferment. Bioeng., 84: 41-46.

- **Kim**, P.I.; Bai, H..; Bai, D.; Chae, H.; Chung, S.; Kim, Y.; Park, R. and Chi, Y.T. (2004). Purification and characterization of a lipopeptide produced by *Bacillus thuringiensis* CMB26. J. Appl. Microbiol., 97:942-949.

- **Kim**, S.D.; Cho, J.Y.; Park, H.J.; Lim, J.H.; Yun, H.I.; Park, S.C.; Kim, S.K.and Rhee, M.H. (2006). Comparison of anti-inflammatory activity of surfactin A, B, C and D from *Bacillus subtilis*. J. Microbiol. Biotechnol., 16:1656–1659.

- **Kim**, S.-Y.; Kim, J.Y.; Kim, S.-H.; Bae, H.J.; Yi, H.; Yoon, S.H.; Koo, B.S.; Kwon,M.; Cho, J.Y.; Lee, C.-E. and Hong, S. (2007). Surfactin from

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:

1.No watermark on the output documents.

2.Can operate scanned PDF files via OCR.

3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Bacillus subtilis displays anti-proliferative effect via apoptosis induction, cell cycle arrest and survival signaling suppression. FEBS Let., 581: 865–871.

- **Kinsinger**, R.F.; Shirk, M.C. and Fall, R. (2003). Rapid surface motility in *Bacillus subtilis* is dependent on extracellular surfactin and potassium ion. J. Bacteriol., 185:5627-5631.

- **Kiran**, G. S.; G.S.; Hema, T.A.; Gandhimathi, R.; Selvin, J.; Thomas, T.A.; Ravji, T.R. and Natarajaseenivasan, K. (2009). Optimization and production of a biosurfactant from the sponge-associated marine fungus *Aspergillus ustus* MSF3. Colloids and Surfaces B: Biointerfaces: 73:250-256.

- **Kitamoto**, D.; Isoda, H. and Nakahara, T. (2002). Functions and potential applications of glycolipid biosurfactants . from energy – saving materials to gene delivery carriers. J. Biosci. Bioeng., 94(3): 187-201.

- **Koglin**, A.; Lohr, F.; Bernhard, F.; Rogov, V.V.; Frueh, D.P.; Strieter, A.R.; Mofid, M.R.; Guntert, P.; Wagner, G. and Walsh, C. (2008). Structural basis for the selectivity of the external thioesterase of the surfactin synthetase. Nature.

- **Kreger**, F.W.; Allen, S.D.; Janda, W.M.; Koneman, E.W. and Procop, W.J.(1997). Color Atlas and Textbook of Diagnostic Microbiology (5th ed.), p. 651-708. Lippincott Williams & Wilkins.

- **Kosaric**, N. (1992). Biosurfactants in industry. Pure Appl. Chem., 64(11):1731-1737.

- **Kowall**, M.; Vater, J.; Kluge, B.; Stein, T.; Franke, P. and Ziessow, D. (1998). Separation and Characterization of Surfactin Isoforms Produced by *Bacillus subtilis* OKB 105. J. Colloid Interface Sci., 204: 1–8.

- **Kraas**, F.I. ; Helmetag, V.; Wittmann, M.; Strieker, M. and Marahiel, M.A.(2010). Functional dissection of surfactin synthetase initiation module reveals insights into the mechanism of lipoinitiation. Chem. Biol., 17: 872–880.

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:

1.No watermark on the output documents.

2.Can operate scanned PDF files via OCR.

3.No page quantity limitations for converted PDF files.

Remove Watermark Now

- **Kracht**, M.; Rokos, H.; Ozel, M.; Kowall, M.; Pauli, G. and Vate, J. (1999). Antiviral and hemolytic activities of surfactin isoforms and their methyl ester derivatives. J. Antibiot., 52**:** 613-619.

- **Kretschner**, A.; Block, H. and Wagner, F. (1982). Chemical and physical characterization of interfacial – active lipids from Rhadococcus erythropolis grown on n- alkane. Appl. Environ. Microbiol., 44:864-870.

- **Kuroda**, J.; Fukai, T.; Konishi, M.; Uno, J.; Kurusu, K. and Nomura, T. (2000). LI-F antibiotics, a family of antifungal cyclic depsipeptides produced by *Bacillus polymyxa* L-1129. Heterocycles, 53:1533-1549.

- **Kuroda**, J.; Fukai. T. and Nomura, T. (2001). Collision-induced dissociation of ring-opened cyclic depsipeptides with a guanidino group by electrospray ionization/ion trap mass spectrometry. J. Mass Spectrom., 36:30-37.

- Kurusu, K.; Ohba, K.; Arai, T. and Fukushima, K (1987). New peptide antibiotics LI-F03, F04, F05, F07, and F08, produced by *Bacillus polymyxa*. I. Isolation and characterization. J. Antibiot., 40:1505-1514.

- Lambalot, R.H.; Gehring, A.M; Flug
  phosphopantetheinyl transferases. Chem. Biol., 3: 923–936.

- **Landy**, M.; Warren, G.H.; Roseman, S.B. and Colio, L.G. (1948). Bacillomycin: an antibiotic from Bacillus subtilis active against pathogenic fungi. Proc. Soc. Exp. Biol. Med., 67: 539-541 cited by: Peypoux, F.; Bonmatin, J.M. and Wallach, J. (1999). Recent trends in the biochemistry of surfactin. Appl. Microbiol. Biotechnol., 51:553-563.

- **Latoud**, C.; Peypoux, F.; Michel, G.; Genet, R. and Morgat, J.L. (1986). Interactions of antibiotics of the iturin group with human erythrocytes. Biochim. Biophys. Acta., 856:526-535.

- **Lang**, G.U.S. and **Wagner**, F. (1984). Sophorose lipid formation by resting cells of *Torulopsis bombicola*. Biotechnol. Lett., 6: 225–30

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:

1.No watermark on the output documents.

2.Can operate scanned PDF files via OCR.

3.No page quantity limitations for converted PDF files.

Remove Watermark Now

- **Latoud**, C.; Peypoux, F. and Michel, G. (1987). Action of iturin A, an antifungal antibiotic from *Bacillus subtilis*, on the yeast *Saccharomyces cerevisiae*: modifications of membrane permeability and lipid composition. J. Antibiot., 40:1588-1595.

- **Latoud**, C.; Peypoux, F. and Michel, G. (1990). Interaction of iturin A, a lipopeptide antibiotic, with *Saccharomyces cerevisiae* cells: influence of the sterol membrane composition. Can. J. Microbiol., 36:384-389.
- **Ledzewicz**, U. (2006). Drug resistance in cancer chemotherapy as an optimal control problem. Discrete and Continuous Dynamical Systems (Series B), 6(1):129-150.

- **Lee**, S.-C.; Kim, S-H.; Park, I.-H.; Chung, S.-Y. and Choi, Y.-L. (2007). Isolation and structural analysis of bamylocin A, novel lipopeptide from *Bacillus amyloliquefaciens* LP03 having antagonistic and crude oil-emulsifying activity. Arch Microbiol., 188:307–312.

- **Lema**, M.W.; Brown, A. and Calkins, J.H. (1994). A g[illegible] method for extraction of DNA from bacteria. J.microbiol methods, 19:17-12[illegible]

- **Li**, [illegible] chi[illegible] *Lindera str*[illegible] [illegible] Med., 31: 857 – 869.

- **Lin**, S.C.; Minton, M.A.; Sharma, M.M. and Georgiou, G. (1994)a. Structural and immunological characterization of a biosurfactant produced by *Bacillus licheniformis* JF-2. Appl. Environ. Microbiol., 60(1): 31-38.

- **Lin**, S.C.; Carswell, K.S.; Sharma, M.M. and Georgiou, G. (1994)b. Continuous production of the lipopeptide biosurfactant of *Bacillus licheniformis* JF-2. Appl. Microbiol. Biotechnol., 41: 281-285.

- **Lin**, S.C. (1996). Biosurfactants : Recent Advances. J. Chem. Tech. Biotechnol., 66:109-120.

- **Lin**, S.C. and **Jiang,** H.J.(1997). Recovery and purification of the lipopeptide biosurfactant of *Bacillus subtilis* by ultrafiltration. Biotechnol Tech., 11:413–6.

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:

1.No watermark on the output documents.

2.Can operate scanned PDF files via OCR.

3.No page quantity limitations for converted PDF files.

Remove Watermark Now

- **Lin**, S-C.; Lin, K-G.; Lo, C-C. and Lin, Y-M. (1998a). Enhanced biosurfactant production by a *Bacillus lichemniformis* mutant. Enzyme Microb. Technol., 23 : 267-273.

- **Lin**, S.C. ; Chen,Y.-C. and Lin, Y.-M. (1998b). General approach for the development of high-performance liquid chromatography methods for biosurfactant analysis and purification. J. Chromatography,825: 149–159.

- **Liras**, P.; Asturias, J.A. and Martin. J.F. (1990) .Phosphates control sequences involved in transcriptional regulation of antibiotic biosynthesis. Tibtech., 8: 184-89.

- **Liu**, Z.-j.; Wang, B.; Yan,Z.-y. ; Qian, D.-m. and Xu-xia Song, X.-x. (2007). Differential proteomics in malignant and normal liver cell lines. Chines J. Cancer Res., 19(2):94-99.

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

- **Liu**, X.-Y.; Wang, S.-Z. and Mu, B.-Z. (2009). Production and characterization of a [illegible] surfactin-C[illegible] producing strain *Bacillus subtilis* HSO1[illegible] 4181[?].

- **Loeb**, L.A. (2001). A mutator phenotype in cancer. Cancer Research, 61: 3230-3239.

- **Lozzio**, C.B. and **Lozzio**, B.B. (1975). Human chronic myelogenous leukemia cell-line with positive Philadelphia chromosome. *Blood*, 45 (3): 321–334.

- **Lozzio**, B.B. and **Lozzio**, C.B. (1979). Properties and usefulness of the original K-562 human myelogenous leukemia cell line. Leukemia Research, 3 (6): 363–370.

- **Lugan**, N.A. (1988). *Bacillus* species of medical and veterinary importance. J. Med. Microbiol., 25 :157-165.

M

- **Maget-Dana**, R.; Heitz, F.; Ptak, M.; Peypoux, F. and Guinand, M. (1985a). Bacterial lipopeptides induce ion-conducting pores in planar bilayers. Biochem. Biophys. Res. Commun., 129:965-971.

- **Maget-Dana**, R.; Ptak, M.; Peypoux, F. and Michel, G. (1985b). Pore-forming properties of iturin A, a lipopeptide antibiotic. Biochim. Biophys. Acta., 815:405-409.

- **Maget-Dana**, R. and **Ptak**, M. (1990). Iturin lipopeptides: interactions of mycosubtilin with lipids in planar membranes and mixed monolayers. Biochim. Biophys. Acta., 1023:34-40.

- **Maget-Dana**, R. and **Peypoux**, F. (1994). Iturins, a special class of pore-forming lipopeptides: biological and physicochemical properties. Toxicology, 87:151-174.

- **Makkar**, R.S. and **Cameotra,** S.S. (1998). Production of biosurfactant at mesophilic and thermophilic conditions by a strain of *Bacillus subtilis*., J. Indust. Microbiol. 20:48-52.

- **Makkar**, R.S. and **Cameotra**, S.S. (2002). Effects of Various Nutritional Supplements on Biosurfactant Production by a Strain of *Bacillus subtilis* at 45°C.J. surfactants and detergents,5(1):11-17.

- **Maneerat**, S. and **Phetrong**, K. (2007). Isolation of biosurfactant-producing marine bacteria and characteristics of selected biosurfactant. Songklanakarin J. Sci. Technol., 29(3) : 781-791.

- **Maniatis**, T.; Fritsch, E. F. and Sambrook, J. (1982). Molecular cloning: A laboratory manual. Gold Spring Harbor Laboratory, New York.

- **Mao**, Q. and **Unadkat**, J.D. (2005). Role of the Breast Cancer Resistance Protein (ABCG2) in Drug Transport. AAPS, 7(1):E118-E133.

- **Marahiel**, M.A.; Stachelhaus, T. and Mootz, H.D. (1997). Modular peptide synthetases involved in nonribosomal peptide synthesis. Chem. Rev., 97: 2651-2673.

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:

1.No watermark on the output documents.

2.Can operate scanned PDF files via OCR.

3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Wondershare™

- **McInerney**, M.J.; Javaheri, M. and Nagle Jr., D.N. (1990). Properties of the biosurfactant produced by Bacillus licheniformis JF-2. J. Ind. Microbiol., 5:95-102.

- **McLean**, K.J.; Marshall, K.R.; Richmond, A.; Hunter, I.S.; Fowler, K.; Kieser, T.; Gurcha, S.S.; Besra, G.S. and Munro, A.W. (2002). Azole antifungals are potent inhibitors of cytochrome P450 mono-oxygenases and bacterial growth in mycobacteria and streptomycetes. J. Microbiol., 148:2937-2949.

- **Menkhaus**, M.; Ullrich, C.; Kluge, B.; Vater, J.; Vollenbroich, D. and Kamp, R.M. (1993). Structural and functional organization of the surfactin synthetase multienzyme system. J. Biol. Chem., 268: 7678–7684.

- **Moore**, A.E.; Sabachewsky, L. and Toolan, H.W. (1955). Culture characteristics of four permanent human cell lines. Cancer Res., 15: 598-605.

- **Morikawa**, M.; Daido, H.; Takao, T.; Murata, S.; Shimonishi, Y.and Imanaka, T. (1993). A new lipopeptide biosurfactant produced by *Arthrobacter sp*. Strain MIS38. J. Bacteriol., 175: 6459–6466.

- **Morikawa**, M.; Hirata, Y. and Imanaka, T. (2000). A study on the structure – function relationship of the lipopeptide biosurfactants. Biochim. Biophys. Acta., 1488:211-218.

- **Morita**, T.; Konishi, M.; Fukuoka, T.; Imura, T. and Kitamoto, D. (2006). Physiological differences in the formation of the glycolipid biosurfactants, Mannosylerythritol lipids, between pseudozyma antarctica and Pseudozyma aphidis. Appl. Microbiol. Biotechnol., 74:307-315.

- **Moteki,** H.; Hibasmai, H.; Yamada, Y.; Katsuzaki, H.; Imai, K. and Komiya, T.(2002).Specific induction of apoptosis 1,8-cineole in two human

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Wondershare™

leukemia cell line ,but not in a human stomach cancer cell lines. Oncology Reports, 9:757-760.

- **Mukherjee**, A.K. and **Das**, K. (2005). Correlation between diverse cyclic lipopeptides production and regulation of growth and substrate utilization by *Bacillus subtilis* strains in a particular habitat. FEMS Microbiol., 54:479-489.

- **Mukherjee**, S.; Das, P. and Sen R. (2006). Towards commercial production of microbial surfactants. Trends Biotechnol., 24(11): 509-515.

- **Mulherkar**, R. (2001). Gene therapy for cancer. Cur. Sci., 81 (5), 555-560.

- **Mulligan**, C.N. and **Gibbs**, B.F. (1990) .Recovery of biosurfactants by ultrafiltration. J. Chem. Technol. Biotechnol., 47: 23-29.

- **Muthusamy**, K.; Gopalakrishnan, S.; Ravi, T.K. and Sivachidambaram, P. (2008). Biosurfactants: Properties, commercial production and application. Cur. Sci., 94(6): 736-747.

- **Nakano**, M.M. and Zuber, P. (1989). Molecular characterization of two loci required for expression of a fengycin-like antibiotic in *Bacillus subtilis*. J. Bacteriol., 170: 5662-5668.

- **Nakano**, M.M.; Magnusson, R.; Myers, A.; Curry, J.; Grossamn, A.D. and Zuber, P. (1991). SrfA is an operon required for surfactin production, competence development and effcient sporulation in *Bacillus subtilis*. J. Bacteriol., 173: 1770-1778.

- **Nakano**, M.M.; Corbell, N.; Besson, J. and Zuber, P. (1992). Isolation and characterization of sfp: a gene that functions in the production of the lipopeptide biosurfactant, surfactin, in *Bacillus subtilis*. Mol. Gen. Genet., 232: 313-321.

- **Naruse**, N.; Tenmyo, O.; Kobaru, S.; Kamei, H.; Miyaki, T.; Konishi, M. and Oki, T. (1990). Pumilacidin, a complex of new antiviral antibiotics. Production, isolation, chemical properties, structure and biological activity. J Antibiot., 43: 267-280.

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:

1.No watermark on the output documents.

2.Can operate scanned PDF files via OCR.

3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Wondershare™

- **Nicholson**, W. L. (2002). Roles of *Bacillus* endospores in the environment. Cell. Mol. Life Sci., 59:410-416.

O

- **Oakenfull**, D. (1981). Saponins in food—A review. Food chem., 6:19–40.

- **Oberbremer**, A.; Muller-Hurtig, R. and Wagner,F. (1990). Effect of the addition of microbial surfactants on hydrocarbon degradation in a soil population in a stirred reactor. App. Microbiol. Biotechnol., 32:485-489.

- **Ohlendorf** ,B.; Lorenzen, W.; Kehraus, S.; Krick, A.; Bode, H.B. and König, G.M. (2009). Myxotyrosides A and B, Unusual rhamnosides from *Myxococcus* sp. J. Nat. Prod., 72(1): 82-86.

- **Olivera**, N.L. ; Nievas, M.L.; Lozada, M.; del Prado, G.; Dionisi, H.M. and Sineriz, F. (2009). Isolation and characterization of biosurfactant-producing *Alcanivorax* strains: hydrocarbon accession strategies and alkane hydroxylase gene analysis. Res. Microbiol., 160:19–26.

- **Ortiz**, A.; Teruel , J.A.; Espuny, M.J.; Marqués, A.; Manresa, A. and Aranda, F.J.(2008). Interactions of a *Rhodococcus* sp. biosurfactant trehalose lipid with phosphatidylethanolamine membranes. Biochim Biophys Acta., 1778(12):2806-2813.

- **Oscariz**, J.C.; Lasa, I. and Pisabarro, A.G. (1999). Detection and characterization of cerein 7, a new bacteriocin produced by *Bacillus cereus* with a broad spectrum of activity. FEMS Microbiol Lett., 178:337-341.

- **Oscariz**, J.C. and **Pisabarro**, A.G. (2000). Characterization and mechanism of action of cerein 7, a bacteriocin produced by *Bacillus cereus* Bc7. J. Appl. Microbiol., 89:361-369.

- **Outtrup**, H. and **Jorgensen**, S.T.(2002). The importance of *Bacillus* species in the production of industrial enzymes. In: Berkeley R, Heyndrickx

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:

1.No watermark on the output documents.

2.Can operate scanned PDF files via OCR.

3.No page quantity limitations for converted PDF files.

Remove Watermark Now

M, Logan N, De Vos P (eds), Applications and systematics of *Bacillus* and relatives. Blackwell publishing, UK, pp 206–218.

P

- **Parkinson**, M. (1985). Biosurfactants. Biotech. Adv., 3:65-83.

- **Patel**, R.N. and **Desai**, A.J. (1997). Surface active properties of rhamnolipids from *Pseudomonas aeruginosa* GS3. J.Basic Microbiol. 32:518-520.

- **Peypoux**, F.; Guinand, M.; Michel, G.; Delcambe, L.; Das, B.C. and Lederer, E. (1978). Structure of iturin A, a peptidolipid antibiotic from *Bacillus subtilis*. Biochem., 17:3992-3996.

- **Peypoux**, F. and **Michel**, G. (1992) .Controlled biosynthesis of [Val7]- and [Leu7]surfactins. Appl. Microbiol. Biotechnol., 36: 515-517.

- **Peypoux**, F.; Bonmatin, J.M. and Wallach, J. (1999). Recent trends in the biochemistry of surfactin. Appl. Microbiol. Biotechnol., 51:553-563.

- Piggott, A.M. and Karuso, P. (2004). Quorum sensing: 579–586.

- **Powalla**, M.; Lang, S.and Wray, V. (1989). Penta– and disaccharide lipid formation by *Nocardia corynebacterioids* grown on n–alkanes. Appl. Microbiol. Biotechnol., 31: 473–479.

- **Priest**, F.G.; Goodfellow, M. and Todd, C. (1988).A numerical classification of the genus *Bacillus*. J. Gen. Microbiol., 134:1847-1882.

- **Priest**, F.G. (2008). *Bacillus*. In: "Rehm, H.J. and Reed, G. (2nd ed.) " . Biotechnology: Biological. Vol.1. Wiley-VCH Verlag GmbH. p.p.368-397.

- **Priya**, T. and **Usharani**, G. (2009). Comparative Study for Biosurfactant Production by Using *Bacillus subtilis* and *Pseudomonas aeruginosa* . Botany Research International , 2 (4): 284-287.

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:

1.No watermark on the output documents.

2.Can operate scanned PDF files via OCR.

3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Q

- **Quentin**, M.J.; Besson, F.; Peypoux, F. and Michel, G. (1982). Action of peptidolipidic antibiotics of the iturin group on erythrocytes. Effect of some lipids on hemolysis. Biochim. Biophys. Acta., 684:207-211.

R

- **Rahman**, K.S.M.; Rahman, T.; Lakshmanaperumalsamy, P.; Banat, I. M. (2002). Occurrence of crude oil degrading bacteria in gasoline and diesel station soils. J. Basic Microbiol., 42 (4): 284–291.

- **Rahman**, K. S. M. ; Rahman, T. J.; Lakshmanaperumalsamy, P.; Marchant, R.and (2003). The Potential of Bacterial Isolates for Emulsification with a Range of Hydrocarbons . Acta Biotechnol., 23(4): 335–345.

- **Rahman**, P. K.S.M. and **Gakpe**, E. (2008). Production, characterization and Applications of Biosurfactants –review. Biotechnol., 7(2):360-370.

- **Reed**, J.C. (2003). Apoptosis-targeted therapies for cancer. Cancer Cell, 3: 17-22.

- **Rodriquez**, R.J. and **Acosta**, D. Jr. (1996). Inhibition of mitochondrial function in isolated rate liver mitochondria by azole antifungals. J. Biochem. Toxicol., 11:127-131.

- **Rosenberg**, E.; Zuckerberg, A.; Rubinovitz, C. and Gulnick, D.L. (1979) . Emulsifier *Arthrobacter* RAG-1: isolation and emulsifying properties. Appl. Environ . Microbiol., 37(3):402-408.

- **Rosenberg**, E. and **Ron**, E.Z. (1999). High and low- molecular – mass microbial surfactants. Appl. Microbiol. Biotechnol. 52 (2): 154- 162.

- **Rudner**, R.; Martsinkevich, O.; Leung, W. and Jarvis, E.D. (1998). Classification and genetic characterization of pattern- forming Bacilli. Mol. Microiol., 27:687-703.

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:

1.No watermark on the output documents.

2.Can operate scanned PDF files via OCR.

3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Wondershare™

- **Rufino**, R.D.; Luna, J.M.; Sarubbo, L.A.;. Rodrigues, L.R.M; Teixeira, J.A.C. and Campos-Takaki, G.M. (2011). Antimicrobial and anti-adhesive potential of a biosurfactant Rufisan produced by *Candida lipolytica* UCP 0988. Colloids and Surfaces B: Biointerfaces, 84:1-5.

S

- **Salager**, J-L. (2002). Surfactants – types and uses . FIRP Booklet # 300A. Cited in http://www.firp.ula.ve.

- **Salihu**, A.; Abdulkadir, I. and Almustapha, M.N. (2009). An investigation for potential development on biosurfactants. Biotechnol. Molecular Bio. Rev., 3(5): 11-117.

- **Sandrin**, C.; Peypoux, F. and Michel, G. (1990). Coproduction of surfactin and iturin A, lipopeptides with surfactant and antifungal properties, by *Bacillus subtilis*. Biotechnol. Appl. Biochem., 12:370-375.

- **Sarney**, D.B. and **Vulfson**, E.N. (1995). Application of enzymes to the synthesis of surfactants. Biotech

- thioesterase domains, integrated in or associated with peptide synthetases, in nonribosomal peptide biosynthesis in *Bacillus subtilis*. Arch. Microbiol. 169:404–410.

- **Schwarzer**, D.; Mootz, H.D.; Linne, U. and Marahiel, M.A. (2002). Regeneration of misprimed nonribosomal peptide synthetases by type II thioesteras*et al.*, 2010).

- **Sen**, R. and **Swaminathan**, T. (2005). Characterization of concentration and purification parameters and operating conditions for the small-scale recovery of surfactin. Process Biochem., 40: 2953–2958.

- **Seo – young**, K.; Joo, Y.K.; Seol-Hee, K.; Hyun, J.B.; Hwaseon, Y.; Sang, H.Y.; Bon, S.K.; Moosik, k.; Jae, Y.C.; Choong-Eun,L. and Sungyoul, H. (2007). Surfactin from *Bacillus subtilis* displays anti-proliferative effect via

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Wondershare™

apoptosis induction, cell cycle arrest and survival signaling suppression. FEBS Letters 581: 865–871.

- **Sepahy**, A.A.; Assadi, M.M.; Saggadian, V. and Noohi, A.(2005). Production of biosurfactant from Iranian oil fields by isolated Bacilli. Int. J. Environ. Sci. Technol., 1(1): 287-293.

- **Seydlová**, G. and **Svobodová**, J. (2008). Review of surfactin chemical properties and the potential biomedical applications. Cent. Eur. J. Med., 3(2):123-133.

- **Shaligram**, N.S. and **Singhal**, R.S. (2010). Surfactin – A Review on Biosynthesis, Fermentation, Purification and Applications. Food Technol. Biotechnol. 48 (2): 119–134.

- **Sheppard**, J.D. and **Cooper**, D.G. (1990). Development of computerized feedback control for the continuous phasing of *Bacillus subtilis*. Biotechnol. Bioeng., 36: 539-545.

- **Sheppard**, J.D. and **Cooper**, D.G. (1991). The response of *Bacillus subtilis* [illegible] during [illegible]. Microbios., 67: 71-76.

- **Sheppard**, J.D., **Jumarie**, C., **Cooper**, D.G. and **Laprade**, R. (1994). Ionic channels induced by surfactin in planar lipid bilayer membranes. Biochim. Biophys. Acta., 1064: 13-23.

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

- **Shimakata**, T. and **Minatogawa** , Y.(2000). Essential Role of Trehalose in the Synthesis and Subsequent Metabolism of Corynomycolic Acid in *Corynebacterium matruchotii*. Archives of Biochemist. Biophysics., 380:331-338.

- **Sieber**, S.A. and **Marahiel**, M.A. *(*2003*)*. Learning from nature's drug factories: nonribosomal synthesis of macrocyclic peptides. J. Bacteriol., 185*:* 7036–7043.

- **Silva**, C.L.; Gesztesi, J.L. and Loneda, T. (1979). Thehalose mycolates from *Nocardia asteroides, Nocardia farcinica, Gordona lentifragmenta* and *Gordona bronchialis.* Chemist. Physi. Lipids., 24:17-25.

- **Singh**, P. and **Cameotra** , S.S. (2004). Potential applications of microbial surfactants in biomedical sciences. Trends in Biotechnol., 22(3): 142-146.

- **Singh**, A; Van Hamme, J.D. and Ward, O.P. (2007). Surfactants in microbiology and biotechnology: Part 2. Application aspects. Biotechnol. Adv., 25: 99–121.

- **Smith**, N.R.; Gordon, R.E. and Clark, F.E. (1952). Aerobic Sporeforming Bacteria (United States Department of Agriculture Monograph no. 16).Washington, DC: USDA. (Cited by Priest *et al*., 1988).

- **Stein**, T.; Vater, J.; Kruft, V.; Otto, A.; Wittmann-Liebold, B.; Franke, P.; Panico, M.; McDowell, R. and Morris , H.R. (1996). The multiple carrier model of nonribosomal peptide biosynthesis at modular multienzymatic templates. J. Biol. Chem., 271: 15428–15435.

- **Stein**, T. (2005). *Bacillus subtilis* antibiotics: structures, syntheses and specific functions. Mol. Microbiol., 56(4): 845–857.

- **Steiner**, S.; Sokoll, A.; Wilde, G.; Bernhard, F.; Franke, P. and Vater, J. (2004). Regulation of cyclic lipopeptide biosynthesis in *Bacillus subtilis*. Appl. Microbiol. Biotechnol., 43: 1133–1138.

- **Stephenson**, F. H. (2003). Calculations in Molecular Biology and Biotechnology. Academic Press, Animprint of Elsevier, USA.

- **Sudo**, T.; Zhao, X.;Wakamatsu, Y.; Shibahara, M. and Nakahara, T. (2000) Induction of the differentiation of human HL-60 leukemia cell line by succinoyl trehalose lipids Cytotechnol., 33: 259–264.

- **Syldatk**, C.; Lang, S.; Matulovic, U. and Wagner, F. (1985).Production of four Interfacial Active Compounds from *n*-Alkanes or Glycerol by Resting Cells of *Pseudomonas* sp. DSM 2874, Z. Naturforsch. 40:61-67. (Cited by Al-Araji *et al*., 2007).

- **Symmank**, H.; Franke, P.; Saenger, W. and Bernhard, F. (2002). Modification of biologically active peptides: production of a novel lipohexapeptide after engineering of *Bacillus subtilis* surfactin synthetase. Protein Engineering, 15:913-921.

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Wondershare™

T

- **Tadros**, F.T. (2005). Applied surfactants , Principles and applications, P.2-12. Wiley- VCH Verlag GmbH and Co. KGaA, Weinheim.

- **Tahara**, Y.; Yamada, Y. and Kondo, K. (1976). A new lysine containing lipid isolated from Agrobacterium tumefaciens. Agric. Biol. Chem., 40:1449-1450.

- **Thaniyavarn**, J.; Roonsawang, N.; Kameyama, T.; Haruki, M.; Imanaka, T.; Morikawa, M. and Kanaya, S. (2003). Production and characterization of biosurfactants from *Bacillus licheniformis* F.2.2. Biosci. Biotech. Biochem., 67(7) :1239-1244.

- **Thimon**, L.; Peypoux, F.; Exbrayat, J.M. and Michel, G. (1994). Effect of iturin A, a lipopeptide from *Bacillus subtilis*, on morphology and ultrastructure of human erythrocytes. Cytobios.,79:69-83.

- **Thomas**, P. (2004). Isolation of *Bacillus pumilus* from in vitro grapes as a long-term contaminant during micropropagation. Plant Cell Rep., 22:813.

- **Thomas**, P. (2006). Reemergence of covert bacteria Bacillus pumilus and Brevibacillus sp. in microbe-freed grape and watermelon stocks attributable to occasional autoclaving-defying residual spores from previous cycles. Plant Cell Tiss. Organ Cult., 87:155–165.

- **Thornberry**, N.A. and **Lazebnik**, Y. (1998). caspases: Enemies within. Sci., 281(5381): 1312-1316.

- **Toolan**, H.W. (1954). Transplantable human neoplasms maintained in cortisone-treated laboratory animals: H.S. No. 1; H.Ep. No. 1; H.Ep. No. 2; H.Ep. No. 3; and H.Emb.Rh. No. 1.Cancer Res., 14(9):660-666.

- **Toraya**, T.; Maoka, T.; Tsuji, H. and Kobayashi, M. (1995). Purification and structural determination of an inhibitor of starfish oocyte maturation from a *Bacillus* species. Appl. Environ. Microbiol., 61:1799-1804.

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Wondershare™

- **Toure**, Y.; Ongena, M.; Jacques, P.; Guiro, A. and Thonart, P. (2004). Role of lipopeptides produced by *Bacillus subtilis* GA1 in the reduction of grey mould disease caused by *Botrytis cinerea* on apple. J. Appl. Microbiol., 96:1151-1160.

- **Tseng**, C.C.; Bruner, S.D.; Kohli, R.M.; Marahiel, M.A.; Walsh, C.T. and Sieber, S.A. (2002). Characterization of the surfactin synthetase C-terminal thioesterase domain as a cyclic depsipeptide synthase. Biochem., 41: 13350–13359.

- **Tugrul**, T. and **Cansunar**, E. (2005). Detecting surfactant- producing microorganisms by the drop- collapse test. World J. Microbiol. Biotechnol., 21:851-853.

- **Van Ginkel**, C. G. (1989). Complete degradation of xenobiotic surfactants by consortium of aerobic microorganisms. Biodegradation, 7(2):151-164.

- **Van Hamme**, J.D.; Singh, A. and Owen P. Ward, O.P. (2006). Physiological aspects Part 1 in a series of papers devoted to surfactants in microbiology and biotechnology. Microbiol. Advances

(2002). Matrix-assisted laser desorption ionization-time of flight mass spectrometry of lipopeptide biosurfactants in whole cells and culture filtrates of *Bacillus subtilis* C-1 isolated from petroleum sludge. Appl. Environ. Microbiol., 68:6210-6219.

- **Veenanadig**, N.K., Gowthaman, M.K. and Karanth, N.G.K. (2000). Scale up studies for the production of biosurfactant in packed column bioreactor. Bioproc. Biosyst. Eng., 22(2):95-99.

- **Visagie**, M. and **Joubert**, A. (2010). Minireview: Immunotherapy and its role in cancer. Biomed. Res., 21(4): 377-381.

- **Vollenbroich**, D.; Mehta, N.; Zuber, P.; Vater, J. and Kamp, R.M. (1994). Analysis of surfactin synthetase subunits in srfA mutants of *Bacillus subtilis* OKB 105. J. Bacteriol., 176: 395-400.

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:

1.No watermark on the output documents.

2.Can operate scanned PDF files via OCR.

3.No page quantity limitations for converted PDF files.

Remove Watermark Now

- **Vollenbroich**, D; Pauli, G; Ozel, M. and Vater, J. (1997). Antimycoplasma properties and application in cell culture of surfactin , a lipopeptide antibiotic from *Bacillus subtilis*. Appl. Environ. Microbiol., 63(1): 44-49.

- **Volpon**, L.; Besson, F. and Lancelin, J.M. (1999). NMR structure of active and inactive forms of the sterol-dependent antifungal antibiotic bacillomycin L. Eur. J. Biochem., 264:200-210.

- **Volpon**. L.; Besson, F.and Lancelin, J.M. (2000). NMR structure of antibiotics plipastatins A and B from *Bacillus subtilis* inhibitors of phospholipase A. FEBS lett., 485:76-80.

W

- **Wakamatsu**, Y.; Zhao, X.; Jin, C.; Day, N.; Shibahara, M.; Nomura, N.; Nakahara, T. and Murata, T. (2001). Mannosylerythritol lipid induces characteristics of neuronal differentiation in PC12 cells through an ERK related signal cascade. Europ. J Biochem., 268: 374–383.

- **Walsh**, C.T.; Gehring, A.M.; Weinreb, P.H.; Quadri, L.E. and Flugel, R.S. (1997). Post-translational modification of polyketide and non-ribosomal peptide synthases. Curr Opin Chem Biol., 1(3): 309-315.

- **Wang**, C.L.; Ng, T.B.; Yuan, F.; Liu, Z.K. and Liu, F. (2007). Induction of apoptosis in human leukemia K562 cells by cyclic lipopeptide from *Bacillus subtilis* natto T-2. Peptides, 28:1344-1350.

- **Wang** , G. and **Wang** T. (2008). Oxidative stability of egg and soy lecithin as effected by transition metal ions and pH in emulsion. J. Agri. Food Chem., 56:11424-11431.

- **Wang**, C.L.; Ng ,T.B.; Cao, X.H.; Jiang , Y.; Liu, Z.K.; Wen, T.Y. and Liu, F. (2009). CLP induces apoptosis in human leukemia K562 cells through Ca2+ regulating extracellular-related protein kinase ERK activation. Cancer Let., 276: 221–227.

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:

1.No watermark on the output documents.

2.Can operate scanned PDF files via OCR.

3.No page quantity limitations for converted PDF files.

Remove Watermark Now

- **Williams**, K. (2011). Biosurfactants for cosmetic application: overcoming production challenges. MMG 445 Basic Biotechnol., 5:78-83.

- **WU**, Z.-j.; LI B.-z. and YUAN H.-l. (2007).Optimization of Biosurfactant-producing Conditions with *Bacillus* sp. W112 and Characterization of the Biosurfactant. Chinese J. Proc. Engin.,7(6): 1175-1180.

X

- **Xu**, Q.; Nakajma, M.; Liu, Z. and shiina, T. (2011). Biosurfactants for micro bubble preparation and application. Int. J. Mol. Sci., 12:462-475.

Y

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:

1.No watermark on the output documents.

2.Can operate scanned PDF files via OCR.

3.No page quantity limitations for converted PDF files.

Remove Watermark Now

- **Yakimov**, M.M.; Abraham, W.R.; Meyer, H.; Laura, Giuliano. and Golyshin, P.N. (1999). Structural characterization of lichenysin A components by fast atom bombardment tandem mass spectrometry. Biochim. Biophys. Acta., 1438:273-280.

- **Yamane**, T. (1987). Enzyme technology for the lipid industry: an engineering overview . J. Am. Oil Chem. Soc., 64:1657-1662.

- **Yang**, S.Z.; Wei, D.Z. and Mu, B.Z.(2006). Determination of the amino acid sequence in a cyclic lipopeptide using MS with DHT mechanism. J. Biochem. Biophys. Methods, 68(1):69-74.

- **Yeh**, E.; Kohli, R.M.; Bruner, S.D. and Walsh, C.T. (2004). Type II thioesterase restores activity of a NRPS module stalled with an aminoacyl-S-enzyme that cannot be elongated. Chem. BioChem., 5:1290–1293.

- **Yin**, H.; Qiang, J.; Jia, Y.; Ye, J.; Peng, H.; Qin, H.; Zhang, N. and He, B. (2009). Characteristics of biosurfactant produced by *Pseudomonas aeruginosa* S6 isolated from oil-containing wastewater. Process Biochem., 44:302-308.

- **Youssef**, N.H.; Duncan, K.E.; Nagle, D.P.; Savage, K.N.; Knapp, R.M. and McInerney, M.J. (2004). Comparison of methods to detect biosurfactnat production by diverse microorganisms. J. Microbiol. Methods., 56: 339-347.

- **Yoshida**, Y. (1988). Cytochrome P450 of fungi: primary target for azole antifungal agents. Curr. Top Med. Mycol., 388-418.

- **Yu**, G.Y.; Sinclair, J.B.; Hartman, G.L. and Bertagnolli, B.L. (2002). Production of iturin A by *Bacillus amyloliquefaciens* suppressing Rhizoctonia solani. Soil Biol. Biochem., 7:955-963.

- **Z**[illegible] Z.; Gutnick, D.L. and Rose[illegible] hydrocarbon – in – water emulsions stabilized by *Acinetobacter* RAG-1emulsan. Biotechnol. Bioeng., 24(2):281-292.

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now

World Wide Web References

Internet (1): World Health Organization: Cancer, 2006 Cited in (http://www.nutrinfo.com/archivos/ebooks/whostat2006.pdf).

Internet (2): Chapter two: detergents Cited in (http://218.6.128.157/excellentcourses/fccae/pdf_textbook/2_jxh_Detergents.pdf).

Internet (3): Nonribosomal peptide synthetases Cited in (http://202.54.226.229/~zeeshan/nrps.html).

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

3. Results and discussion

3.1 Isolation and identification of biosurfactant – producing *Bacillus* species

3.1.1 Isolation and primary characterization of *Bacillus* spp.

In order to isolate biosurfactant producing *Bacillus*, 45 heavily oil contaminated soil samples were collected from fuel stations and Al- Dorah oil refineries in Baghdad Province, from which 109 bacterial isolates were isolated . Oil contaminated soil were chosen for isolation of biosurfactant producing *Bacillus* since they create a selective niche to such microorganisms that have enhanced ability to utilize hydrocarbons as nutrition source (Rahman *et al*., 2002 ; Rahman *et al*., 2003; Priya and Usharani, 2009).

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now

When these isolates were subjected to morphological and cultural [illegible] 88 isolates where suspected to be *Bacillus* isolates since they were gram positive , spore forming bacilli , some of them occur singly , others arranged in pairs but [illegible] wrinkled appearance , they vary greatly in translucence, opaqueness and in color so according to Collins and Lyne (1985), Claus and Berkeley (1986) and Fritz (2004) all these features were characteristics of *Bacillus* spp.

Such high percentage of *Bacillus* isolation (80.7 % of the total isolates) was expected due to the predominant of *Bacillus* spp in the soil (Earl *et al*.,2008) and to the selective isolation method was used that impose high temperature (80°C) on the sample which will select for *Bacillus* that can survive due to their spores formation (Claus and Berkeley ,1986).

.

3.1.2 Screening of biosurfactant producing isolates

To detect the biosurfactant production ability of the 88 *Bacillus* isolates, two detection methods were used , the surface tension measurements and haemolytic activity on sheep blood agar medium .

Results indicated in table (3.1) showed that 87 bacterial isolates were capable of lowering surface tension (ST) of the cell free supernatant after cultivation in E medium, while only 49 isolates were able to haemolyse blood when grew on blood agar plates. Although some negative isolates with the later detection method were capable of lowering (ST) with noticeable percentages (29-30) %, such observation was also detected by Youssef *et al.* (2004) who found that 49 % of the studied strains were unable to haemolyse blood but capable of lowering (ST), while [illegible] % of the isolates were able to haemolyse blood [illegible] lowering (ST). These results can be explained by the fact that not all kinds of biosurfactants can haemolyse blood especially [illegible] diffusible one (Iqbal *et al.* [illegible] found that b[illegible] other microbial products such as virulence factors.

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now

Upon quantitative screening for the highest biosurfactant producing isolates in E medium, ten isolates were chosen for further studies named (B1, B2, B3, B4, B5 , B6 , B7, B8, B9 , B10) as shown in table 3.2.

The ability of these ten isolates in lowering (ST) was also studied by another detecting method (oil spreading method), results in table (3-3) showed that diameter of the oil spreading was proportional with lowering of surface tension by tensiometer, such results were also recorded by Priya and Usharani, (2009).

.

Table (3-1) Detection of biosurfactant activity by measurement (ST) of E medium by Du Nouy ring tensiometer and blood haemolysis methods after cultivation in E medium for 24 hrs.

Number of isolates	Percentage of lowering the surface tension%*	Number of isolates	Blood haemolysis
18	28-30	14	+
		4	-
23	25-27	11	+
		12	-
40	14-24	19	+
		21	-
6	4.5-13	5	+
		1	-

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

* Percentage of lowering surface tension by using Du Nouy ring

Table (3-2) Percentage of lowering surface tension of B isolates in E medium after cultivation in E medium for 24 hrs. determined by Du Nouy ring tensiometer.

Symbol of isolates	Percentage of lowering surface tension%
B1	29.10
B2	29.54
B3	29.72
B4	28.60
B5	29.55
B6	29.56
B7	29.55
B8	28.40
B9	30.00
B10	29.20

.

Table (3-3) Detection of biosurfactant activity by measuring lowering of (ST) of Jacques medium by Du Nouy ring tensiometer and oil spreading method after cultivation in Jacques medium for 72 hrs (180 rpm).

Symbol of isolates	Percentage of lowering surface tension%	Oil spreading method (cm)
B1	43.3	6.3
B2	42.3	6.0
B3	41.5	5.8
B4	46.2	7.0
B5	44.6	6.5
B6	43.5	6.3
B7	43.7	6.3
B8	45.0	6.7
B9	42.5	6.1
B10	42.1	6.0
Standard surfactin	50.0	9.0

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Wondershare™

3.1.3 Molecular Identification of *Bacillus* species

3.1.3.1 Isolation of Genomic DNA

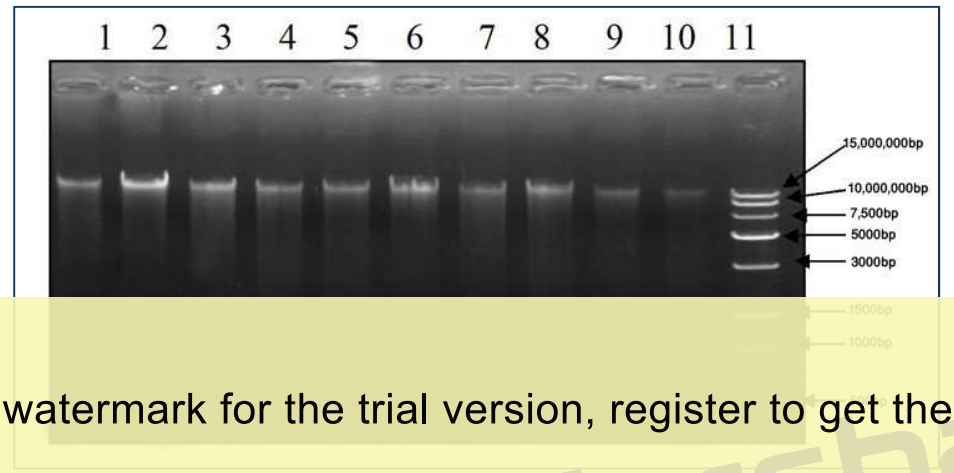
In order to amplify16S rDNA for local isolates of *Bacillus* spp., genomic DNA of the selected isolates was extracted to provide a PCR template for the amplification.

Purity ratios of the extracted DNA samples were (1.8-1.9) which indicates a high purity since a pure DNA preparation has expected A260/A280 ratio of ≈1.8. Which are based on the extinction coefficients of nucleic acids at 260 nm and 280 nm (Maniatis *et al*., 1982).

.

Such results were also observed when the DNA samples were analyzed by gel electrophoresis, in which sharp DNA bands were detected indicating purified DNA samples as shown in figure (3-1) .

The concentrations of the DNA samples were range between (24-79) µg/µl.



This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Bacillus B4, Lane (8): *Bacillus* B3 , Lane (9): *Bacillus* B2 , Lane (10): *Bacillus* B1, Lane (11): 15Kb DNA landmark ladder

3.1.3.2 Amplification of 16S rDNA

Two sets of primers were used to amplify the 16S rDNA .

Forward Primers:

(B16SF) 5′-TGTAAAACGACGGCCAGTGCCTAATACATGCAAGTCGAGCG-3′
(27F) 5′-AGAGTTTGATCCTGGCTCAG-3′

Reverse Primers:

(B16SR) 5′-CAGGAAACAGCTATGACCACTGCTGCCTCCCGTAGGAGT-3′

.

(1492R) 5′-ACGGTTACCTTGTTACGACTT -3′

The first set (B16SF and B16SR) were used to amplify the 5′ end region (approx. 275 bp) which was the hypervariant region (HV region) in the16S rDNA gene and was highly specific for each type of bacterial strain of *Bacillus* spp.(Goto *et al*., 2000).

In order to check the specifity of the amplification by the used primers, gel electrophoresis was carried out as shown in figure (3.2) which revealed single amplicon band with proximal size 300 bp while the other band of about 100bp size that appeared reflect primers polymerization.

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

(Figure 3.2) Agarose gel electrophoresis for a PCR amplification of 16S rDNA by the set of primers (B16SF and B16SR) of the *Bacillus* isolates on (0.8%) agarose gel for 15 minutes, 72 voltage.

Lane (1) : *Bacillus* B10, Lane (2): *Bacillus* B9, Lane (3): *Bacillus* B8, Lane (4): *Bacillus* B7 , Lane (5): *Bacillus* B6, Lane (6): *Bacillus* B5 , Lane (7): *Bacillus* B4, Lane (8): *Bacillus* B3 , Lane (9): *Bacillus* B2 , Lane (10): *Bacillus* B1, Lane (11): 2Kb DNA landmark ladder.

.

PCR products were purified and sequenced as illustrated in figure (3-3) and (appendices 1-9) which showed the complete nucleotide sequence of the HV region of the 16S rDNA gene.

GAATGGGAGCTTGCTCCCTGATGTTAGCGGCGGACGGGTGAGTAACAC
GTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGGGGCT
AATACCGGATGCTTGTTTGAACCGCATGGTTCAAACATAAAAGGTGGC
TTCGGCTACCACTTACAGATGGACCCGCGGCGCATTAGCTAGTTGGTGA
GGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTG
ATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCA

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Wondershare™

Figure (3-3) Sequence of PCR product of amplification of 16S rDNA by the universal primers of the *Bacill*

Isolates identities were established by comparing the sequences obtained with the NCBI data base, results showed that all the selected isolates were belong to the *Bacillus* species with (99- 100) % similarities with query coverage (92-93) % ,such results were also obtained by Goto *et al.*(2000) who revealed that this (HV) region was highly conserved within the species.

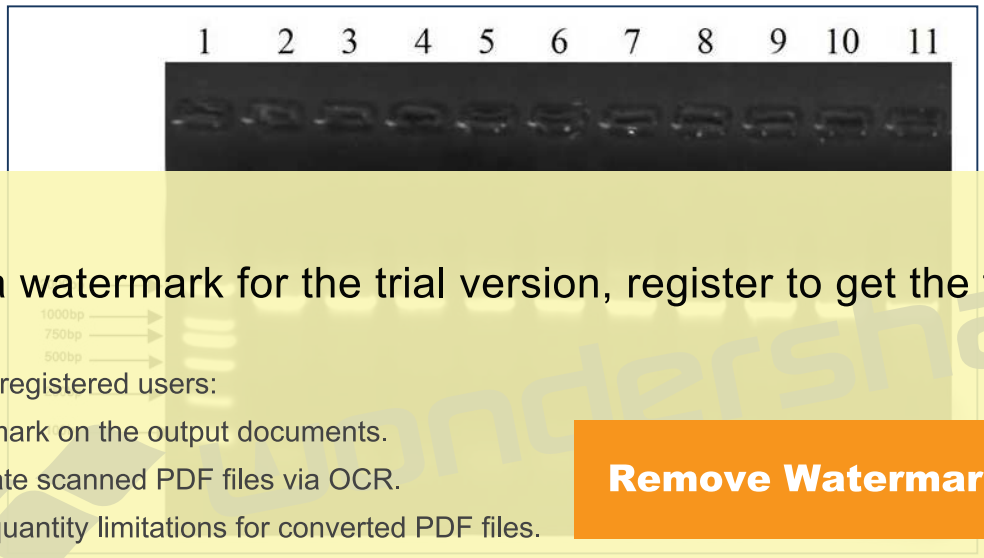
Also it was found that as shown in table (3-4) the partial 16S rRNA gene sequences of all isolates show sequence similarity to certain *Bacillus* strains mostly to *B. subtilis.*

One other set of primers was used (27F and1492R) which are universal primers for amplifying complete 16S rRNA gene of *Bacillus* to identify the

.

selected isolates to the strain level (Akhmaloka *et al*., 2006 ;Thomas, 2004; 2006 ; Cerritos *et al*.,2008).

PCR products for the selected isolates were observed on agarose gel electrophoresis. The result showed an amplicon band for each one with the size of 1.5 kb respectively (Figure 3.4).



This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

(Figure 3.4) Agarose gel electrophoresis for a PCR amplification of 16S rDNA by the set of primers (27F and1492R) of the *Bacillus* isolates on (0.8%) agarose gel for 15 minutes, 72 voltage. Lane (1) : 2Kb DNA landmark ladder, Lane (2): *Bacillus* B10 isolate , Lane (3): *Bacillus* B9, Lane (4): *Bacillus* B8 , Lane (5): *Bacillus* B7, Lane (6): *Bacillus* B6 , Lane (7): *Bacillus* B5, Lane (8): *Bacillus* B4 , Lane (9): *Bacillus* B3 , Lane (10): *Bacillus* B2, Lane (11): *Bacillus* B1.

.

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Wondershare™

.

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Wondershare™

.

Results from sequencing (Appendices10-11) showed the full length of the specified piece of 16S rDNA gene for two isolates (B6 and B9) while the other eight isolates were partially sequenced due to loop structure in the amplified amplicon so molecular analysis was done by using partial 16S rDNA sequences from the 5′ (632bases) and 3′ ends (725 bases) of first strand to these isolate, such results were also obtained by Thomas (2006).

Alignment of these sequences with NCBI data base revealed that strain B6 show sequence similarity 100% to *B. subtilis (* Genebank accession no. AB526464.1 and AB383135.1) with query coverage 100% and with the higher score, other strains show similarities range (96-100) % with (41) *B. subtilis,* (7) *Bacillus mojavensis,*(2) *Bacillus amyloliquefaciens,* (2) *Bacillus axarquiensis,* [illegible] (illustrated in table 3-4).

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

All of these species are members of the *Bacillus subtilis* group which are closely related and thus not easily distinguishable. All members of the group are placed in 16S rRNA ONA similarity. The two species *B. subtilis* and *B. amyloliquefaciens* are not distinguishable phenotypically so far. The same is true for *B. licheniformis* and B*acillus sonorensis*. (Fritz ,2004).

Other sequences of the rest studied isolates also show the greatest sequence similarity to a large number of *B. subtilis* as shown in table (3-4) in comparing with other *Bacillus* spp.

Lee *et al*., (2007) depended partial sequencing of the 16S rRNA gene and partial *gyrA* gene to identify a strain of *B. amyloliquefaciens* , they found that the partial 16S rRNA gene of *B. amyloliquefaciens* LP03 exhibited over 98% homology with most *Bacillus* species, but the partial *gyrA* gene showed greater homology with *B. amyloliquefaciens* (95%) than other *Bacillus* species (less than 83%). While Joshi *et al*. (2008) got 98% sequence similarity to *B. subtilis* as per

.

NCBI, BLAST (Genbank accession no. DQ 922951) when align the partial 16S rRNA gene of the strain *B. subtilis* 20B which was already identified by biochemical tests as *B. subtilis.*

3.2 Selection of the best biosurfactant production media

In order to select the best production media for biosurfactant production by the selected isolates, two media were used; E medium and Jacques Medium (Youseef *et al.*,2004 ; Jacques *et al.* ,1999).

Results shown in table (3-5) illustrated that Jacques medium was the best one for biosurfactant production compared to E medium since a high percentage of lowering surface tension by produced biosurfactant was achieved.

However, to screening biosurfactant production, E medium was found to be better than Jacques medium since it require shorter time with no agitation.

For further experiment steps Jacques medium was selected as the production medium.

Table(3-5) Percentage of lowering surface tension by biosurfactant produced in two production media.

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Symbol of Isolates	Percentage of lowering (ST) in Jacques medium (%)	Percentage of lowering surface tension in E medium (%)
B1	43.3	29.10
B2	42.3	29.54
B3	41.5	29.72
B4	46.2	28.60
B5	44.6	29.55
B6	43.5	29.56
B7	43.7	29.55
B8	45.0	28.40
B9	42.5	30.00
B10	42.1	29.20

.

3.3 Optimum period for biosurfactant production

The selected *B. subtilis* isolates (B1, B2, B3,B4 ,B5, B6, B7, B8,B9,B10) were propagated in Jacques medium for different periods of time in order to determine the production time for biosurfactant production from each isolate.

Results from figure (3-5) revealed that maximum yields (g/l) of the surface active agents produced by *B. subtilis* isolates B1 ,B2, B6, B7,B9 and B10 were achieved after 48 hours of incubation , followed by drop in the yield which may result from the consumption of biosurfactant as a carbon source after depletion of nutrients in the broth (Shaligram and Singhal, 2010). It was also found that surfactin acts as end product inhibitor which lead to lower yield (Drouin and Cooper, 1992).While *B. subtilis* isolates B3, B4,B5 and B8 were able to produce the surface active agent with the yield after 72 hrs of incubation.

Also it was found that the highly produced biosurfactant isolate was *B. subtilis* B4 with 3.6 g/l yield after 72 hrs of incubation.

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

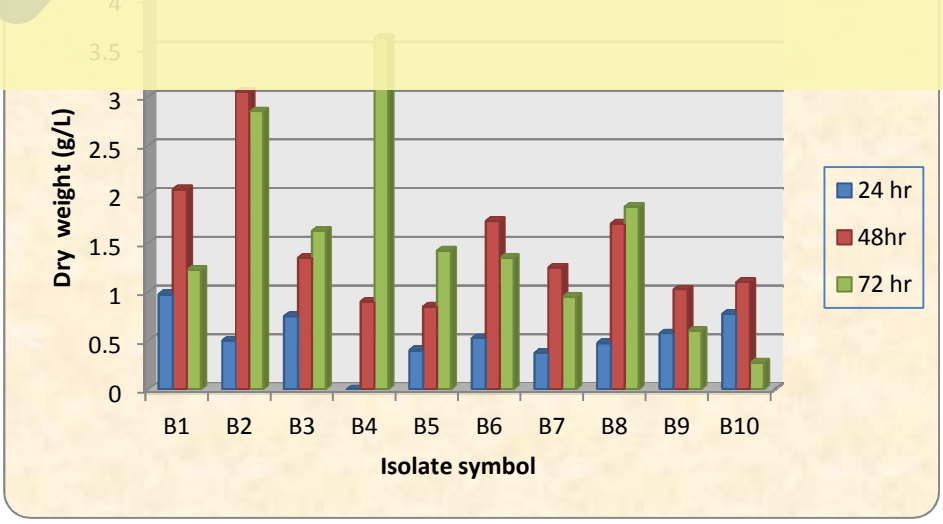


Figure (3-5) Dry weight of biosurfactant produced by *Bacillus subtilis* isolates propagated in Jacques medium for different incubation time.

.

3.4 Purification of biosurfactant produced by the selected isolates

Combination of techniques were used to purify biosurfactants produced by the ten selected *B. subtilis* isolates , beginning with acid precipitation of biosurfactant in cell – free supernatant to crystallize biosurfactant since such compounds tends to become insoluble at low pH due to charge neutralization and protonation of carboxylic acid side chains of aspartic or glutamic acids in the peptide portions of these molecules (Maneerat and Phetrong, 2007).

The obtained brown precipitates were solubilized in alkaline D.W to retain their surface active lowering activity followed by lyophylization and weighing to calculate the primary yield of biosurfactant (g/L of broth) as shown in table (3-6).

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

These results were inagreement with Cooper *et a.l* (1981) who found that acidification of the supernatant of the strain *B. subtilis* ATCC 21332 lowered the surface tension from 27 mN/m to 62 but when neutralized without removing the precipitate, the surface tension dropped to the original value of 27 mN/m and the original CMC

Table (3-6) Weight of partially purified biosurfactant from the selected *Bacillus* isolates after cultivation in Jacques medium for the optimum period of incubation.

Isolates Symbol	Incubation periods (hrs.)	Partially purified biosurfactant (g/l)
B1	48	2.050
B2	48	3.050
B3	72	1.625
B4	72	3.600
B5	72	1.425
B6	48	1.725
B7	48	1.250
B8	72	1.875
B9	48	1.025
B10	48	1.100

.

For further purification, a two-step membrane ultrafiltration process was performed for each sample; the first step to remove low molecular weight contaminant, while the high molecular contaminant were removed during the second step of ultrafiltration ((Lin *et al*, 1998a, Isa *et al*.,2007).

Results in table (3-7) showed the yield of purified biosurfactant, Chen *et al.* (2007b) got 97% recovery of the initial concentration of surfactin by this method.

Table (3-7) Weight of purified biosurfactant from the selected *B. subtilis* isolates by two steps ultrafiltration method.

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Symbol of isolates	Purified biosurfactant (g/l)
B1	0.175
B2	0.175
B3	0.12
B4	
B5	
B6	
B7	0.075
B8	0.100
B9	0.125
B10	0.150

3.5 Characterization of purified biosurfactant

3.5.1 By thin layer chromatography (TLC)

Characterization of biosurfactants produced by the ten selected *B. subtilis* isolates was done by TLC analysis which was frequently employed for the characterization of biosurfactants (Javaheri *et al*., 1985; McInerney *et al*., 1990).

.

Each sample of purified biosurfactant was dissolved in absolute methanol and analyzed on silica gel plates by using chloroform-methanol-water (65:25:4v/v/v) as the mobile phase.

Thin-layer chromatography of the purified biosurfactant and standard surfactin revealed white spots when the plate was sprayed with water as indication of hydrophilic compound (Figures (3-6) and (3-7)). Same spots were appeared with the rhodamine B when examined under UV light, indicating the presence of lipids in the compounds. As a conclusion, purified biosurfactants were belonged to the lipopeptide family.

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

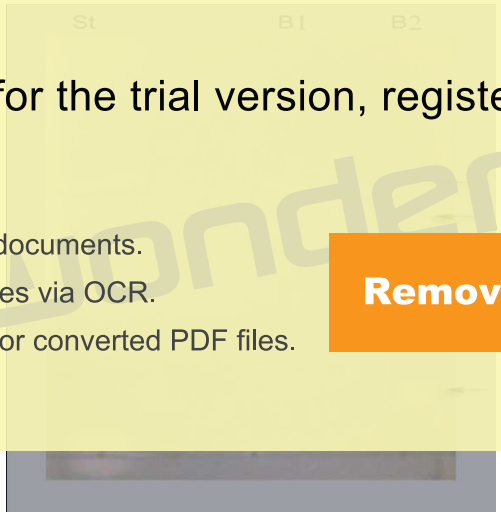
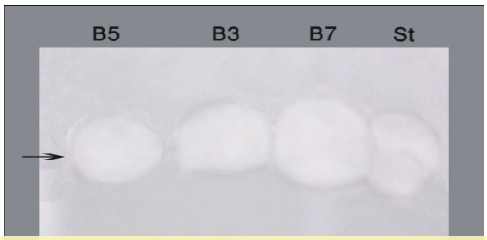


Figure (3-6) TLC analysis of biosurfactants produced by *B. subtilis* isolates (B1,B2) ,st: standard surfactin

Results of table (3- 8) showed that the relative mobility (R*m*) of standard surfactin was (0.6) with single spot. Regarding the isolates, purified biosurfactants from isolates (B1, B2) were separated into three and four spots, resprctively, and biosurfactants from isolates (B3.B7,B8,B5) were separated into two spots, while

.

(B4,B6,B9,B10) were separated into a single spot. However, almost all isolates were capable to produce one spot that align with the spot of standard surfactin , indicating that all isolates were capable in producing surfactin. This fact was also supported by the haemolytic activity of all the selected isolates, a characteristic of the surfactin (Moran *et al*., 2002).



This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Figure(3-7) TLC analysis of biosurfactant produced by *B. subtilis* isolates (B3, B5,B7)

Separation of other spots indicated the presence of different species or isoforms of biosurfactants which were detected in many strains of *Bacillus* such as *B. subtilis* complex BC1212 that produced four surfactin isoforms (A,B,C and D) differed slightly in their physiological properties (Kim *et al*., 2006). Sandrin *et al*. (1990) isolated *B. subtilis* S 499 strain that produced surfactin and antifungal lipopeptides belonged to the iturin family.

.

Table (3-8) Relative mobility of the purified surface active agents produced by the *B. subtilis* isolates (B1,B2,B3,B4,B5,B6,B7,B8,B9,B10) after TLC Separation .

sample	Number of spots	Relative mobility (R*m*)
Standard surfactin	1	0.6
B1	3	0.4,0.5, 0.6
B2	4	0.1,0.4, 0.5, 0.6
B3	2	0.1,0.6
B4	1	0.6
B5	2	0.1, 0.6
B6	1	0.6
B7	2	0.1,0.6
B8	2	0.45 ,0.6
B9	1	0.6
B10	1	

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Wondershare™

followed by ms/ms analysis to study the amino acids sequence in the purified biosurfactants.

Under same purification conditions , the purified biosurfactant produced by the isolate *B. subtilis* B6 were resolved into main five fractions on HPLC with the same retention time as standard surfactin (figures (3-8) and (3-9)).

Separation of standard surfactin into several fractions was due to the fact that natural surfactin produced by *B. subtilis* was a mixture of isoforms with slightly different properties as a result of substitutions in amino acids and the aliphatic chain (Grangemard *et al*., 1997).

.

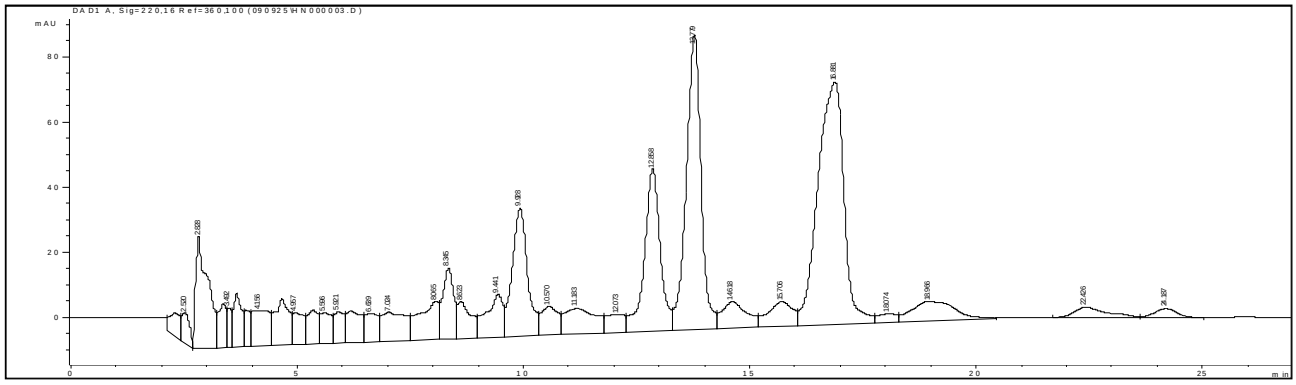


Figure (3-8) HPLC spectrogram of standard surfactin from Sigma-Aldrich

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Figure (3- 9) HPLC spectrogram of the purified lipopeptide produced by *B. subtilis* B6.

In order to compare the molecular mass of standard surfactin and the purified lipopeptide, Ion extracted chromatogram (IEC) of fractions retained in the HPLC were studied by HPLC- mass .

Results of table (3-9) and chromatograms in appendices (21-30) showed that both standard surfactin and purified lipopeptide had the same molecular weight.

.

Table (3-9) Results of IEC of the standard surfactin and purified lipopeptide .

Retantion time (minutes) of peaks of standard surfactin	**Retantion time (minutes) of peaks of purified lipopeptide**	***m/z* $[M+H]^+$**	***m/z* $[M+Na]^+$**	**Molecular weight (Dalton)**
8.4	8.4	995	1017	993.92
9.9	9.8	1009	1031	1007.9
13	12.8	1023	1045	1021.9
16.9	16.6	1037	1059	1035.9
22.4	22.7	1051	1073	1049.9

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

give important information about the connecting relationship of the peptide since the difference between any two peaks was the mass of the lost fragments so the differences can be used to determine the connection of some amino acid residue in a peptide chain (Wang *et al*.,2007 ; Cao *et al*.,2009b).

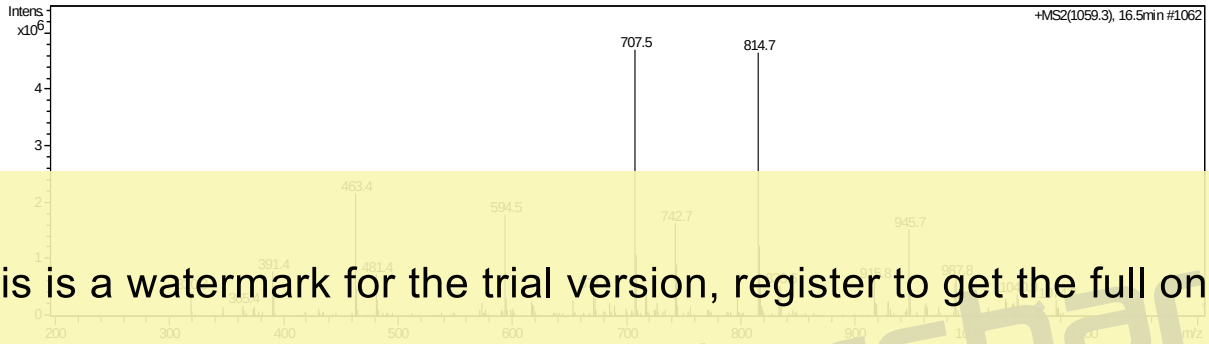
So each peak in table (3-6) was further fragmented by MS2 which generating an identifying mass spectrum for each component in the mixture.

The mass spectrum of $[M+Na]^+$ at m/z 1059 (figure 3-10) showed the connection of the following amino acids Leu -Leu -Asp –Val (as a result of peak

.

1059.3 → 945.7 → 833.6 → 718.5 → 619) while the peaks (707.5 → 594.5 → 481.4 → 365.4) suppose the connection of Leu- Leu –Asp.

In addition , according to the mechanism of (double hydrogen transfer), the C-terminus of the peptide was formed at peak 800.6 which represent the loss of Leu-Leu-OH_2, which suggests that a leucine residue serves at the C-terminus of the peptide chain (Yang *et al*.,2006).



This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Wondershare™

Figure (3.10) Mass spectrum obtained from [M+Na]$^+$ of the peak 1059 of *Bacillus* B6 lipopeptide

Analysis of the other peaks of [M+Na]$^+$ at m/z (1017, 1031,1045,1073) revealed the same amino acids connection for the studied lipopeptide as shown in appendices (31-34).

3.6 Optimization of biosurfactant production by isolate *B. subtilis* B6

3.6.1 Effect of carbon source

Carbon source represents an important factor in biosurfactant production; therefore, several kinds of carbon sources were tested to optimize the production of biosurfactant.

.

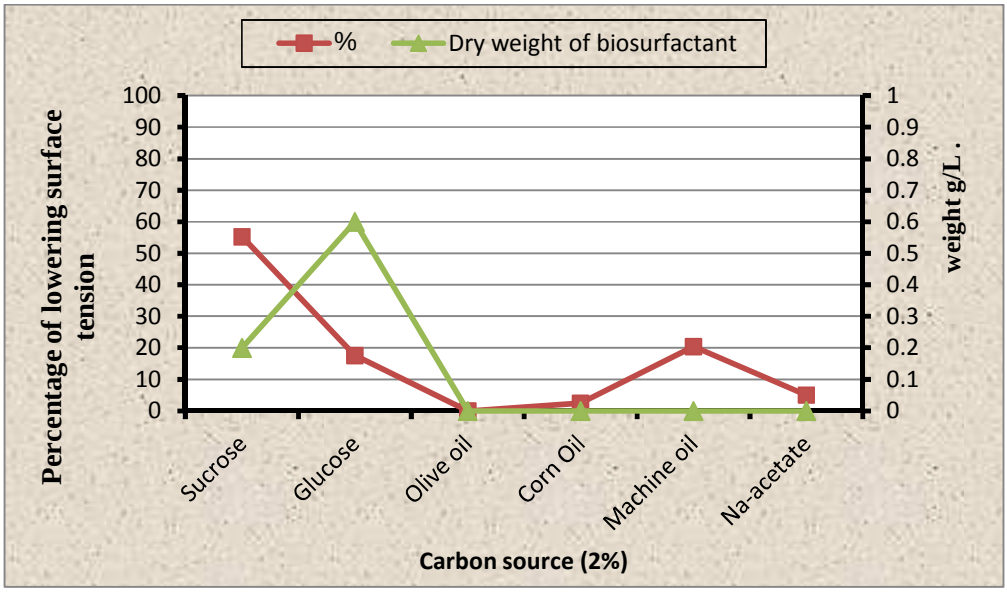


Figure (3-11) Effect of carbon source (2%) on biosurfactant production from *B. subtilis* B6, grown in Bushnell-Hass medium at 30 °C in a shaker incubator (180 rpm) for 72 hrs.

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

As mentioned in figure (3-12) illus[illegible] sources (glucose and sucrose) were the best substrates for synthesis of biosurfactant in reducing the surface tension (ST). Although glucose was better than sucrose in increasing the surfactin yield, the later was better in enhancing the percentage of (ST) reduction.

Addition of water insoluble carbon source (Olive oil, Corn oil, machine oil) didn't support the production of biosurfactant.

This characteristic for the carbon sources utilization was in accordance with Sandrin *et al*., (1990) and Kim's *et al*, (1997) results that found *B. subtilis* prefer the water-soluble carbon sources especially glucose, fructose and sucrose as carbon sources to produce biosurfactants while, addition of the hydrocarbons to culture

.

medium completely inhibited surfactin production by *B. subtilis* (Davis *et al.*,1999) .

Makkar and Cameotra (2002) found that *Bacillus subtilis* MTCC 2423 was unable to utilize sodium acetate as a carbon source for biosurfactant production, while Liu *et al.*, (2009) found that in medium containing olive oil , *B. subtilis* can grow and produce biosurfactant but was unable to lower the (ST). In contrast, glucose, sucrose and sodium supported the production of biosurfactant

When the biomass and biosurfactant production by *B. subtilis B6* grown on different carbon sources were compared, great variations in the O.D. of cells and concentration of lipopeptides were recorded. Maximum O.D. values were observed for glucose, corn oil and sucrose (1.373, 1.36,1.266), respectively. Whereas the lowest production in olive oil, soybean oil and sodium acetate recorded to only O.D. values (0.66 ,0.61and 2.47), respectively.

Such variations in results were also achieved by Haddad *et al.* (2009) who found that [illegible] carbon source [illegible] especially glucose.

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now
Wondershare™

As a consequence of these results sucrose was subsequently used.

3.6.2 Effect of carbon source concentration

Different concentrations (0.5, 1, 2, 3, 4, 5%) of sucrose after was chosen as the best carbon source for surfactin production ,were used to propagate *B. subtilis* B6 to determine the optimum concentration.

.

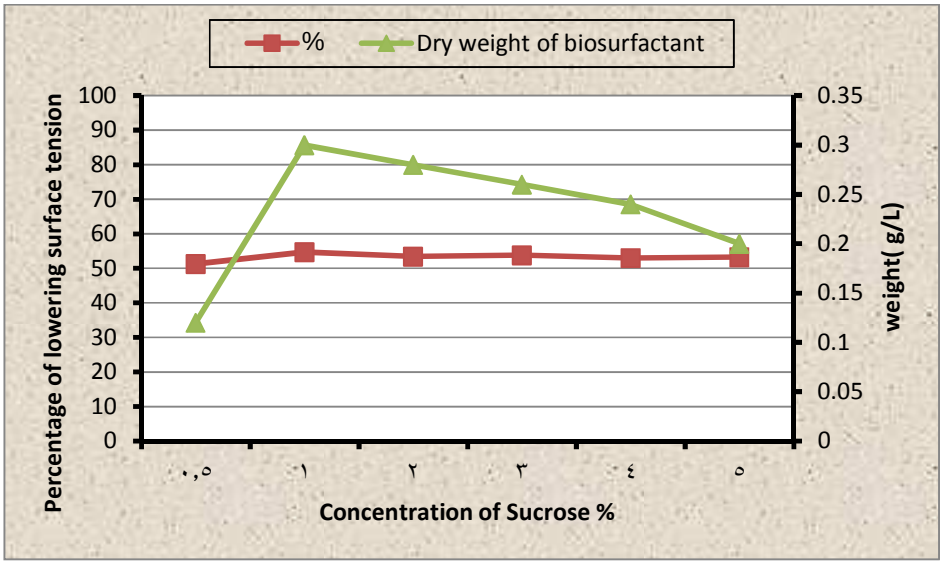


Figure (3-12) Effect of sucrose (as carbon source) concentration on biosurfactant production from *B. subtilis* B6, grown at 30 °C in a shaker incubator (180 rpm) for

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

producing isolates *Bacillus* B6, since the O.D of the bacterial growth dropped from 1.6 at 1% sucrose concentration to 1.3 at 2%.

Regarding lowering ST, highest percentage was obtained at sucrose concentration of (1%), while higher or lower concentrations caused decreased in the percentage.

These results were in accordance with Hartoto and Mangunwidjaja (2002) who studied the importance of sucrose and its concentration in surfactin production by *Bacillus sp.*BMN14 in a shaker flask. They found that an optimum surfactin production with lower ST and higher bacterial mass was reached at sucrose

.

concentration of 2%, but dropped when the concentrations increased or decreased from that.

3.6.3 Effect of nitrogen source

Biosurfactant production was depending on the nitrogen source used in the fermentation. As seen from figure (3-13), inorganic nitrogen sources were more efficient in enhancing the isolate for production of biosurfactant than organic sources. Also, it was observed that among the inorganic nitrogen sources, potassium nitrate was the best as it caused the production of highest yield of biosurfactant (0.6 g/l) along with highest reduction in ST (54%) ,while ammonium nitrate and sodium nitrate were less efficient in producing biosurfactant.

Regarding organic sources, peptone was the most efficientwhile the other sources gave relatively lower results.

In contrast with biosurfactant production results, organic nitrogen sources [illegible] with the efficiency [illegible] which [illegible] the growth of the bacterial group [illegible] inorganic sources : patassium nitrate , sodium nitrate, ammonium sulphate, ammonium nitrate and ammonium chloride resulted in the following O.D. (4.051,4.018,2.045,1.69,1.67).

This is a watermark for the trial version, register to get the full one!

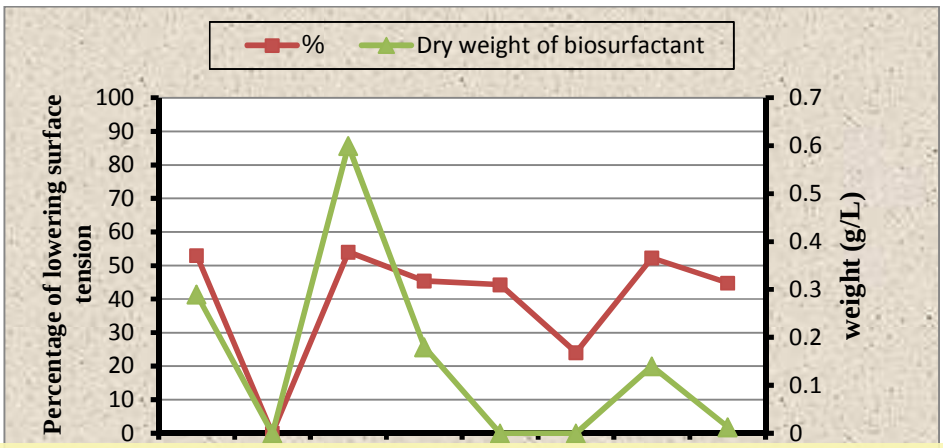
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

As a consequence of these results potassium nitrate was chosen for next experiments.

These results were in agreement with Makkar and Cameotra (2002)) who noted potassium nitrate salts as a preferable nitrogen source for the biosurfactant production by *B. subtilis* MTCC 2423. However, Haddad *et al.* (2009) found itammonium sulphate ,while, Abushady *et al.*(2005) mentioned ammonium nitrate, and Ghribi and Ellouze-Chaabouni (In press) mentioned urea as the best nitrogen source by different isolates of *B. subtilis,* furthermore, another group (Wu

et al.,2007) use a mixture of two nitrogen sources (ammonium sulphate :ammonium nitrate) in a ratio (1:2) to produce biosurfactant by *Bacillus* spp.



This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

3.6.4 Effect of nitrogen concentration

The effect of different concentrations of potassium nitrate on biosurfactant production which was chosen as the best nitrogen source was studied.

Results illustrated in figure (3-14) showed that the optimum concentration was 0.3% for both biosurfactant yield and lowering ST, which drop dramatically above this concentration while, lower concentration had lower effect on biosurfactant production .

.

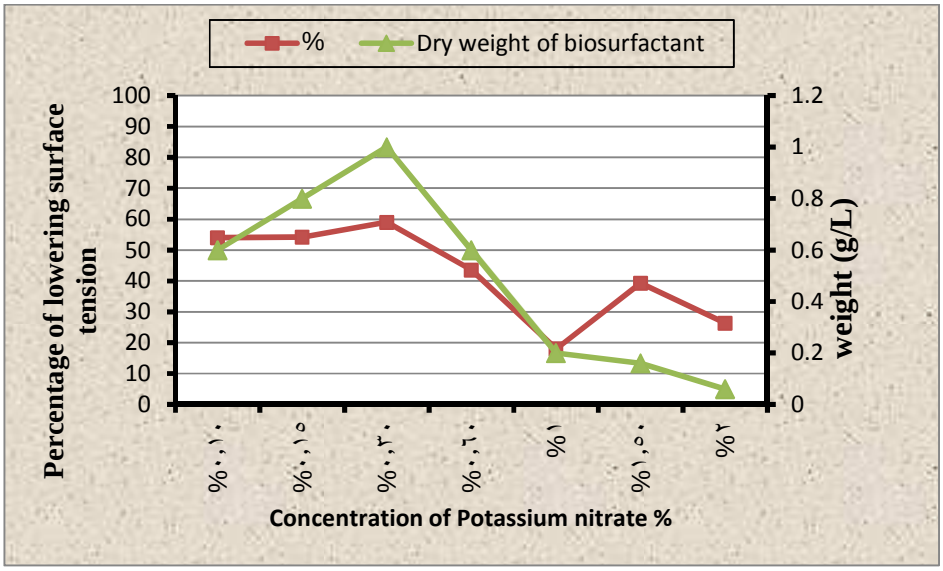


Figure (3-14) Effect of potassium nitrate (as a nitrogen source) concentration on biosurfactant production from *B. subtilis* B6 grown with 1% sucrose at 30 °C in a shaker incubator (150 rpm) for 24 h.

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

3.6.5 Effect of phosphate concentration

Effect of phosphate concentration on biosurfactant production was studied.

Results in figure (3-15) showed that optimum concentration was 1g/l for biosurfactant production which decreased when phosphate concentration change above or below the optimum concentration.

.

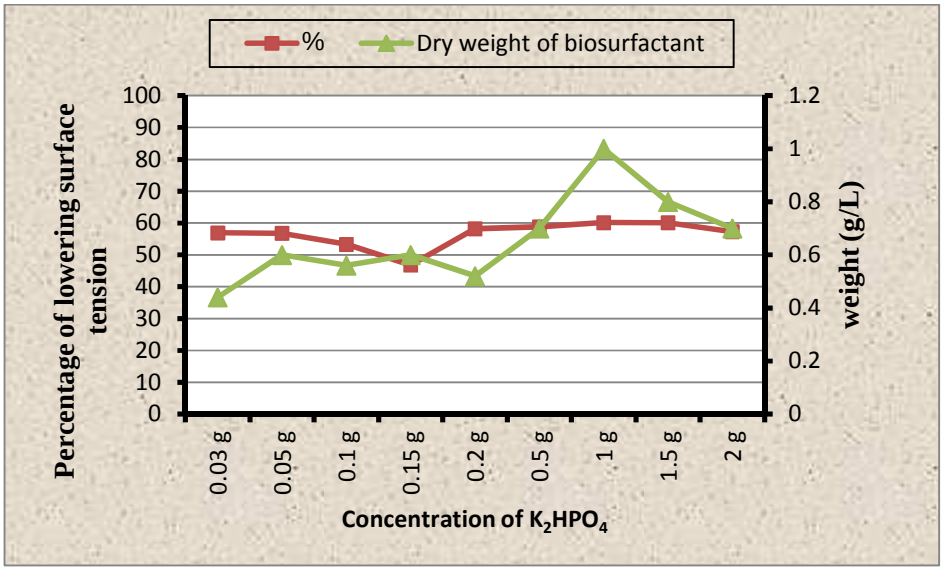


Figure (3-15) Effect of KH_2PO_4 concentration on biosurfactant production from *B. subtilis* RS grown with 4% sucrose and 0.3% KNO_3 at 30 °C in shake incubator (180 rpm) for 72 hrs.

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:

1.No watermark on the output documents.

2.Can operate scanned PDF files via OCR.

3.No page quantity limitations for converted PDF files.

Remove Watermark Now

biosynthesis at high concentration is that phosphate repression might play a role in the regulation of surfactin production, which is consistent with the negative effect of easy utilizable phosphate source on the biosynthesis of antibiotics and other secondary metabolites at the level of transcription (Liras *et al*.,1990).

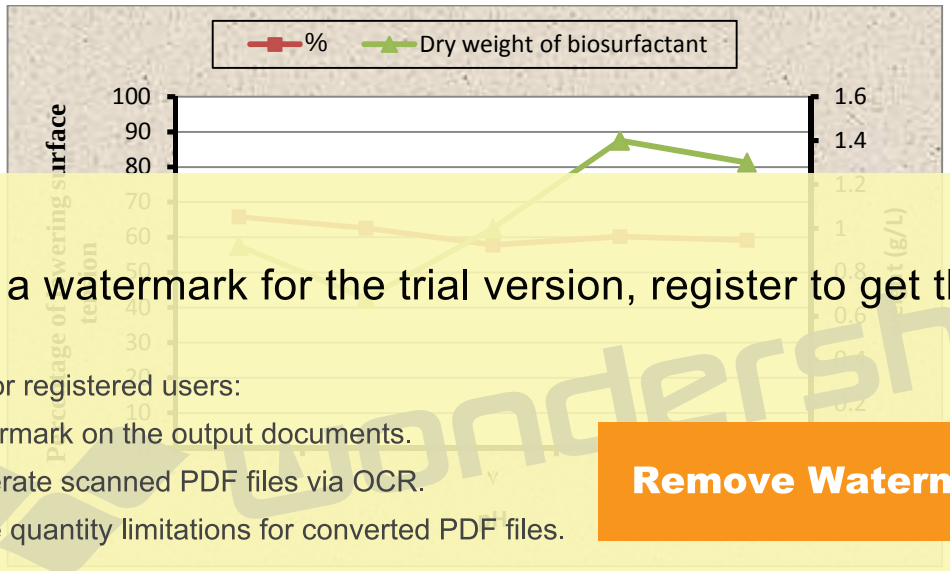
While in some *B. subtilis* strains, high phosphate concentration was demanded to increase the yield of biosurfactant as found by Kim *et al*. (1997), *B. subtilis* C9 required as much as 13.5% K_2HPO_4 for production of high yield biosurfactant.

.

3.6.6 Effect of pH

The pH of medium plays an important role in biosurfactant production, so to different pH values the production media was adjusted.

Results from figure (3-16) revealed that the optimum pH for biosurfactant production was 8 and the yield drop when pH decreased or increased from that value.



This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now

Figure (3-16) Effect of pH on biosurfactant production from *B. subtilis* B6, grown with 1% sucrose, 0.3% KNO_3 and 1g/L K_2HPO_4 at 30 °C in a shaker incubator (180 rpm) for 72 hrs.

These results were in accordance with that of a study conducted on *B. licheniformis* byAli *et al*. (2010) and Huszcza and Burczyk (2003) when they studied the biosurfactant production by *B. coagulanse*.

Sepahy *et al*. (2005) found that very low yield of surfactin was recorded when the production medium of *B. subtilis* was adjusted at pH values lower than 6.5, and the maximum yields were achieved at a pH range of (6.5- 8.5).

.

Makkar and Cameotra (2002) found that *B. subtilis* strain MTCC 2423 was able to produce biosurfactant in a pH range of (4.5- 10.5), although the maximal yield of the biosurfactant was obtained at pH 7.0 (, 2002).

3.6.7 Effect of temperature

Different temperatures of incubation were investigated to study their effect on biosurfactant production.

The optimal incubation temperature was 30°C for biosurfactant production as illustrated in figure (3-17) followed by 37 °C and dropped significantly at 40°C and 28°C while the OD. of the bacterial growth was higher at 30°C followed by 37°C then 28°C , with less value at 40°C as follows (4.1 , 3.5, 3.1, 2.0).

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

above 40 °C (Makkar and Cameotra, 2002; Sepahy *et al*., 2005).

3.6.8 Effect of incubation period

The surfactin production medium was inoculated with *B.subtilis* B6 and incubated for different incubation periods from 12- 96 hrs, figure (3-18) show that surfactin concentration increased with the increased in incubation period up to 72 hrs but surfactin concentration was decreased with the other incubation periods.

Also it was observed that reduction in ST was not highly effected by the incubation period.

.

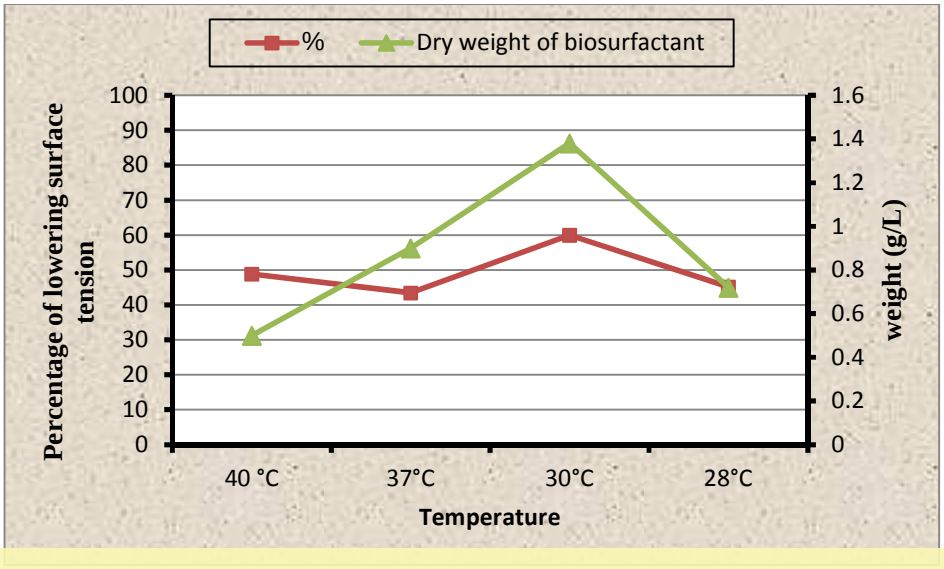


Figure (3-17) Effect of temperature on the growth and biosurfactant production from *B. subtilis* B6, grown with 1% sucrose, 0.3% KNO_3 and 1g/L K_2HPO_4, pH 8, at 30 °C in a shaker incubator (180 rpm) for 72 hrs.

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now

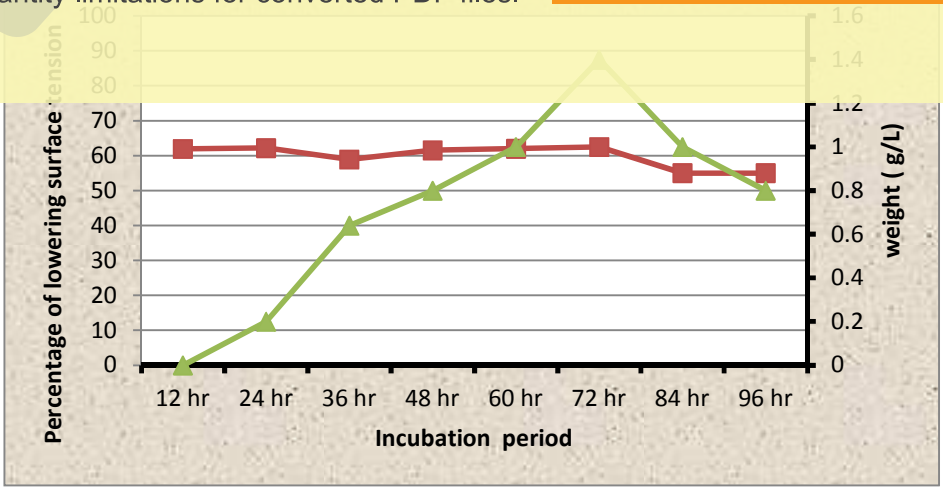


Fig (3-18) Effect of incubation period on biosurfactant production from *B. subtilis* B6, grown with 1% sucrose, 0.3% KNO_3 and 1g/L K_2HPO_4, pH 8, at 30 °C in a shaker incubator (180 rpm) for 72 hrs.

.

In this regard Abushadi *et al*. (2005) found that there was no biosurfactant production before 24 hrs,. but it increased until reached its maximum at 72 hrs. Above that, no further increases in the concentration was observed.

Ali *et al*. (2010) repoted that best incubation time for a strain of *B. licheniformis* was 72 hrs of incubation and biosurfactant production was decreased in other incubation periods used.

3.7 Biological activity of purified biosurfactants

3.7.1 The cytotoxicity assay by MTT kit

In order to evaluate the potential antitumor activity of the purified lipopeptide from *B. subtilis* isolates (B1,B2,B3,B4,B5,B6,B7,B8,B9,B10) in vitro, [illegible] concentrations of these lipopeptide samples for 24, 48, 72 hours. Viable cells were detected by using the MTT dye, which forms blue formazan crystals that were reduced by mitoc[illegible] The resultant effect for each cell line was explained as the following:-

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now
Wondershare™

A. Cytotoxic effect on K562 cell line.

The results in tables (3-11),(3-12) and(3-13) revealed that lipopeptide from *B. subtilis* isolates (B2,B3,B4,) showed low effect on the proliferation of human leukemia (K562) cell line during all periods of exposures, while lipopeptide produced by *B. subtilis* isolates (B1,B5,B6,B7,B8,B9,B10) had cytotoxic effect on K562 proliferation as were shown in tables (3-10), (3-14), (3-15),(3-16), (3-17) ,(3-18) and (3,19) with high significant inhibition rate at highest concentrations which were being decreased gradually as concentrations decreased too..

.

These results were in accordance with Cao *et al.* (2009a) results which showed that lipopeptide produced by *Bacillus* natto TK-1induce significant reduction in K562 cell line viability in a dose dependent manner at 48 hrs. of exposure.

It was observed from tables (3-15), (3-16) and (3-18) that there were no significant differences at (P<0.05) between inhibition rate when lipopeptide concentrations 240mg/l and 200 mg/l were used from *B. subtilis* isolates (B6,B7,B9).

Table (3-10) Cytotoxic effect of the purified lipopeptide produced by *B. subtilis* B1 on K562 cell line.

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now

Exposure time / Concentration mg/l	Inhibition rate% (mean ±SD)		
	24 hrs.	**48 hrs.**	**72 hrs.**
[illegible]	A,a [illegible]	[illegible] 88.43 [illegible]	[illegible]
200	B,a 62.77 ±1.5	A,[illegible] 85.497 ± 1.861	[illegible] 73.917 ± 2.255
160	C,a 49.625 ±4.589	A,b 80.97 ± 0.65	B,c 72.323 ± 2.654
80	D,a 5.58 ±0.535	B,b 13.167 ± 3.495	C,a 7.753 ± 0.645
40	E,a 0.15 ±0.05683	C,a 0.192 ± 0.118	D,a 0.119 ± 0.019
20	E,a 0.172 ±0.09678	C,a 0.11903 ± 0.09119	D,b 0.3424 ± 0.03704

Different upper case letters: significant difference (P<0.05) between means of rows
Different lower case letters: significant difference (P<0.05) between means of columns

.

Table (3-11) Cytotoxic effect of the purified lipopeptide produced by *B. subtilis* B2 on K562 cell line.

Exposure time / Concentrations mg/l	Inhibition rate% (mean ±SD)		
	24 hrs.	**48 hrs.**	**72 hrs.**
240	A,a 0.24740±0.09787	A,b 0.0975±0.1147	A,c 0.11613±0.07716
200	A,a 0.31703±0.05919	A,b 0.1468±0.1274	A,c 0.08280±0.05631

Different upper case letters: significant difference (P<0.05) between means of rows
Different lower case letters: significant difference (P<0.05) between means of columns

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

The effect of exposure time was also studied, it was observed that there was no significant differences at (P<0.05) among the inhibition rate at the exposure time (24,28 and72) hrs with high concentration (240 mg/l) of lipopeptide produced by isolates (B1,B5,B7) were used, while at concentrations (200, 160, 80,40,20) mg/l, there were significant differences.

Generally it was observed that inhibition rate was increased gradually with prolonged incubation period till 72 hrs. ,however , with prolonged incubation, lipopeptide from *B. subtilis* isolates (B1,B5,B6,B8,B10) became less inhibitor for malignant cell proliferation ,this may be due to the development of resistance cells since it was found that some malignant cells can either had repair system that became activated to overcome the damage done by the antitumor agent (Ledzewicz , 2006) or may be the antitumor agent was susceptible to ABC transport proteins which remove molecules out of the cell, thus an over –expression by gene amplification for example in these molecules is an important mechanism for resistance to various drugs and antitumor agents (Mao and Unadkat, 2005).

.

Malignant cancer cell populations were highly heterogeneous - the number of genetic errors present within one cancer cell lies in the thousands and fast duplications combined with genetic instabilities provided just one of several mechanisms which allow for quickly developing acquired resistance to anti-cancer drugs (Loeb,2001).

When all the results of cytotoxic effect exhibited by lipopeptide from *B. subtilis* isolates (B1,B5,B6,B7,B8,B9) on K562 cell line were compared together , *B.* B7, was found to be the most effective with IC50 at 80mg/l for 72 hrs. of exposure.

While lipopeptide from isolates (B1, B6, B9) and (B5,B8) induce IC50 at 160 mg/l and 200mg/l respectively.

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

(wang *et al.*,2009).

Surfactin has also been reported to have an antitumor activity against Ehrlich's ascite carcinoma cells (Kameda *et al.*, 1974), another study on the effect of surfactin on the proliferation of a human colon carcinoma cell line showed that surfactin strongly blocked the cell proliferation which was due to apoptosis induction and cell cycle arrest via the suppression of cell survival regulating signals such as ERK and PI3K/Akt (Kim *et al.*,2007).

.

Table (3-12) Cytotoxic effect of the purified lipopeptide produced by *B. subtilis* B3 on K562 cell line.

Exposure time / Concentrations mg/l	Inhibition rate% (mean ±SD)		
	24 hrs.	**48 hrs.**	**72 hrs.**
240	A,a 0.7117±0.5187	A,a 0.1762±0.2045	A,a 0.1223±0.0558
200	A,a 0.7±0.5456	A, a 0.1658±0.1254	A,a 0.1148±0.1655

Different upper case letters: significant difference (P<0.05) between means of rows
Different lower case letters: significant difference (P<0.05) between means of columns

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Table (3-13) Cytotoxic effect of the purified lipopeptide produced by *B. subtilis* B4

Exposure time / Concentrations mg/l	Inhibition rate% (mean ±SD)		
	24 hrs.	**48 hrs.**	**72 hrs.**
240	A,a 0.24410±0.05604	A,b 0.10483±0.08625	A,c 0.6378±0.1081
200	A,a 0.28580±0.04655	A,b 0.19417±0.06576	A,c 0.5567±0.1369

Different upper case letters: significant difference (P<0.05) between means of rows
Different lower case letters: significant difference (P<0.05) between means of columns

.

Table (3-14) Cytotoxic effect of the purified lipopeptide produced by *B. subtilis* B5 on K562 cell line.

Exposure time / Concentrations mg/l	Inhibition rate% (mean ±SD)		
	24 hrs.	**48 hrs.**	**72 hrs.**
240	A,a 83.12 ±5.85	A,a 91.307 ±3.36	A,a 85.27 ±2.29
200	B,a 25.993 ±6.655	B,b 75.42 ±4.958	B,b 66.26 ±8.793
160	C,a 0.183 ±0.08904	C,b 17.547 ±3.697	C,a 1.450±0.268
80	C,a 0.2752 ±0.13912	C,b 10.513 ±3.177	C,a 0.1725 ± 0.03128
40	C,a 0.1273 ±0.7718	D,b 1.263±0.592	C,a 0.0798 ±0.05928
	C,a	D,a	C,a

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now

Different upper case letters: significant difference (P<0.05) between means of rows
Different lower case letters: significant difference (P<0.05) between means of columns

Table (3-15) Cytotoxic effect of the purified lipopeptide produced by *B. subtilis* B5 on MCF-7 cell line.

Exposure time / Concentrations mg/l	Inhibition rate% (mean ±SD)		
	24 hrs.	**48 hrs.**	**72 hrs.**
240	A,a 73.58±3.758	A,b 92.29±1.066	A,b 84.113±0.357
200	B,a 64.1±3.09	A,b 89.597±1.974	A,b 84.823±1.372
160	C,a 38.3±0.6	B,b 77.177±6.121	B,b 68.36±6.05
80	D,a 8.547±2.021	C,b 20.493±2.532	C,ab 13.714±3.293
40	E,a 0.0908±0.03904	D,b 7.097±0.616	D,b 3.377±3.129
20	E,a 0.06347±0.05484	E,a 0.1315±0.00537	D,a 0.09243±0.0479

Different upper case letters: significant difference (P<0.05) between means of rows
Different lower case letters: significant difference (P<0.05) between means of columns

.

Table (3-16) Cytotoxic effect of the purified lipopeptide produced by *B. subtilis* B7 on K562 cell line.

Exposure time / Concentrations mg/l	Inhibition rate% (mean ±SD)		
	24 hrs.	**48 hrs.**	**72 hrs.**
240	A,a 85.99 ±0.54	A,a 90.84 ±4.96	A,a 90.94 ±0.253
200	B,a 77.15 ±5.996	A,a 87.31 ±5.44	A,a 86.87 ±3.626
160	C,a 26.443 ±3.301	B,a 30.273 ±3.091	B,b 70.907 ±2.057
80	D,a 7.813 ±4.033	C,a 6.53 ±5.65	C,b 57.17 ±1.254
40	DE,a 0.588±0.38	C,a 0.531±0.448	D,a 0.462±0.207
	E,a	C,a	D,a

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now

Different upper case letters: significant difference (P<0.05) between means of rows
Different lower case letters: significant difference (P<0.05) between means of columns

Table (3-17) Cytotoxic effect of the purified lipopeptide produced by *B. subtilis* B7 on HepG2 cell line.

Exposure time / Concentrations mg/l	Inhibition rate% (mean ±SD)		
	24 hrs.	**48 hrs.**	**72 hrs.**
240	A,a 79.103±2.96	A,b 89.86±1.65	A,a 82.11±0.139
200	B,a 40.42±8.08	B,a 61.61±2.99	B,a 50.49±0.6
160	C,a 0.023±0.008	C,b 11.797±3.939	C,a 1.581±0.648
80	C,a 0.908±0.119	D,b 4.467±1.569	C,a 0.2889±0.1182
40	C,a 0.130±0.008	D,a 0.19413±0.02441	C,a 0.152±0.1685
20	C,a 0.0897±0.04753	D,a 0.05697±0.05932	C,a 0.0187±0.0251

Different upper case letters: significant difference (P<0.05) between means of rows
Different lower case letters: significant difference (P<0.05) between means of columns

.

Table (3-18) Cytotoxic effect of the purified lipopeptide produced by *B. subtilis* B9 on K562 cell line.

Exposure time / Concentrations mg/l	Inhibition rate% (mean ±SD)		
	24 hrs.	**48 hrs.**	**72 hrs.**
240	A,a 71.58±1.45	A,a 70.723±1.397	A,b 87.52±4.04
200	B,a 60.945±6.739	A,a 65.873±2.079	B,b 80.91±2.935
160	C,a 48.307±4.159	B,a 48.035±3.939	C,b 63.58±2.01
80	D,a 4.133±0.531	C,b 10.860±3.762	D,c 23.07±0.762
40	D,a 2.526±0.508	D,b 7.460±1.81	E,b 9.467±2.207
20	D,a 0.410±0.037	E,a 0.633±0.15564	F,b 1.842±0.364

Different upper case letters: significant difference (P<0.05) between means of rows
Different lower case letters: significant difference (P<0.05) between means of columns

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now

Table (3-19) Cytotoxic effect of the purified lipopeptide produced by *B. subtilis* B9 on L1210 cell line.

Exposure time / Concentrations mg/l	Inhibition rate% (mean ±SD)		
	24 hrs.	**48 hrs.**	**72 hrs.**
240	A,a 17.54±2.89	A,b 55.865±1.6242	A,c 28.75±0.22
200	B,a 0.23003±0.04322	B,b 21.23±3.23	B,b 14.57±0.28

Different upper case letters: significant difference (P<0.05) between means of rows
Different lower case letters: significant difference (P<0.05) between means of columns

B. Cytotoxic effect on L1210 cell line.

The effect of the purified lipopeptide on malignant animal cell line proliferation was studied through treating mice leukemia (L1210) cell line with

.

different concentrations of lipopeptides produced by *B. subtilis* isolates (B1, B2, B3, B4, B5,B6,B7,B8,B9,B10) for 24,48,72 hrs.

Results illustrated in tables (3-21) and (3,23) revealed that lipopeptides from isolates *B.* (B2, B4) were ineffective in inhibition the growth rate of the tested cell line for all the exposure times while lipopeptides from *B. subtilis* isolates (B1,B3, B5,B6, B7, B8, B9, B10) inhibited the growth rate in a dose dependent manner as it increased towards the higher concentrations with significant differences at ($P<0.05$) as illustrated in tables (3-20), (3-22), (3-24), (3-25), (3-26), (3-27) ,(3-29).

Highest inhibition effect by *B. subtilis* isolates (B1,B3,B5,B6,B7,B8,B9) was achieved 48 hrs. of exposure as were shown in appendices (36, 42, 45, 49, 52, 55 and 59).

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Table (3-20) Cytotoxic effect of the purified lipopeptide produced by *B. subtilis* B1 on L1210 cell line

Concentrations mg/l	Inhibition rate% (mean ±SD) 24 hrs.	48 hrs.	72 hrs.
240	A,a 87.68 ±1.64	A,a 93.74 ±0.12	A,b 54.14 ±8.32
200	B,a 69.297 ±8.831	A,b 88.72 ±1.98	AB,c 45.57 ±4.1079
160	C,a 44.24±1.834	B,b 52.913 ±7.437	B,a 37.92 ±1.133
80	D,a 1.153 ±0.508	C,a 2.123 ±0.799	C,a 1.857 ±0.4397
40	D,a 0.009 ±0.00052	C,a 0.0143 ±0.02181	C,a 0.0201±0.00469
20	D,a 0.00703 ±0.00431	C,a 0.01463 ±0.01488	C,a 0.03873 ±0.03791

Different upper case letters: significant difference ($P<0.05$) between means of rows
Different lower case letters: significant difference ($P<0.05$) between means of columns

.

Table (3-21) Cytotoxic effect of the purified lipopeptide produced by *B. subtilis* B2 on L1210 cell line.

Exposure time / Concentrations mg/l	Inhibition rate% (mean ±SD)		
	24 hrs.	**48 hrs.**	**72 hrs.**
240	A,a 0.07353±0.02832	A,b 0.1681± 0.1104	A,c 0.6039±0.2718
200	A,a 0.15283±0.03682	B,b 0.7993±0.1078	A,a 0.1171±0.0560

Different upper case letters: significant difference (P<0.05) between means of rows
Different lower case letters: significant difference (P<0.05) between means of columns

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Table (3-22) Cytotoxic effect of the purified lipopeptide produced by *B. subtilis* B2

Exposure time / Concentrations mg/l	Inhibition rate% (mean ±SD)		
	24 hrs.	**48 hrs.**	**72 hrs.**
240	A,a 60.113 ±8.887	A,a 72.338 ±5.8	A,b 21.68 ±13.025
200	B,a 1.480±0.156	B,b 6.765±1.888	A,a 1.5 ±0.427

Different upper case letters: significant difference (P<0.05) between means of rows
Different lower case letters: significant difference (P<0.05) between means of columns

.

Table (3-23) Cytotoxic effect of the purified lipopeptide produced by *B. subtilis* B4 on L1210 cell line.

Exposure time / Concentrations mg/l	Inhibition rate% (mean ±SD)		
	24 hrs.	**48 hrs.**	**72 hrs.**
240	A,a 11.09 ±4.401	A,b 32.18 ±7.99	A,c 0.3866±0.4223
200	A,a 6.724±5.608	B,a 6.61 ±2.669	A,b 0.1612 ±0.1182

Different upper case letters: significant difference (P<0.05) between means of rows
Different lower case letters: significant difference (P<0.05) between means of columns

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Exposure time / Concentrations mg/l	Inhibition rate% (mean ±SD)		
	24 hrs.	**48 hrs.**	**72 hrs.**
240	A,a 84.09±6.886	A,a 86.63±2.498	A,b 69.35 ±13.18
200	B,a 59.383 ±4.211	B,b 75.46 ±3.317	B,c 45.57 ±8.96
160	C,a 36.748±2.539	C,a 37.55 ±6.11	B,a 34.087±3.301
80	D,a 1.003 ±0.718	D,b 19.68 ±2.041	C,a 0.887±0.105
40	D,a 0.174±0.17	E,b 1.753±0.767	C,a 0.329±0.103
20	D,a 0.02±0.02	E,b 1.032 ±1.429	C,a 0.014±0.008

Different upper case letters: significant difference (P<0.05) between means of rows
Different lower case letters: significant difference (P<0.05) between means of columns

.

Table (3-25) Cytotoxic effect of the purified lipopeptide produced by *B. subtilis* B6 on L1210 cell line.

Exposure time / Concentrations mg/l	Inhibition rate% (mean ±SD)		
	24 hrs.	**48 hrs.**	**72 hrs.**
240	A,a 71.685±5.805	A,b 88.51 ±3.5	A,b 76.86 ±4.8624
200	B,a 51.84 ±7.44	B,b 82.62 ±0.44	B,a 61.97 ±7.894
160	C,a 37.717 ±5.958	C,b 72.273 ±1.945	B,c 53.813±2.564
80	D,a 3.86±0.255	D,b 24.717 ±1.168	C,c 11.955±2.898
40	D,a 0.56 ±0.08385	D,b 20.6 ±3.949	D,a 1.443±0.289
20	D,a 0.286±0.154	E,b 2.037±0.665	D,a 0.079±0.069

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now

Different upper case letters: significant difference (P<0.05) between means of rows
Different lower case letters: significant difference (P<0.05) between means of columns

Exposure time / Concentrations mg/l | Inhibition rate% (mean ±SD) | 24 hrs. | 48 hrs. | 72 hrs.

240	A,a 80.38 ±2.84	A,b 91.22 ±1.32	A,a 79.71 ±2.1905
200	B,a 68.163 ±1.709	B,b 80.375 ±2.114	B,c 46.33±6.675
160	C,a 43.333 ±4.748	C,b 58.277 ±2.33	B,c 31.097 ±8.815
80	D,a 2.415±0.53	D,a 2.875±0.813	C,b 0.495±0.49
40	D,a 0.143±0.078	D,a 0.272±0.224	C,a 0.741±0.192
20	D,a 0.0254 ±0.0021	D,a 0.0199 ±0.01345	C,a 0.06575 ±0.00247

Different upper case letters: significant difference (P<0.05) between means of rows
Different lower case letters: significant difference (P<0.05) between means of columns

.

Table (3-27) Cytotoxic effect of the purified lipopeptide produced by *B. subtilis* B8 on L1210 cell line.

Exposure time / Concentrations mg/l	Inhibition rate% (mean ±SD)		
	24 hrs.	**48 hrs.**	**72 hrs.**
240	A,a 39.35 ±1.81	A,b 84 ±5.16	A,b 71.33 ±8.567
200	B,a 32.97 ±0.575	B,b 73.8 ±2.1	B,c 48.36 ±9.7
160	B,a 14.34±4.257	B,b 24.826±1.127	C,b 20.173±1.728
80	D,a 4.285±1.676	D,b 13.863±2.734	D,a 3.223±1.011
40	D,a 0.084 ±0.034	E,b 1.771±0.190	D,a 0.312±0.211
20	D,a 0.0	E,a 0.0	D,a 0.0

Different upper case letters: significant difference (P<0.05) between means of rows
Different lower case letters: significant difference (P<0.05) between means of columns

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now

Table (3-28) Cytotoxic effect of the purified lipopeptide produced by *B. subtilis* B8 on Vero cell line.

Exposure time / Concentrations mg/l	Inhibition rate% (mean ±SD)		
	24 hrs.	**48 hrs.**	**72 hrs.**
240	A,a 86.457±1.662	A,b 92.9±0.42	A,a 87.36 ±1.261
200	A,a 85.24 ±1.869	A,b 92.04±1.129	A,a 84.323 ±1.312
160	B,a 55.08 ±2.855	A,b 86.76 ±0.95	A,b 87.12 ±0.562
80	C,a 1.477±0.376	B,b 58.62 ±0.666	B,b 50.15 ±1.463
40	C,a 1.063±0.501	C,b 5.593 ±0.5391	C,a 2.518±1.293
20	C,a, 0.01813 ±0.003	C,b 1.651±0.257	C,c 0.495±0.075

Different upper case letters: significant difference (P<0.05) between means of rows
Different lower case letters: significant difference (P<0.05) between means of columns

.

Table (4-29) Cytotoxic effect of the purified lipopeptide produced by *Bacillus* B10 on L1210 cell line.

Exposure time / Concentrations mg/l	Inhibition rate% (mean ±SD)		
	24 hrs.	**48 hrs.**	**72 hrs.**
240	A,a 33.75 ±1.89	A,b 79.7 ±4.97	A,b 74.59 ±9.23
200	B,a 8.88 ±3.78	B,a 17.563 ±5.58	B,a 6.34 ±1.03

Different upper case letters: significant difference (P<0.05) between means of rows
Different lower case letters: significant difference (P<0.05) between means of columns

Comparison among the inhibition effect of all tested lipopeptides on L1210 cell line showed that lipopeptide from isolate *B.* B9 was the most effective one followed by *B. subtilis* isolates (B1, B6, B7).

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

C. Cytotoxic effect on Hep-2 cell line.

In order to study the effect of the purified lipopeptide on human cell line, human epidermal [illegible] was tested.

Results from tables (3-31) and (3-33) showed that lipopeptide from the two *B.* isolates B2 and B4 had very low effect on the cell line proliferation, this may due to the fact that several surfactin isoforms usually coexist in the cell as a mixture of several peptidice variants (Kowall *et al*,. 1998; Bonmatin *et al*.,2003) with a different aliphatic chain length (Hue *et al*., 2001) that differ in their biological activity (Seydlová and Svobodová, 2008).

The results from tables (3-30) - (3-39) showed that cell survival in Hep-2 treated cultures were progressively decreased with increasing the concentration and it was time dependent as increased with prolonged incubation this may explained

.

by the release of intracellurar components from dead cells that induce more cells to die as a results of toxicity increasing .

Cao *et al*., (2009) found that surfactin from *B. natto* TK-1 was dose and time dependent in inhibiting the proliferation of human breast cancer cell line (MCF-7).

However, statistically , there were no significant differences (P<0.05) between the inhibition rate when lipopeptides from *B. subtilis* isolates (B1,B3, B6,B7,B8,B9) were used at concentrations 240 mg/l and 200 mg/l (dose independent) or between the two exposure times 48 and 72 hrs. of same concentrations as showed in tables (3-30), (3-32), (3-35), (3-36), (3-37) and (3-38).

This may due to the heterogeneity within the tumor cell line which lead to the rising of drug resistant cells that will multiply and remain as a surviving cells in spite of increasing the lipopeptide concentration (L[illegible] 2011).

This is a watermark for the trial version, register to get the full one!

The previous results showed that inhibition rate depends on the type of cell line , since Hep-2 cell line was more sensitive than K562 or L1210 to lipopeptide treatment. The differences in Hep-2 respon[illegible] to[illegible] indicate a[illegible] cellular receptors in each type of cell lines; making the cells interacts at same concentration in different manners. Moreover the metabolic pathways in response to each treatment differed from one line to another(Kim *et al*., 2007). This fact was mentioned in different studies which investigated at different bacterial and plants extracts in treating several types of cell lines (Li *et al*., 2003; Al-dulami , 2006; Darwish, 2007).

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now

.

Table (3-30) Cytotoxic effect of the purified lipopeptide produced by *B. subtilis* B1 on Hep-2 cell line.

Exposure time / Concentrations mg/l	Inhibition rate% (mean ±SD)		
	24 hrs.	48 hrs.	72 hrs.
240	A,a 70.917±0.815	A,b 86.573±2.026	A,b 88.19±0.908
200	A,a 69.863±1.917	A,b 86.313±2.293	A,b 87.587±1.035
160	A,a 66.8±1.46	A,b 81.83±4.713	A,b 86.437±0.182
80	B,a 51.375 ±1.082	AB,b 70.64±4.952	A,c 85.287±0.657
40	C,a 6.02±4.031	C,a 7.85±6.536	B,b 23.835±7.135
20	C,a [illegible]	C,a [illegible]	BC,a [illegible]

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now

Exposure time / Concentrations mg/l	Inhibition rate% (mean ±SD)		
	24 hrs.	48 hrs.	72 hrs.
240	A,a 8.433±0.41	A,ab 13.783±3.726	A,a 8.043±2.202
200	A,a 5.34±4.831	A,a 10.707±3.327	A,a 7.96±0.178

Different upper case letters: significant difference (P<0.05) between means of rows
Different lower case letters: significant difference (P<0.05) between means of columns

.

Table (3-32) Cytotoxic effect of the purified lipopeptide produced by *B. subtilis* B3 on Hep-2 cell line.

Exposure time / Concentrations mg/l	Inhibition rate% (mean ±SD)		
	24 hrs.	**48 hrs.**	**72 hrs.**
240	A,a 74.685±4.054	A,ab 87.179±1.555	A,b 90.433±0.519
200	A,a 70.42±8.622	A,ab 87.133±2.513	A,b 89.967±0.047

Different upper case letters: significant difference (P<0.05) between means of rows
Different lower case letters: significant difference (P<0.05) between means of columns

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Table (3-32) Cytotoxic effect of the purified lipopeptide produced by *B. subtilis* B3 on Hep-2 cell line.

Exposure time / Concentrations mg/l	Inhibition rate% (mean ±SD)		
	24 hrs.	**48 hrs.**	**72 hrs.**
240	A,a 5.613±3.65	A,a 4.165±0.346	A,a 3.47±0.8485
200	A,a 3.155±3.444	B,b 39.335±0.445	B,a 0.67±0.00

Different upper case letters: significant difference (P<0.05) between means of rows
Different lower case letters: significant difference (P<0.05) between means of columns

.

Table (3-34) Cytotoxic effect of the purified lipopeptide produced by *B. subtilis* B5 on Hep-2 cell line.

Exposure time / Concentrations mg/l	Inhibition rate% (mean ±SD)		
	24 hrs.	**48 hrs.**	**72 hrs.**
240	A,a 52.04±4.8	A,b 82.533±0.242	A,b 87.097±0.447
200	B,a 28.81±3.118	A,b 73.51±0.339	A,c 85.947±0.6
160	C,a 9.93±1.247	B,b 19.855±0.474	B,c 75.085±4.632
80	C,a 8.233±4.536	C,a 7.645±3.472	B,b 9.513±2.331
40	C,a 7.11±1.6	C,ab 4.04±2.425	C,ac 10.087±1.599
20	C,a 6.36±2.818	C,b 10.45±1.852	D,b 14.337±3.888

Different upper case letters: significant difference (P<0.05) between means of rows
Different lower case letters: significant difference (P<0.05) between means of columns

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now

Table (3-35) Cytotoxic effect of the purified lipopeptide produced by *B. subtilis* B5 on Hep-2 cell line.

Exposure time / Concentrations mg/l	Inhibition rate% (mean ±SD)		
	24 hrs.	**48 hrs.**	**72 hrs.**
240	A,a 69.183±1.079	A,b 85.963±0.162	A,b 88.307±0.359
200	A,a 66.187±1.022	A,b 85.77±0.29	A,b 87.47±0.501
160	A,a 65.377±2.228	B,ab 71.537±4.642	A,b 85.003±0.309
80	B,a 30.477±6.751	C,b 57.757±7.199	B,b 69.219±1.419
40	C,a 3.3±1.748	D,b 14.955±3.062	C,b 15.46±4.627
20	C,a 6.36±2.818	D,ab 10.45±1.852	C,b 14.337±3.888

Different upper case letters: significant difference (P<0.05) between means of rows
Different lower case letters: significant difference (P<0.05) between means of columns

.

Table (3-36) Cytotoxic effect of the purified lipopeptide produced by *B. subtilis* B7 on Hep-2 cell line.

Exposure time / Concentrations mg/l	Inhibition rate% (mean ±SD)		
	24 hrs.	**48 hrs.**	**72 hrs.**
240	A,a 79.13±2.161	A,b 84.473±2.483	A,c 90.6± 0.67
200	A,a 75.257±4.012	A,ab 80.595±1.336	A,b 86.27±3.6
160	B,a 52.12±4.78	A,b 74.177±10.842	B,a 61.5±4.37
80	C,a 4.387±2.011	B,a 5.36±1.895	C,b 22.797±1.798
40	C,a 4.927±1.163	B,b 0.813±0.147	D,b 0.447±0.202
20	C,a 0.038±0.06	B,a 0.048±0.017	D,a 0.048±0.004

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Different lower case letters: significant difference (P<0.05) between means of columns

Table (3-37) Cytotoxic effect of the purified lipopeptide produced by *B. subtilis* B6 on Hep-2 cell line.

Exposure time / Concentrations mg/l	Inhibition rate% (mean ±SD)		
	24 hrs.	**48 hrs.**	**72 hrs.**
240	A,a 74.593±1.979	A,b 86.737±3.055	A,b 88.91±4.14
200	A,a 73.483±5.137	A,b 85.78±2.889	A,b 88.757±4.181
160	A,a 72.677±5.596	A,b 84.967±3.71	A,b 88.357± 3.886
80	B,a 11.710±4.2	B,b 28.22±4.992	A,c 86.043±1.529
40	B,a 9.820±2.036	C,a 10.047±3.605	B,a 16.87±5.94
20	C,a 0.106±0.074	D,a 0.125±0.046	C,b 3.723±0.297

Different upper case letters: significant difference (P<0.05) between means of rows
Different lower case letters: significant difference (P<0.05) between means of columns

.

Table (3-38) Cytotoxic effect of the purified lipopeptide produced by *B. subtilis* B9 on Hep-2 cell line.

Exposure time / Concentrations mg/l	Inhibition rate% (mean ±SD)		
	24 hrs.	**48 hrs.**	**72 hrs.**
240	A,a 75.497±3.581	A, ab 85.78± 2.889	A,b 90.6±0.28
200	A,a 71.575±0.827	A,b 84.407±0.802	A,c 89.7±0.608
160	AB,a 63.843±9.122	A,ab 74.03±0.4	A,ab 84.67±1.6
80	C,a 6.16±0.663	B,b 20.21±0.99	B,c 64.6±7.07
40	C,a 5.407±2.813	BC,a 8.857±8.252	C,a 6.77±5.8
20	C,a 4.743±4.437	C,a 4.64±4.233	C,a 10.86±0.09

Different upper case letters: significant difference (P<0.05) between means of rows
Different lower case letters: significant difference (P<0.05) between means of columns

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:

1.No watermark on the output documents.

2.Can operate scanned PDF files via OCR.

3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Wondershare™

Table (3-39) Cytotoxic effect of the purified lipopeptide produced by *B. subtilis* B10 on Hep-2 cell line.

Exposure time / Concentrations mg/l	Inhibition rate% (mean ±SD)		
	24 hrs.	**48 hrs.**	**72 hrs.**
240	A,a 64.083±1.948	A,b 84.42±2.421	A,b 85.947±0.313
200	B,a 4.34±0.792	B,ab 30.96±8.319	A,b 59.483±4.75

Different upper case letters: significant difference (P<0.05) between means of rows
Different lower case letters: significant difference (P<0.05) between means of columns

.

Table (3-40) Cytotoxic effect of standard surfactin on Hep-2 cell line.

Exposure time / Concentrations mg/l	Inhibition rate% (mean ±SD)		
	24 hrs.	**48 hrs.**	**72 hrs.**
240	A,a 83.873 ±1.872	A,ab 88.048 ±0.512	A,b 90.542 ±1
200	A,a 83.555 ±2.201	A,b 88.76 ±0.104	A,b 88.014 ±0.836
160	A,a 79.872 ±4.158	A,b 87.98 ±1.689	A,b 87.3406 ±0.663
80	A,a 72.031±13.032	A,a 79.603 ±7.411	B,a 78.07 ±0.52
40	B,a 39.142 ±8.482	B,a 39.573±4.236	C,b 56.67 ±1.049
20	BC,a 21.365 ±3.767	C,a 21.437±9.524	D,a 23.247±2.509
	C,a	D,b	E,c

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Different upper case letters: significant difference ($P<0.05$) between means of rows
Different lower case letters: significant difference ($P<0.05$) between means of columns

Cytotoxic effect on L02 cell line.

Purified lipopetide (surfactin) produced by the isolate *B.* B6 was selected to study its cytotoxic effect on normal human cell line since it inhibited the proliferation of K562, L1210, Hep-2 cell lines with high efficiency as were shown in appendices (44, 45,46).

Results from table (3-41), revealed very low cytotoxic affectivity of this surfactin on the normal cell line even at the highest concentrations. This considered as indication of the relative safety of *B.* B6 lipopeptide towards normal cells.

Such selective toxicity towards malignant cell lines was due to the differences in the malignant cellular physiology such as the present of some metabolic factors that found in the cancer cell lines but not found in normal cells , like the

.

angiogenic promoters and inhibitors (Folkman,2000 ,Moteki *et al.*,2002). In addition DNA of tumor cell found in relaxant shape ,and the DNA molecule was found in a unstable figure because the far away between the H-bond which connect the both strand of DNA and this make easy for compound to interfere or associated to both strands of DNA, while DNA of normal cell has a strong H-bond connect the both strands to each other and make it more stable, so the compounds cannot interfere or associated with DNA strand (Belijanski, 2000).

The Hep-2 cell line was chosen for further studies since there is no known studies about the effect of *Bacillus* lipopeptide on this cell line.therefore, purified lipopeptide from *B. subtilis* B6 isolate (surfactin) was chosen at concentration 40 mg/ L and 80 mg/L that cause IC50 to study the mechanism of proliferation inhibition of Hep-2 cell line. The results were represented in 3-8-2

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Table (3-41) Cytotoxic effect of the purified surfactin produced by *B. subtilis* B6 on Hep2 cell line

Concentrations mg/l	Inhibition rate% (mean ±SD) 24 hrs.	48 hrs.	72 hrs.
240	A,a 6.9767±2.0011	A,b 30.707±5.927	A,c 0.6991 ±0.228
200	B,a 1.2133±0.9775	B,b 6.215±0.377	B,c 0.0793±0.013
160	B,a 0.3583±0.2852	C,a 0.550±0.043	B,b 0.0787±0.008
80	B,a 0.0538±0.0562	C,a 0.051±0.002	B,a 0.0396±0.035
40	B,a 0.2152 ±0.1515	C,a 0.4842±0.044	A,a 0.1113 ±0.091
20	B,a 0.0124±0.0014	C,a 0.026±0.003	A,b 0.1972±0.1322

Different upper case letters: significant difference (P<0.05) between means of rows
Different lower case letters: significant difference (P<0.05) between means of columns

.

4.7.2 Mitochondrial membrane potential assay JC-1

In order to study mechanism of action of the purified surfactin from the isolate *B.* B6 in the inhibition of Hep-2 cell proliferation, mitochondrial membrane potential assay were conducted.

Results from figures (3-19) and (3-20) showed gradual loss of J-aggregates which represent the accumulation of the membrane permeate JC-1 dye within healthy mitochondria when cell line treated with it because the dye was bearing a delocalized positive charge that tend to enter into the negatively charged intact mitochondrial membrane and accumulate to form J-aggregates which become fluorescent red while In apoptotic cells, the mitochondrial membrane potential collapses, and the JC-1 cannot accumulate within the mitochondria (Cossarizza,1993), hence cells JC-1 remains in the cytoplasm in the form of fluorescent monomeric form, then finally diffused to form cytoplasmic diffusion of green monomer. This indicates that treating Hep-2 cell line with surfactin cause depolarization of mitochondrial which finally [illegible] mitochondrial membrane at concentration as low as 5000ng/ml.

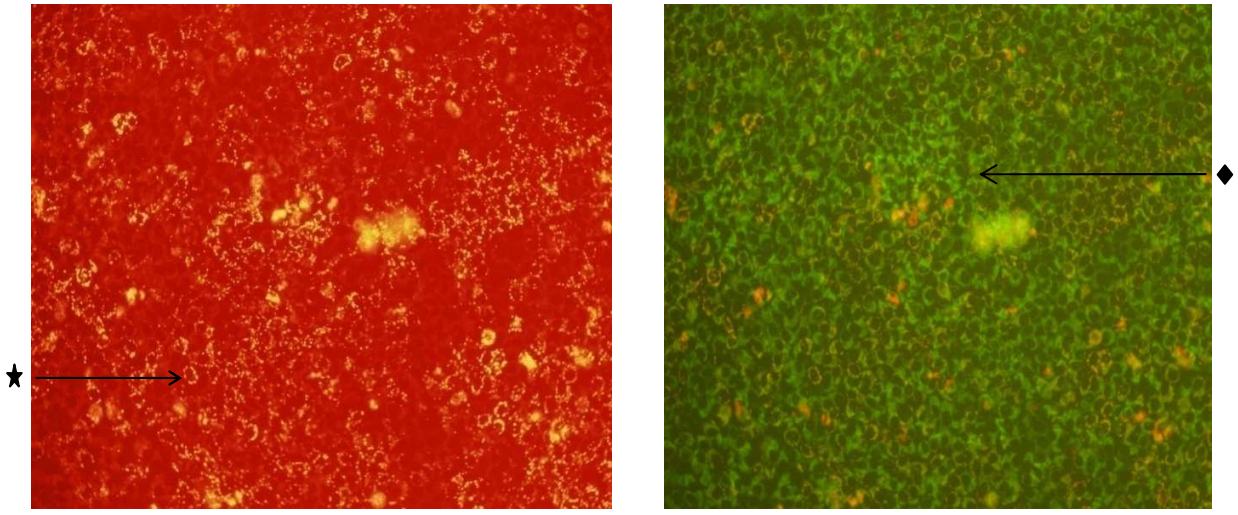
This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now
Wondershare™

3.7.3 Caspase – G10 3/7 assay

Caspase –G10 3/7 assay were used to study the involvement of apoptosis in cellular inhibition when treated with *B.* B6 surfactin.

Results shown in table (3-42) revealed increasing caspase 3 activity with prolonged incubation with surfactin which indicate the onset of cellular apoptosis.

.



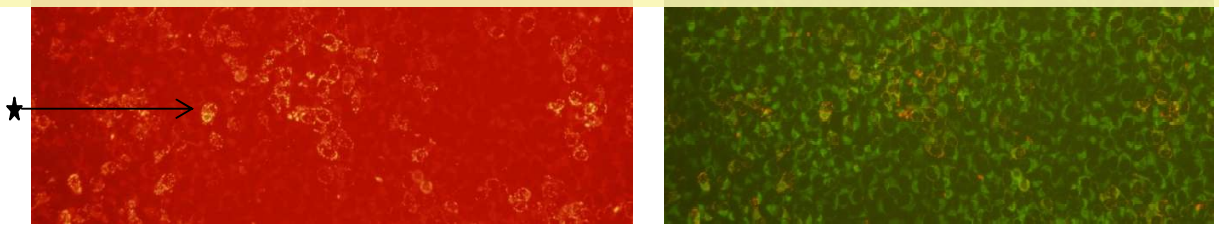
A B

Figure (3-19) Hep-2 cell line treated with 40 mg/l of *B. subtilis* B6 surfactin for 8 hrs and stained with JC-1 under A: red light fluorescence microscope showed ★ intact cells with J-aggregates, B: under green light fluorescence microscope showed ♦ apoptotic cells that loss J-aggregates to the cytoplasm then diffuse to form green monomer (X40).

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now



C D

Figure (3-20) Hep-2 cell line treated with 40 mg/l of *B. subtilis* B6 surfactin for 18 hrs and stained with JC-1 under C: red light fluorescence microscope showed ★ decreased number of intact cells with J-aggregates, D: green light fluorescence microscope showed♦ increased number of apoptotic cells that loss J-aggregates to the cytoplasm then diffuse to form green monomer (X40).

.

Table (3-42) Caspase activity in Hep-2 cell line treated with 40mg/L of purified surfactin produced by *B. subtilis* B6 for different incubation time.

sample	**Caspase activity (unit/well)**
control	9690.00
Treated cell for 6 hours	24848.0
Treated cell for 12 hours	48367.0
Treated cell for 18 hours	56407.3

Caspase 3 and caspase 7 form part of effector caspases which when activated cleave a large number of substrates in the cell that lead to disassembly of cellular structures and the characteristics cellular changes occurring during apoptosis, also they cleave proteins that were normally protect cell from apoptosis and inactivate them (Thornberry and Lazebnik, 1998). [illegible] et al., (2007) found that surfacti[illegible] Human colon carcinoma (Lovo cells) by inducing pro-apoptotic activity and arresting the cell cycle according to several lines of evidence on DNA fragmentation, Annexin V staining, and altered levels of poly (ADP-ribose) polymerase, caspase-3 activation, while Cao *et al*.,(2010) found that surfactin produced by *Bacillus natto* TK- 1 induce apoptosis in Human breast cancer (MCF-7) 1 by caspase cascade activation through induction of ROS/JNK-mediated mitochondrial/caspase pathway.

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now

.

Summary

Eighty eight local bacterial isolates of *Bacillus* spp. were obtained from 45 oil contaminated soil samples from different fuel stations in addition to Al- Dorah oil refinery in Baghdad Province /Iraq.

All these isolates were screened for their ability for biosurfactant production by surface tension measurement of cell free supernatant after cultivation in E medium , which referred that 81 of the 88 isolates were biosurfactant producer , while screening depended on the blood haemolysis activity on sheep blood agar indicated that only 49 isolates were biosurfactant producers.

Molecular identification based on the 16S rDNA by two sets of primers (27F and 1492R) , (B16SF and B16SR) of the best ten biosurfactant producer isolates (B1,B2, B3, B4, B5, B6 , B7, B8, B9, B10) revealed that all isolates were belonged to *Bacillus* spp. with 100% similarity to *Bacillus subtilis* group, [illegible] (100)% [illegible]

All the selected [illegible] isolates were capable [illegible] highest efficiency (40 -43.1)% in Jacques medium than in E medium (28-30)%.

Biosurfactants produced by the selected isolates were purified by acid precipitation followed by ultrafiltration with centricons.

Purified biosurfactants were found to be member of the lipopeptide family when characterized by the TLC that sprayed with water or with Rhodamine B, and all isolates were capable of producing surfactin with or without other types of biosurfactants depending on the isolate as showed by comparison with standard surfactin.

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:

1.No watermark on the output documents.

2.Can operate scanned PDF files via OCR.

3.No page quantity limitations for converted PDF files.

Remove Watermark Now

HPLC/MS analysis of the purified biosurfactant from *B. subtilus* B6 revealed that it is a surfactin with partial sequence determination as follows: Val-Asp -Leu-Leu-OH_2.

Cytotoxicity assay of the purified biosurfactant from the selected isolates on (K562, Hep-2, L1210) were tested , results showed that lipopeptides cytotoxicity were dependent on the type of tumor cell line, type of isolate, biosurfactant concentration and exposure time

Purified biosurfactant produced by *Bacillus* B7 was found to be the most cytotoxic one to K562 cell line that caused 57.17% inhibition of the cell line growth at concentration (80) mg/l, while purified biosurfactant produced by isolate *Bacillus* B9 was the most cytotoxic one to L1210 cell line that caused 58.63% inhibition of the cell line growth at the same concentration , however purified biosurfactans produced by (*B. subtilis* isolates B6, B8, B9 were the most cytotoxic on Hep-2 proliferation with inhibition rate (69.219,86.043,64.6)% at concentration (80) mg/l while purified biosurfactant

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now

It was found that it caused depolarization of mitochondria membrane which finally led to cell death. In addition, the caspase activity assay revealed that surfactin induced apoptosis in Hep-2 cells that associated with caspase 3.

Optimum conditions for biosurfactants production from *B. subtilis* B6 were determined . Results indicated that maximum biosurfactant production from this isolate was achieved by using Sucrose (1%) v/v , Potassium nitrate (0.3%) and Potassium dihydrogen phosphate (1 g/l), at pH 8, 30 °C, 180 rpm of shaking for 72 hrs. The biosurfactant yield was 1.4 g/l of culture medium under the optimum conditions.

Table (3-4) : Results of NCBI alignment of PCR amplification of 16S rDNA by two sets of primers (B16SF and B16SR) and (27F and1492R) of the isolates (B1,B2,B3 ,B4,B5,B6,B7,B8,B9,B10).

Primer	*B. spp*	*Bacillus subtilis*	*Bacillus tequilensis*	*Bacillus licheniformis*	*Bacillus amyloliquefaciens*	*Bacillus methylotrophicus*	*Bacillus mojavensis*	*Bacillus axarquiensis*	*Bacillus malacitensis*
B1*	25	68	4	0	2	0	1	0	0
B1 (27F)	17	76	3	1	0	0	0	0	0
B1 (1492R)	10	53	17	0	7	0	2	0	0
B2*	46	41	0	0	2	0	0	2	0
B2 (27F)	46	36	0	0	0	0	8	4	2
B2 (1492R)	47	24	2	0	0	0	0	0	2
B3*	29	59	4	0	2	0	1	0	0
B3 (27F)	17	٧٦	3	1	0	0	0	0	0
B3 (1492R)	16	٤٢	17	0	9	6	3	2	0
B4*	29	59	4	0	2	0	1	0	0
B4 (27F)	18	٧٥	3	1	0	0	0	0	0
B4 (1492R)	15	٤٢	17	0	8	6	3	4	0
B5*	20	23	16	0	6	0	5	4	0
B5 (27F)	48	٤٧	1	2	0	0	1	1	0

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now
Wondershare™

B5 (1492R)	11	٥٦	16	0	6	6	2	2	0
B6 *	46	41	0	0	2	0	7	2	0
B6♦	47	26	2	2	0	0	13	6	0
B7*	20	23	16		6	0	5	4	0
B7 (27F)	43	48	1	1	0	0	1	3	0
B7 (1492R)	9	55	16	0	7	6	2	2	0
B8*	27	61	4	0	2	0	1		0
B8 (27F)	15	57			0	0		0	0
B8 (1492R)	9	69	16	0					0
B9*	20	23	16	0	6	0	5	4	0
B9♦	38	47	4	2	0	0	1	1	0
B10*	20	23	16	0	6	0	5	4	0
B10 (27F)	49	46	1	2	0	0	1	1	0
B10 (1492R)	20	59	15	0	2	0	2	2	0

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now
Wondershare™

*: primer set (B16SF and B16SR), ♦: primer set (27F and 1492R)

الملخص

عزلت ٨٨ عزلة محلية عائدة للجنس *Bacillus* من ٤٥ عينة تربة ملوثة بالمشتقات النفطية من مختلف محطات الوقود إضافة إلى مصفى الدورة.

تبين من إخضاع جميع هذه العزلات للتحري عن قابليتها في إنتاج المستحلب الحيوي بواسطة قياس الشد السطحي لوسط النمو E- medium الخالي من الخلايا بعد نموها فيه ان ٨١ عزلة كان منتجة بينما اظهر التحري عن الإنتاجية بواسطة الكشف عن تحلل الدم قابلية ٤٩ عزلة فقط على إنتاج المستحلب الحيوي . وعند تشخيصها بالاعتماد على دراسة 16S rDNA باستخدام زوجين من البرايمرات (27F and 1492R) و (B16SF and B16SR)عائدية العزلات المختارة ذات الإنتاجية العالية للمستحلبات

This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now

السطحي عند تنميتها في وسط Jacques medim و بنسبة (40-43)% بالمقارنة مع وسط E. medium بنسبة (٢٨-٣٠)%

نقيت المستحلبات الحيوية من العزلات البكتيرية المختارة بواسطة الترسيب بالحامض والترشيح بالاغشية ثم وصفت باستخدام كروماتوغرافيا الطبقة الرقيقة والتي اظهرت عائديتها لعائلة الببتيدات الدهنية، اذ كانت موجبة لفحص الماء وكاشف الرودامين B ، وعند مقارنتها مع السرفاكتين القياسي تبين انها منتجة للسرفاكتين والذي أنتج بصورة مفردة أو كمزيج مع غيره من الببتيدات الدهنية اعتمادا على نوع العزلة المنتجة.

عند التنقية والتوصيف باستخدام جهاز كروماتوغرافيا السائل عالي الكفاءة لتحديد الكتلة للمستحلب الحيوي المنتج من قبل العزلة *Bacillus* B6 تاكد بأنه سرفاكتين كما تم تحديد تتابع الأحماض الأمينية المكونة له بصورة جزئية وكان بالتسلسل Val-Asp-Leu-Leu-OH_2 .

أظهرت نتائج فحص السمية للمستحلبات الحيوية المنقاة المنتجة من قبل العزلات المختارة على خطوط الخلايا السرطانية (K562,Hep-2, L1210)، أن تأثير المستحلبات الحيوية اعتمد على نوع حط الحلايا السرطانية ونوع العزلة المنتجة وعلى تركيز المستحلب الحيوي ومدة التعريض.

لوحظ إن المستحلب الحيوي المنقى المنتج من العزلة *Bacillus* B٧ بأنه الأكثر تثبيطا لنمو خط الخلايا السرطانية K562 اذ تسبب بنسبة تثبيط بلغت 57.17% بتركيز ٨٠ ملغم /لتر) بينما كان

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

أظهرت دراسة الظروف المثلى لإنتاج المستحلب الحيوي من قبل العزلة *Bacillus* B6 إن أعلى إنتاجية للمستحلب الحيوي بلغت (1.4 غم/لتر) في وسط يحتوي على السكروز (١%) كمصدر كاربوني وعلى نترات البوتاسيوم (0.3%) كمصدر نايتروجيني وفوسفات الصوديوم ثنائية الهيدروجين (١غم/لتر) كمصدر فوسفاتي و pH مساوي لـ ٨ بدرجة حرارة حضن ٣٠°م وبمعدل اهتزاز ١٨٠ دورة/دقيقة لمدة حضن ٧٢ ساعة.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة النهرين
كلية العلوم

الفعالية البيولوجية للمستحلبات الحيوية المنتجة من بكتيريا *Bacillus* spp. المعزولة محلياً

اطروحة
مقدمة الى كلية العلوم جامعة النهرين
This is a watermark for the trial version, register to get the full one!
Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.
Remove Watermark Now
ماجستير تقانة احيائية / جامعة بغداد/ كلية العلوم/٢٠٠٦

باشراف

د. حميد مجيد جاسم
(أستاذ)

د.ناهي يوسف ياسين
(أستاذ)

محرم ١٤٣٣هـ

تشرين الثاني ٢٠١١

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:

1.No watermark on the output documents.

2.Can operate scanned PDF files via OCR.

3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Wondershare™

Chapter One

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:

1.No watermark on the output documents.

2.Can operate scanned PDF files via OCR.

3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Chapter Two

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:

1.No watermark on the output documents.

2.Can operate scanned PDF files via OCR.

3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Chapter Three

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:

1.No watermark on the output documents.

2.Can operate scanned PDF files via OCR.

3.No page quantity limitations for converted PDF files.

Remove Watermark Now

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Chapter Four

Conclusions and recommendations

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:
1.No watermark on the output documents.
2.Can operate scanned PDF files via OCR.
3.No page quantity limitations for converted PDF files.

Remove Watermark Now

Wondershare™

This is a watermark for the trial version, register to get the full one!

Benefits for registered users:

1.No watermark on the output documents.

2.Can operate scanned PDF files via OCR.

3.No page quantity limitations for converted PDF files.

Remove Watermark Now