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Nucleotide sequence of a 1,3-1,4-β-glucanase-encoding gene in *Bacillus circulans* WL-12

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We have previously reported (1) the molecular cloning of a DNA fragment from *Bacillus circulans* WL-12 which directs the synthesis in *E. coli* of an enzyme that specifically degrades lichenan (mixed-linkage 1,3-1,4-β-glucan). The nucleotide sequence of the 1987-bp BamHI-HindIII fragment containing the 1,3-1,4-β-glucanase gene (BGC), has been determined. The BGC gene comprises an open reading frame of 1227-bp coding for a 409-amino acid precursor polypeptide with a calculated molecular weight of 44812 daltons, in close agreement with the value of 40500 previously estimated for the mature extracellular form by SDS-polyacrylamide gel electrophoresis. The Kyte and Doolittle hydropathy plot of the deduced amino acid sequence shows a protein in which the N-terminus is the most hydrophobic region, with structural features resembling those found in signal peptides of secreted proteins. A search for putative promoters upstream from the proposed translation initiation codon identified two hexamers related to the −35 and −10 consensus sequences, located at the appropriate distance (17 bp): TTGACG (positions −190 to −185) and GATGAT (positions −167 to −162). A presumed ribosome-binding sequence AAAGGAGCGU, which is complementary to the 3′ end of *B. subtilis* 16S rRNA, is found immediately preceeding (6 bp) the initiation codon. Beyond the translation stop codon, a sequence of 33 nucleotides (positions +1328 to +1360) can be folded into a stem-loop structure which might function as a transcriptional terminator. No significant amino acid sequence homology was found between the BGC product and other 1,3-1,4-β-glucanases from different *Bacillus* species, such as *B. subtilis* (2) and *B. amyloliquefaciens* (3), or with a 1,3-β-glucanase from the same microorganism *B. circulans* WL-12 recently reported (4).

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        GGATCCGAACAAAAACGTAAAATCATCGGTAACGAGTTTATTTACGTATTTGACGAAGAGTCCAAGCAGTTTGATGATTTTGCATTCCTGGCGCAAGGTACACTGTACACGGATAT
     CGTGGAAAGTGGCACAGCGACAGCTCAAACCATCAAATCCCACCAGCAATGTGGGCGGATTGCCTGAGGACATGAACTTCAAGCTGATCAAAACCCATTTGGACCTAAAAGGAGCGTAAATTT
   1 ATGAGAAAAAATAGGGGATTTTCGTTTAGCAGCAAGGCTGTTATGATGTGTTGTCTCGCTTTTCTTCTGATTCCGGCGAGCTTTGCTTTCGCCGCTCCAAACAAGCCGTTCCCTCAGCACACG
     M  R  K  N  R  G  F  S  F  S  S  K  A  V  M  M  C  C  L  A  F  L  L  I  P  A  S  F  A  F  A  A  P  N  K  P  F  P  Q  H  T
 124 ACATATACCAGCGGCTCGATCAAGCCTAACCATGTTACTCAAAGTGCGATGGACAGCTCTGTGAAAGCGAAATGGGACAGCTGGAAATCGGCTTATCTGAAAACGGCGGGCACAGGCAAGTAT
     T  Y  T  S  G  S  I  K  P  N  H  V  T  Q  S  A  M  D  S  S  V  K  A  K  W  D  S  W  K  S  A  Y  L  K  T  A  G  T  G  K  Y
 247 TATGTAAAGTATCAGTCCAACGGGGATACCGTATCTGAGGCACACGGGTACGGAATGCTGGCAACGGTTATAATGGCCGGTTATGACGGCAATGCGCAGACCTATTTCGACGGACTTTATCAA
     Y  V  K  Y  Q  S  N  G  D  T  V  S  E  A  H  G  Y  G  M  L  A  T  V  I  M  A  G  Y  D  G  N  A  Q  T  Y  F  D  G  L  Y  Q
 370 TATTATAAAGCGCATCCGAGCGCCAACAATTCGAAATTGATGGCATGGAAACAAAACAGCAGCTTCCAGAACATTGAAGGCGCCGACTCGGCCACGGATGGAGACATGGACATTGCTTATTCG
     Y  Y  K  A  H  P  S  A  N  N  S  K  L  M  A  W  K  Q  N  S  S  F  Q  N  I  E  G  A  D  S  A  T  D  G  D  M  D  I  A  Y  S
 493 CTCCTGCTTGCGGACAAGCAGTGGGGTAGCAGCGGCAGTATCAACTACCTTCAGGCCGGCAAGGACATCATCAATGCCATCATGCAAAGTGACGTGAATCAGTCTCAATGGACGCTGCGTCTT
     L  L  L  A  D  K  Q  W  G  S  S  G  S  I  N  Y  L  Q  A  G  K  D  I  I  N  A  I  M  Q  S  D  V  N  Q  S  Q  W  T  L  R  L
 616 GGCGACTGGGCAACAGACAACACGTTCAAAAATGCTACCCGTCCATCGGACTTTATGCTGAATCACTTGAAGGCGTTCCAGGCGGCAACTGGTGATGCCAGATGGGCGAACGTGATCGACAAA
     G  D  W  A  T  D  N  T  F  K  N  A  T  R  P  S  D  F  M  L  N  H  L  K  A  F  Q  A  A  T  G  D  A  R  W  A  N  V  I  D  K
 739 ACCTATACTATTATCAACTCCTTATACAGCGGCTACAGCTCATCAACCGGATTACTTCCAGACTTCGTCGTTCTGTCGGGCTCGACGTACAAGCCGGCTTCAGCCGATTTTCTGGAAGGGGCA
     T  Y  T  I  I  N  S  L  Y  S  G  Y  S  S  S  T  G  L  L  P  D  F  V  V  L  S  G  S  T  Y  K  P  A  S  A  D  F  L  E  G  A
 862 AACGACGGCAGCTACGATTACAACTCATGCCGCACACCTTGGCGGATCGCAACGGATTACCTGATGACCGGTGACAGCCGGGCGCTCAATCAGCTCAATCAAATGAATAGCTGGATCTCAGCC
     N  D  G  S  Y  D  Y  N  S  C  R  T  P  W  R  I  A  T  D  Y  L  M  T  G  D  S  R  A  L  N  Q  L  N  Q  M  N  S  W  I  S  A
 985 AAAGTAAGCGGTAACCCTAGCAACGTAAAGGATGGTTATAAACTCAACGGCACGGTTACAGGTTCCGGCGGCAGCGGCGCGTTCTATGCACCGTTTGGTGTCAGCGCTATGACCTCGTCCGTG
     K  V  S  G  N  P  S  N  V  K  D  G  Y  K  L  N  G  T  V  T  G  S  G  G  S  G  A  F  Y  A  P  F  G  V  S  A  M  T  S  S  V
1108 AACCAAAACTGGCTGAATTCCGTCTGGACCAAAACGGCCGGCAGCTCCAATGAAGGCTACTATGAAGACAGCATCAAGCTGTTCTCCATGATCGTGATGTCCGGTAACTGGTGGACATACTAA
     N  Q  N  W  L  N  S  V  W  T  K  T  A  G  S  S  N  E  G  Y  Y  E  D  S  I  K  L  F  S  M  I  V  M  S  G  N  W  W  T  Y  *
     GCAACTTATAATCCGTTGATTGCTCATATGCAAGCAGGCAGGTAGATTAGTGAAGTGACCCCTTAAAGTTAGACAAATATTTTTATGCAACTTGTTTGGCATGAGCTCGGTATTGTACCGGGC
     TCATGCCTTTTAATTTTGACTTGATGCGTTTGTGATTGTAGTAATCCATGTATCGTTCTAGTTCTTGCTTGAAGTGATCTACGCTTTCAAACTCATGAAGATAAAGAAACTCTGACTTCATAA
     TGCCAAAGAAGTCTCCATTACTGCGTTATCGTAACAATTCCCTTTGCGTGACATGCTCTGGGTAATCGCTTGTTTCTTTAAAGCCTGTCGATACTGCTTCATCTGGTAGTGCCAGCCTTGATC
     CGAATGCAGGAGGAGCTGATCTTCGCTGGATAAGCGTTTAAAGGCTTTATTTAGCATTTCGGAGACTAATGAATACGTAGGGCGAGACCCTACTGTGTAAGTGATAATTTCACTGTTAAACAA
     GTCCATAACAGTGATAAATAAAGCTT
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