Notes

NUCLEOTIDE SEQUENCE OF THE PHENOMYCIN GENE FROM *Streptoverticillium baldacci* Ma564-C1

NOBUO SAKATA*, TOMOICHIRO OKA, SOUICHI IKENO and MAKOTO HORI

Showa College of Pharmaceutical Sciences, Machida, Tokyo 194, Japan

KENJI YAMAGUCHI†, YOSHIO INOUYE and SHOSHIRO NAKAMURA

Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine, Minami-ku, Hiroshima 734, Japan

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Phenomycin is a basic polypeptide antitumor antibiotic discovered by screening for inhibition of protein synthesis, using a cell-free system derived from Ehrlich mouse carcinoma[1]. The antibiotic

Fig. 1. pPH1.

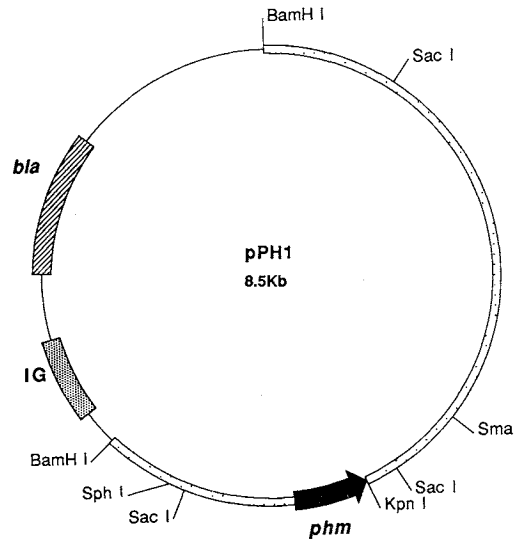

Fig. 2. Nucleotide sequence of *phm*.

See text for underlined sequences. The nucleotide sequence data of *phm* will appear in the DDBJ, EMBL and GeneBank Nucleotide Sequence Databases with the following accession number D17759.

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                                        40                                      80
CGCAAAACATCCTTTTGTCGCGTGATCGCCATGCAGCAGGCTGGTCGGCGTATCGAACCGGAATGCTTGATTCGAACTGA
                                       120                                     160
AGAGGTATCGCATGAAGCTCATCGTCCGGACCGGGCTCGCCGCCGCCGTGGTGCTCGGAGCCGCTGCTGCCGCCGTCGTT
            M  K  L  I  V  R  T  G  L  A  A  A  V  V  L  G  A  A  A  A  A  V  V
                      -40                                     -30
                                       200                                     240
CCCGCCTCCGCCGCTGTCGTCACCGACGACCCGCGCCCGGTGGCCGGTGCGCACGCGGTGGTGCCCAACCCGAAGACGAT
 P  A  S  A  A  V  V  T  D  D  P  R  P  V  A  G  A  H  A  V  V  P  N  P  K  T  I
    -20                                     -10                                -1/ 1
                                       280                                     320
CAAGGCCGCCGCGTACAACCAGGCCCGCAGCACCCTGGCCGACGCGGGCAGCCGGACGGCGGCCAAGTCGCACCCGATCC
  K  A  A  A  Y  N  Q  A  R  S  T  L  A  D  A  G  S  R  T  A  A  K  S  H  P  I
           10                                      20                              30
                                       360                                     400
ATGGGAAGACCGACGTGCCGGTCAGCTACGGCACCAGCCTGCTGGCCGCCGCCCGCGACGAGTTCCGGCAGGCCGACAAG
H  G  K  T  D  V  P  V  S  Y  G  T  S  L  L  A  A  A  R  D  E  F  R  Q  A  D  K
                     40                                      50
                                       440                                     480
AAGCTGCCGGCGAAGGACAAGAAGTCCGACATGTCGATCGCGCACTACAACGCCGTTCACAGCGCGGCGAAGACCATGGG
 K  L  P  A  K  D  K  K  S  D  M  S  I  A  H  Y  N  A  V  H  S  A  A  K  T  M  G
   60                                      70                                  80
                                       520                                     560
GATCGACACGTGGTGACGCACCGCTAGCCGGTACCGGTGCGCCCCGCCCCCGCGAACGGGGGCGGGGCGCACGTGCGTCA
  I  D  T  W  *
        89
      570
GCCCTCGACGC
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† Present Address: Kikuchi Research Center, The Chemo-Sero-Therapeutic Research Institute, Kumamoto 861-15, Japan.

exhibits significant antitumor activities against Ehrlich carcinoma, sarcoma 180 and adenocarcinoma in mice, but does not have any detectable antimicrobial activity[2]. Recent progress in the clinical use of immunosuppresants encouraged us to re-evaluate phenomycin, especially for its effectiveness against solid tumors. The amino acid sequence of phenomycin, which had been reported[3], enabled us to clone the phenomycin gene (*phm*), from *Streptoverticillium baldacci* Ma564-C1 (ATCC 29809), a producer of the antibiotic. By sequencing *phm*, we found that phenomycin derived from a precursor (pre-phenomycin) that should be cut into a leader peptide (M^{-45}–P^{-1}) and phenomycin (N^{1}–W^{89}). Here we describe these results.

A genomic clone (pPH1, shown in Fig. 1) encoding pre-phenomycin was isolated from an *S. baldacci* total DNA library by screening with the PCR fragment corresponding to R^{14} to A^{72} of the protein as a probe. Nucleotide sequence analysis was performed on both strands, using *Bca*BEST DNA polymerase (Takara Biomedicals). As shown in Fig. 2, the sequence included an ORF that could code a precursor protein (pre-phenomycin) comprising a leader peptide and phenomycin. The deduced amino acid sequence of phenomycin was identical to the previously reported one that had been based on analysis of the purified protein[3]. The GC content of the 3rd letters of codons was 94% while that of the gene (throughout the ORF) was 69.5%, consistent with data on streptomycetes ORFs reported so far. AAGAGG preceding the initiator ATG with a 6 base space was considered to be the Shine-Dalgarno sequence of the ORF. Behind the end of ORF, there was a sequence for a hairpin structure with -53.8 kcal/mol, possibly acting as a terminator.

The successful cloning of *phm* opened the way to the genetic engineering of better phenomycins with modified amino acid sequences. It would also be possible to fuse *phm* with the gene for a cell-targeting molecule. This type of strategy has been attempted for recombinant chimeric toxins[4].

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References

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