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NOTE

Efficient Cloning of the Genes for RNA Polymerase Sigma-like Factors from Actinomycetes

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Abstract We have cloned the RNA polymerase sigma-like factors from a wide range of actinomycetes by using specific primers with the polymerase chain reaction (PCR). The specific oligonucleotide primers were designed on the basis of amino acid sequences of conserved regions from HrdA, B, D of *Streptomyces griseus* as well as from the *rpoD* box of many eubacteria. The consensus sequences were from the *rpoD* box and helix-turn-helix motif involved in -35 recognition. The designed primers were successfully applied to amplify the DNA fragments of the *hrd* homolog genes from 8 different strains of actinomycetes which produce a wide variety of important antibiotics. The 480 bp of the DNA fragment was amplified from all 8 strains, and it was identified as a part of *hrdA* and *hrdB* as we designed. The deduced amino acid sequence of PCR-amplified DNA fragments were highly homologous to those of other known RNA polymerase sigma factors of *S. griseus* and *Streptomyces aureofaciens*. Therefore, this study with specifically designed primers will support rapid cloning of the RNA polymerase sigma factors which recognize different classes of promoters from actinomycetes, and it will also be helpful in understanding the relationship of promoters and sigma factors leading to heterogeneity of RNA polymerases in actinomycetes.

Keywords: Sigma factor, *hrdB*, *rpoD*, RNA polymerase, promoter, actinomycetes

Actinomycetes is a gram-positive soil bacteria with a complex differentiation of morphology and physiology that is associated with the production of antibiotics, pigments, and other secondary metabolites. Most prokaryotic genes are regulated at the level of transcription by the controlled expression of different RNA polymerase sigma factors, which recognize different classes of promoters [9, 12, 14]. Regulation of transcription is a critical role for biochemical changes leading to differentiation. Initiation of transcription is associated with the RNA polymerase which consists of five subunits, $\alpha_2\beta\beta'\sigma$, in bacteria. Promoter recognition by RNA polymerase is determined by the sigma factor associated with the core enzyme, $\alpha_2\beta\beta'$. In *Bacillus subtilis*, it is known that sporulation is controlled by the substitution of the sigma factor of RNA polymerase, which is different from σ^A for vegitative growth [8].

Since G+C contents of the actinomycetes genome is as high as 70%, and the morphological differentiation associated with secondary metabolites including commercially important antibiotics is complex, the gene expression of these organisms is a very interesting subject. Many promoters of *Streptomyces* genes were characterized and revealed that the nucleotide sequences of promoters were very diverse. Therefore, it was difficult to classify the *Streptomyces* promoters by nucleotide sequence unlike those of *Escherichia coli*, and several kinds of sigma factors which recognize different classes of promoter have been discovered [2, 7, 9, 14].

RNA polymerase heterogeneity in *Streptomyces* was first reported by Westpheling *et al.* [17], and four distinct genes, *hrdA, hrdB, hrdC*, and *hrdD*, homologous to *E. coli rpoD* were discovered in *Streptomyces coelicolor* A3(2) by Tanaka *et al.* [15]. They used a probe, based on 13 conserved amino acids in the principal sigma factor of *E. coli* and *B. subtilis*. Following the pioneering work, *hrdA, hrdB, hrdD*, and *hrdE* from *S. aureofaciens* [6], and *hrdA, hrdB, hrdD*, and *hrdT* from *S. griseus* [9, 12, 13, 14] were also identified. However, the most studied strain about sigma factor is *S. coelicolor* [2, 3, 5, 7].

The nucleotide sequence analysis showed that the *hrd*-encoded proteins were extremely similar to each other and to the products of the *rpoD* genes of *E. coli* [10], *B. subtilis* [16], and *Myxococcus xanthus* [4].

The *hrdB* gene product is essential for normal growth and is involved in the transcription of housekeeping

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genes [1, 2, 11], therefore, it could be a counterpart of σ^{70} in *E. coli* and σ^{A} in *B. subtilis*, but the functions of the other *hrd* gene products are unclear at present. It is interesting to know which of the expression of these *hrd* genes are involved in which stage of the cell cycle. Here, we report the PCR-amplified *hrd* homolog from a wide range of actinomycetes, which is very likely a part of *hrdA* or *hrdB* genes. Similarity of the deduced amino acids of the cloned region with other known HrdB is as high as 98% in BLAST search.

These results will support rapid isolation of the genes for principal sigma factor and cloning of the other sigma factors (subfamily of sigma factor) which are working at different stage of cell cycle or physiology.

Design of PCR Primers for the Amplification of the Sigma-like Transcription Factor

The PCR method is a very valuable and powerful tool for the cloning of related genes in DNA sequences. For the cloning of the RNA polymerase sigma-like factor from 8 different actinomycetes strains in hand, we designed and prepared specific primers from amino acid consensus sequences which can be found in the *rpoD* box in the major sigma factors of eubacteria

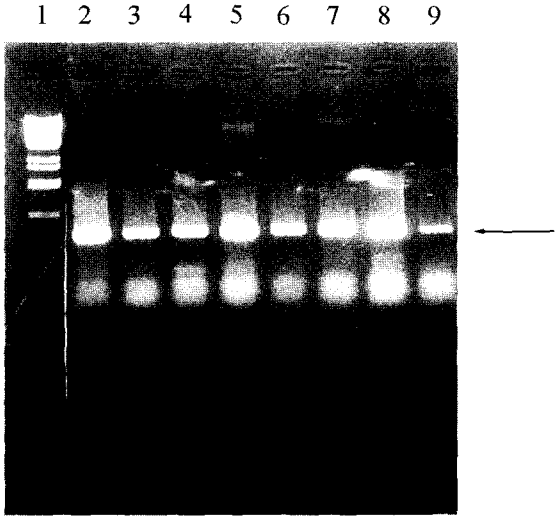

Fig. 1. PCR products from 8 different actinomycetes.
About a 480-bp DNA fragment was amplified with designed primers from all the chromosomal DNA of used strains, and it revealed that all the amplified DNA fragments were a part of *hrdA*, *hrdB*, or *hrd* homologous genes. The lane 1 is λ-*Bst*EII as a size marker. The second lane to the ninth lane were PCR-amplified DNA from the chromosomal DNA. Lane 2: *S. lividans* TK24; lane 3: *S. glauceuces*; lane 4: *S. bluensis*; lane 5: *S. peucetius*; lane 6: *S. spectabilis*; lane 7: *M. purprea*; lane 8: *M. olivasterospora*; and lane 9: *A. autotrophica*.

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S. aureofaciens HrdA  NLRLVVSVAKRYVGRGLTMLDLVQEGNLGLIRAVEKFDYARGYKFSTYATWWIRQAMSRALADQARTIRVPVHVVELINRVVRVQ--MLQERG-YEPTAEEVAVHLELTPERVLEVLRLA
S. aureofaciens HrdB  NLRLVVSLAKRYTGRGMLFLDLIQEGNLGLIRAVEKFDYTKGYKFSTYATWWIRQAITRAMADQARTIRIPVHMVEVINKLARVQRQMLQDLG-REPTPEELAKELDMTPEKVIEVQKYG
S. aureofaciens HrdD  NLRLVVAVVRRYPRSGLPLLDLIQEGNAGLVRAVEKFDYAKGFKFSTYATWWIRQAITRSIADQSRTIRLPVHLVEELGRIRRVQREFNRENG-RDPEHAEIAAELDSTEKRVGDVLDWA
S. griseus HrdA       NLRLVVSTAKRYVGRGLTMLDLVQEGNLGLIRAVEKFDYARGYKFSTYATWWIRQAMSRALADQARTIRVPVHVVELINRVVRVQRRLLQERG-VEPTAEDIAVDLDLTPERVTDILRLA
S. griseus HrdB       NLRLVVSLAKRYTGRGMLFLDLIQEGNLGLIRAVEKFDYTKGYKFSTYATWWIRQAITRAMADQARTIRIPVHMVEVINKLARVQRQMLQDLG-REPTPEELAKELDMTPEKVIEVQKYG
S. griseus HrdD       NLRLVVAVARRYPRSGLPLLDLIQEGNAGLVRAVEKFDYAKGFKFSTYATWWIRQAITRSIADQSRTIRLPVHLVEELGRIRRVQREFNRENG-RDPEPAEIAAELSSTPERVTDVLDWA
S. griseus HrdT       NLRLVVSVAKRYTGRGLTMLDLVQEGNLGLIRAVEKFDYTRGFKFSTYATWWIRQAMSRALADQARTIRVPVHVVELINRVVRTQRALLQEAG-REPAAEEVGERLGLSAEKVEEVLRLA
S. lividans TK24    ----------YATWWIRQAMSRALADQARTIRVPVHVVELINRVVRVQRRMLQERG-CEPTPQEVAAHLDLAPERVGEVLRLA
S. glauceuces         ----------YATWWIRQAMSRALADQADTIRVPVHVVELINRVVRVQRRLLQERG-VEPTAEDIGVDLDLTPERVTDILRLG
S. bluensis           ----------YATWWIRQAITRAMADQARTIRIPVHMVEVINKLARVSRQMLQDLG-REPTPEELAKELDMTPEKVIEVQKYG
S. peucetius          ----------YATWWIRQAMSRAMADQARTIRIPVHMIETINKLNRISRQMLQDQMGREPTPEELGERMEMPEDKIRKVLKIA
S. spectabilis        ----------YATWWIRQAMSRAMADQARTIRVPVHVVELINRVVRVQRRMLQERG-YEPSPEEVAAHLDLPHERVRG-LRLA
M. purprea            ----------YATWWIRQAITRAMADQARTIRIPVHMVEVINKLGRIQRELLQDLG-REPTPEELAKEMDITPEKVLEIQQYA
M. olivasterospora    ----------YATWWIRQAITRAMADQARTIRIPVHMVEVINKLGRIQREFFQDLG-REPTPEELAKEMDITPEKVLEIQQYA
A. autotrophica       ----------YATWWIRQAITRAMADQARTIRIPVHMVEVINKLGRIQRELLQDLG-REPTPEELAKEMDITPEKVLETQQYA
                                **********..*..***. ***.***..* .... *    ..   .*  ..  .   ..

S. aureofaciens HrdA  QEPVSLHAPVGEEDDVALGDLIEDGDAASPVESAAFFLRCEHLEAVLSTLGERERKVVQLRYGLADGRPRTLEEIGRIFGVTRERIRQIESKTLNKLRDHAFADQLRGYLD---
S. aureofaciens HrdB  REPISLHTPLGEDGDSEFGDLIEDSEAVVPADAVSFTLLQEQLHSVLDTLSEREAGVVSMRFGLTDGQPKTLDEIGKVYGVTRERR-QIESKTMSKLRHPSRSQVLRDYLDRSP
S. aureofaciens HrdD  RDPVSLNMSVDDEGDTQFGDLLEDTSAISPEQ-SVSLLRSEELEDLLGKLDQRTASIIKMRYGIEDGRERTLTEVGKQHGLTRERIRQIEKHALLELKRMARDTGFDAVAEE--
S. griseus HrdA       QEPVSLHAPVGEEDDVSFGDLIEDGDAASPVESAAFLLLREHLEAVLSTLGERERKVVQLRYGLEDGRPRTLEEIGRIFGVTRERIRQIESKTLSRLRDHAFADQLRGYLD---
S. griseus HrdB       REPISLHTPLGEDGDSEFGDLIEDSEAVVPADAVSFTLLQEQLHSVLDTLSEREAGVVSMRFGLTDGQPKTLDEIGKVYGVTRERIRQIESKTMSKLRHP-RSQVLRDYLD---
S. griseus HrdD       RDPVSLNMSVDDEGETQFGDLLEDTS--SPEQSVLTLLRSEELDDLIGQLDDRTASIIKMRYGIEDGRERTLTEVGKQHGLTRERIRQIEKHALLELKRMAHDTGFDAAA----
S. griseus HrdT       REPS-LHAPVGGEDDVFLGDLIEDADAASPAESAALLLLRQHLDAVLATLGDRERQVVQLRYGFDDGRPRTLEEIGRLFGVTRERVRQIESKTLRRLREHTAAGQLRGYL----
S. lividans TK24     QEPVSLHAPVGEEDDVALGDLIEDGDAASPVESAAFLLLRQHLEAVLSTLGERERKVVQLRYGLADGRPRTLEEIGRLFGVTRERIRQIE----------
S. glauceuces         QEPVSLHAPVGEEDDVSFGDLIEDGDAASPVESAVFLLLREHLEAVLSTLGERERKVVQLRYGLEDGRPRTLEEIGRIFGVTRERIRQIE----------
S. bluensis           REPISLHTPLGEDGDTQFGDLIEDSEAVVPADAVSFTLLQEQLHSVLDTLSEREAGVVSMRFGLTDGQPKTLDEIGKVYGVTRERIRQI----------
S. peucetius          KEPISMETPIGDDEDSHLGDFIEDSTMQSPIDVATVESLKEATRDVLSGLTAREAKVLRMRFGIDMNTDHTLEEVGKQFDVTRERIRQIE----------
S. spectabilis        QEPVSLHAPVGEEDDVALGDLIEDGDAASPVESAAFLLLREHLEAVLSTLGERERKVVQLRYGLADGRPRTLEEIGRIFGVTRERIRQIE----------
M. purprea            REPISLDQTIGDEGDSQLGDFIEDSEAVVAVDAVSFSLLQDQLQQVLQGSPS-VRRVWSMRFGLTDGQPRTLDEIGQVYGVTRERIRQIE----------
M. olivasterospora    REPISLDQTIGDEGDSSLGDFIEDSEAVVAVDAVSFSLLQDQLQQVLQTLSEREAGVVRLRFGLTDGQPRTLDEIGQVYGVTRERIRQIE----------
A. autotrophica       REPISLDQTIGDEGDSQLGDFIEDSEAASPSDAVSFTLLQDQLQSTLGTLSEREAGVVRLRFGLTDGRPRTLDEIGQVY----------
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Fig. 2. Comparison of the PCR products with the HrdA and HrdB proteins of *S. griseus* and *S. aureofaciens*.
Multiple sequence alignment of the deduced amino acid sequences of the PCR products with HrdA and HrdB of *S. griseus* and *S. aureofaciens* was done using the Clustal-W method. This data showed that most of these amplified DNA fragments from the 8 different strains of actinomycetes were a part of Hrd homolog proteins. The seqnences reported in this paper have been assigned AF071790 for *S. lividans* TK24, AF071791 for *S. glauceuces*, AF071792 for *S. blueusis*, AF071793 for *S. peucetius*, AF071794 for *S. spectabilis*, AF071795 for *M. purprea*, AF071796 for *M. olivasterospora* and AF071797 *A. autotrophica*.

Table 1. Similarity of the amino acid sequences of PCR products with the HrdA, HrdB, HrdD, and HrdT.

Percent Divergence	Percent Similarity														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
1		51.5	45.7	50.5	46.8	67.7	60.5	88.3	60.2	49.7	90.7	54.0	58.6	57.6	1
2	46.4		43.9	84.9	43.8	51.4	99.4	59.9	98.1	57.1	59.0	76.4	80.9	79.5	2
3	50.3	53.3		43.9	86.3	44.5	49.4	51.9	50.3	39.3	50.3	45.3	48.1	47.7	3
4	46.8	11.2	53.0		43.5	50.8	99.4	59.9	98.1	57.1	59.0	76.4	80.9	79.5	4
5	48.5	54.0	12.0	53.9		45.6	50.0	51.2	50.9	38.0	49.1	46.6	48.8	47.7	5
6	29.2	46.5	51.5	46.5	49.2		56.8	77.8	56.5	50.3	77.6	52.2	55.6	56.3	6
7	39.8	0.6	50.3	0.6	50.3	43.5		59.3	98.8	58.0	58.4	75.8	80.2	78.8	7
8	11.7	40.1	47.8	40.1	48.4	22.2	41.0		59.0	46.9	85.7	52.2	56.8	55.0	8
9	39.8	1.9	49.4	1.9	48.8	43.5	1.2	41.0		57.1	57.8	75.2	79.5	78.8	9
10	48.1	40.7	58.6	40.7	59.9	47.5	40.4	51.2	41.0		49.7	51.6	54.3	53.0	10
11	7.5	39.4	47.5	39.4	49.4	20.6	39.8	12.5	40.0	47.5		51.6	56.5	55.0	11
12	44.1	21.7	52.5	21.7	51.9	46.0	22.5	46.0	22.5	44.7	44.7		90.7	84.8	12
13	41.4	19.1	51.6	19.1	50.9	44.4	19.9	43.2	20.5	43.8	41.9	7.5		91.4	13
14	42.4	20.5	52.0	20.5	52.0	43.7	21.2	45.0	21.2	45.0	42.7	12.7	8.6		14
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	

1: *S. aureofaciens* HrdA; 2: *S. aureofaciens* HrdB; 3: *S. aureofaciens* HrdD; 4: *S. griseus* HrdB; 5: *S. griseus* HrdD; 6: *S. griseus* HrdT; 7: *S. lividans* TK24; 8: *S. glauceuces*; 9: *S. bluensis*; 10: *S. peucetius*; 11: *S. spectabilis*; 12: *M. purprea*; 13: *M. olivasterospora*; and 14: *A. autotrophica*.

and helix-turn-helix motif from *S. griseus* HrdA, HrdB, and HrdD.

Designed primers were 5'-TACGCSACCTGGTG-GATCCGSCAGGC-3', which was from the conserved regions 2.3 and 2.4 in the *rpoD* box, and 5'-CTCGA-TCTGGCGGATSCGYTCGCGGGT-3', which was from the complementary sequence of the conserved region 4.2 in helix-turn-helix motif involved in -35 recognition. With these primers, it is possible to amplify about a 480-bp DNA fragment from all 8 actinomycetes strains of *Streptomyces lividans* TK24, *Streptomyces glauceuces, Streptomyces bluensis, Streptomyces peucetius, Streptomyces spectabilis, Micromonospora purprea, Micromonospora olivasterospora, and Amycolata autotrophica* (Fig. 1).

Analysis of Nucleotide Sequences of the Genes for Sigma Factor Homologs

A PCR amplified DNA fragment was cloned into the PCR vector, pGEMT-easy, and nucleotide sequences of cloned regions were obtained using an automatic DNA sequencer (ABI model 373, Applied Biosystems, U.S.A.). The deduced amino acid sequence of the cloned fragments revealed remarkable similarity to each other and to those of the other known *hrd* genes of *S. griseus* and *S. aureofaciens* (Fig. 2). With multiple sequence alignment of deduced amino acids, it was found that six of the amplified DNA fragments were homologous with *hrdB* genes that are the principal sigma factors for the transcription of housekeeping genes, and the others were homologous with the *hrdA* gene that is in a subfamily of the sigma factor which is working at a different stage of cell cycle and physiology. However, the function of these minor sigma factors is not yet clear. Similarities among the amino acid sequences revealed that the cloned fragments were highly homologous with *hrd* genes for sigma factors (Table 1).

Our primers were designed from the highly conserved regions in all kinds of sigma factors known to date, therefore, the amplified DNA fragment would most probably be a part of *hrdA, hrdB*, or other *hrd* genes, which are minor sigma factors related with the differentiation of morphology or physiology. Therefore, our designed primers proved to be a very efficient and useful PCR primer or probe for Southern hybridization in obtaining genes for principal sigma factors or subfamily of sigma factors. It would be especially helpful for the rapid isolation of *hrd* homologous genes from actinomycetes strains.

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