Molecular cloning and nucleotide sequence of the gene encoding phosphate-inducible pectin lyase of *Bacillus subtilis*

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Abstract The gene encoding the pectin lyase (PNL; EC 4.2.2.10) of *Bacillus subtilis* has been cloned, sequenced, and characterized. A coding sequence for the PNL composed of 345 amino acids including a 24-amino-acid signal peptide was assigned. No sequence resembling a LexA binding site was found upstream of the structural gene. Furthermore, PNL activity of the gene product expressed in *Escherichia coli* DH5α was detected intracellularly, which might suggest that expression of the gene was not controlled by RecA. Regulation of the gene expression seemed to be quite different from that of other bacterial PNL genes previously reported.

Key words: Pectin lyase; Protopectinase; Nucleotide sequence; Phosphate-dependent expression; *Bacillus subtilis*

1. Introduction

Enzymes that catalyze the solubilization of protopectin, which is a water-insoluble parent pectic substance found in plant tissues, have tentatively been named protopectinases [1–4]. We recently purified a pectin lyase (PNL) with protopectinase activity from the culture broth of *Bacillus subtilis* IFO 3134 [5]. This enzyme, which we called protopectinase-R (PPase-R), was the first PNL to be isolated from a *Bacillus* species.

Previously, bacterial PNLs had been found only in strains belonging to *Erwinia* and *Pseudomonas*. The production of bacterial PNLs is dependent on the culture conditions: PNLs from *Erwinia carotovora* [6], *E. chrysanthemi* [7], *E. aroideae* [8], and *Pseudomonas marginalis* [9] are induced using DNA-damaging agents such as mitomycin C, and that of *P. fluorescens* [10] is induced by the addition of glycerol to the culture medium. Genetic studies on the synthesis of these PNLs reveal that RecA, which is a key protein in the SOS regulon of *E. coli*, is necessary for their gene expression [11,12]. However, neither mitomycin C nor glycerol enhanced the production of PPase-R in *B. subtilis* IFO 3134 in the same culture conditions as reported for the above-mentioned bacteria. The production of PPase-R is induced under high-phosphate conditions and little enzyme activity is found without the addition of phosphate [5]. Previous studies on phosphate-controlled gene expression in *E. coli* and *B. subtilis* have focused on phosphate starvation [13–15] and little is known about the mechanism of phosphate stimulation. In order to study the genetic mechanism of the phosphate induction, we attempted to isolate the gene encoding PPase-R.

This paper reports the first cloning and characterization of a PNL gene, *ppr*, from the *Bacillus* genus and the deduced amino acid sequence is compared with those of other lyases.

2. Materials and methods

2.1. Bacterial strain, plasmid, media, and growth conditions

In the study of PPase-R production in *B. subtilis* IFO 3134, the microorganism was cultivated in test tubes (i.d. 24 mm) containing 10 ml of 1.5% defatted soybean flour extract supplemented with different compounds, pH 7.0, at 37°C for 22 h.

In the experiments involving gene manipulation, *B. subtilis* IFO 3134 was used as a source of the *ppr* gene. *E. coli* DH5α and a plasmid, pUC119, were used to construct a *B. subtilis* genomic library. Plasmid pUC119 was also used as a vector in the nucleotide sequencing. Microorganisms were grown in LB medium consisting of 1% peptone, 0.5% yeast extract, and 0.5% NaCl, pH 7.0, with shaking at 37°C. When required, the medium had ampicillin (50 μg/ml) added and was solidified by the addition of agar (1.5%).

2.2. DNA manipulations

Extraction and purification of plasmids, cleavage and ligation of DNA, transformation of *E. coli* with plasmids, and other general procedures for DNA manipulations were done as described by Maniatis et al. [16].

2.3. Construction of B. subtilis genomic library

The *B. subtilis* genomic DNA was extensively digested with *Hin*dIII and then electrophoresed in a 0.8% agarose gel. The DNA fragments in the range 2.5–4.0 kb were extracted using a DEAE paper (Toyo Roshi Kaisha, Ltd., Tokyo) and ligated with the *Hin*dIII-cleaved pUC119 vector. The recombinant plasmid DNA was used to transform *E. coli* DH5α cells, which were spread and grown on LB plates containing ampicillin to construct the *B. subtilis* genomic library.

2.4. Southern hybridization

Genomic or plasmid DNA cut with appropriate restriction endonucleases was electrophoresed in a 0.8% agarose gel and transferred by capillary blotting to Hybond-N^+ membranes (Amersham International plc, Bucks, UK). Oligonucleotide probes were synthesized (5′-(C/T)TT(C/T)TC(A/G)TT(C/T)TTIGC(A/G)TTICCIGC(A/G)AAIC-C-3′) on the basis of positions 14 to 23 of the amino acid sequence of PPase-R [5], and were non-radioactively labeled using an ECL 3′-Oligolabelling system (Amersham). Hybridization was carried out as recommended by the manufacturer using a hybridization temperature of 37°C and final washing in 1×SSC–0.1% SDS at 42°C.

2.5. Nucleotide sequencing

DNA fragments were subcloned into pUC119 and a series of deletion mutants of each subclone was constructed using a Kilo-Sequence Deletion Kit (Takara Shuzo Co., Ltd., Kyoto, Japan). Double-stranded DNA sequencing of pUC119 subclones was carried out by the dideoxy-chain termination method of Sanger et al. [17] using the Auto Sequencer Core Kit (Toyobo Co., Ltd., Osaka, Japan) on an automated sequencer (A.L.F. DNA Sequencer, Pharmacia LKB Biotech., Tokyo). The sequences were analyzed with the program GENETYX (Software Development Co., Ltd., Tokyo).

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Abbreviations: IPTG; Isopropyl-1-thio-β-D-galactopyranoside

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2.6. Enzyme assay

PNL activity was assayed by measurement of the increase in absorbance at 235 nm of the reaction mixture. A reaction mixture containing 2 ml of 0.1% methoxylated pectic acid in 100 mM sodium acetate buffer, pH 6.0, and an appropriate amount of enzyme solution was incubated at 50°C for 60 min. The reaction was stopped by boiling the mixture for 5 min. One unit of enzyme activity was defined as the activity that liberates 1 μmol of unsaturated galacturonate per milliliter of the reaction mixture under the above-mentioned conditions. The molar extinction coefficient of the unsaturated galacturonate at 235 nm is 4600 M^{-1}.

3. Results and discussion

High concentrations of KH_2PO_4 suppressed the growth of *B. subtilis* IFO 3134 but appeared to enhance the production of PPase-R (Table 1). On the other hand, production of PPase-R was not substantially affected by KCl (a control for KH_2PO_4) or glycerol, which is known to induce PNL in a certain strain of *Pseudomonas* [10]. Addition of NaH_2PO_4 or $NH_4H_2PO_4$ also showed stimulating effects on the enzyme productivity in this strain (Table 1). In the case of mitomycin C, growth is so low that the effect cannot be discussed on the PPase-R production. From these results, the phosphate anion seemed to be involved in the stimulation of PPase-R production in *B. subtilis* IFO 3134. However, several other factors might have been involved such as local pH, osmolarity, and concentration of divalent cation to which phosphate is a strong chelator.

In order to study the regulation of a phosphate-inducible PPase-R at the DNA level, the molecular cloning of this gene was carried out. Southern hybridization analysis was done with the fragments of chromosomal DNA of *B. subtilis* digested with 11 kinds of restriction endonucleases using the oligonucleotide probes described in Section 2.4. A single band at the 3.3-kb position was detected in the case of DNA fragments digested with *Hin*dIII. The *B. subtilis* genomic library was constructed using *Hin*dIII-digested DNA fragments in the range 2.5–4.0 kb.

Screening for *ppr* by colony hybridization was done with approximately 2000 colonies from the library. Two positive clones were selected and they carried the same recombinant plasmid, designated pUC9-2, having a 3.3-kb fragment. The plasmid was digested with several restriction endonucleases and Southern hybridization was carried out to determine the gene location. The region hybridized with the probe described in Section 2.4 existed in a 0.9-kb *Sma*I–*Pst*I site in the insert DNA. The 0.9-kb *Sma*I–*Pst*I fragment was subcloned into pUC119, and used for sequencing. The fragment had a nucleotide sequence corresponding to the N-terminal amino acid sequence of PPase-R, near the *Pst*I site.

The structural gene of *ppr* and the flanking regions in the pUC9-2 was sequenced in both strands using 30 deletion subclones (Fig. 1). A coding sequence (CDS) of 1035 bp starts with an ATG codon at nucleotide (nt) 182 and stops with a TGA termination codon at nt 1217. The CDS encodes a polypeptide of 345 amino acids with a calculated molecular weight of 37994 Da and a calculated isoelectric point of 9.54. The deduced 25-amino-acid sequence from residue 25 to residue 49 corresponds to that of the N-terminus of the purified *B. subtilis* PPase-R.

Generally, a bacterial signal peptide contains one to three positively charged amino acid residues at the N-terminal region, followed by a long hydrophobic sequence of about 15 amino acid residues. The N-terminus of the deduced PPase-R protein appears to contain a traditional cleavable signal sequence consisting of 24 amino acid residues. The precursor of PPase-R might be cleaved at Gly–Ala at positions 24 and 25. The calculated molecular weight (37994 Da) is slightly higher than that of *B. subtilis* PPase-R (35000 Da). This difference can be explained by the presence of a 24-amino-acid signal peptide at the N-terminus of the recombinant protein.

There is a purine-rich sequence (GAAAGGAAG) in the 5′-flanking region of the ATG initiation codon of the structural gene. This sequence seems to contain the ribosome binding site. Nine of the 12 bases from nt 48 to nt 53 and from nt 74 to nt 79 match the sigma 70 binding consensus sequence of *E. coli*, suggesting that these regions might be the promoter for the *ppr* gene. Expression of the clone harboring pUC9-2, in which the *ppr* gene is linked downstream of the pUC119 *lac* promoter, was not affected by addition of the inducer IPTG to the culture medium. Furthermore, clones harboring plasmids with opposite orientations of the 3.3-kb *Hin*dIII fragment in pUC119 produced pectin lyase at the same level. From the results, the *ppr* gene seemed to be expressed using its own promoter on the 3.3-kb DNA fragment.

There are 12 enzymes involved in the pectin-degradation pathway in *Erwinia chrysanthemi* strain 3937 [18]. Although the genes encoding these enzymes are distributed over five differet regions of the chromosomal DNA, expression of the

Table 1
Effects of different inducers on PPase-R production in *B. subtilis* IFO 3134

Compound	Concentration (%)	(mM)	Growth (dry weight in mg)	Total activity (U)	Productivity (U/mg dry weight)
None	–		19	2	0.11
KH_2PO_4	1.0	73	20	25	1.3
	3.0	220	17	56	3.3
	5.0	370	15	63	4.2
NaH_2PO_4	2.5	160	17	45	2.6
$NH_4H_2PO_4$	2.5	220	19	51	2.7
KCl	2.5	340	21	12	0.57
NaCl	2.5	430	22	12	0.55
NH_4Cl	2.5	470	25	18	0.72
Glycerol	1.0	–	15	6	0.40
Mitomycin C[a]	5×10^{-5}	–	2	0	0

Each compound was added to the soybean extract medium in the concentrations shown. Supernatants of the broth after 22 h of culture were used for the enzyme assays.
[a]Mitomycin C was added to the broth after cultivation of 6 h and incubation was continued until 22 h.

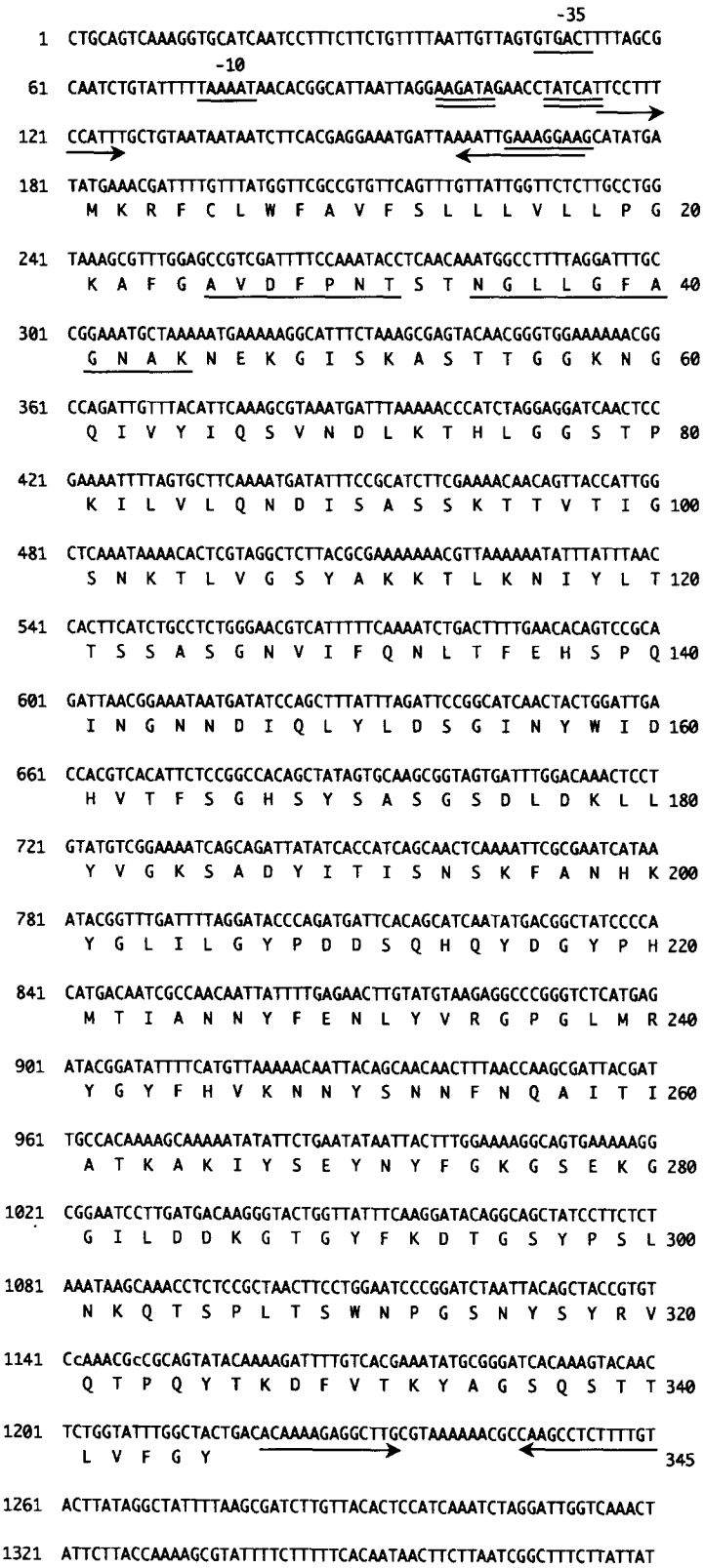

Fig. 1. Nucleotide sequence of *ppr* from *B. subtilis* IFO 3134. The deduced amino acid sequence is shown beneath the nucleotide sequence. The putative ribosome binding site and the N-terminal amino acid sequence of the mature *B. subtilis* PPase-R, determined by Edman degradation, are underlined. The putative KdgR binding site is double underlined, and the −35 and −10 regions are labeled as such. Inverted repeat sequences are indicated by arrows. This sequence data will appear in the DDBJ, EMBL, and GenBank nucleotide sequence databases with the following accession number: D83791.

genes is under the control of the KdgR repressor. The sequence of the KdgR binding site in regulatory regions of these genes has been proposed to be ATGAAAXXXXXTTTCAT, where X is an unspecified base. The region (nt 98–114) located

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                                        *     *  ***          *  ***  *  *
Bs     1:MKRFCLWFAVFSLLLVLLPGKAFGAVDFPNTSTNGLLGFAGNAKNEKGISKASTTGGKNGQIVYIQSVNDL
Pm     1:                            MSYPESKLTGLTGFA---------LAAKVTGGWAGPVVSITNLDQL
Ec71   1:                            MAYPTTNLTGIIGFA---------KAANVTGGTGGKVVTVNSLADF
EcEr   1:                            MAYPTTNLTGLIGFA---------KAAKVTGGTGGKVVTVNSLADF

         *          *       *  * *  * ***  **       * ** *     * * * *** * *
Bs    72:KTHLGGSTPKILVLQNDISASSKTTVTIGSNKTLVGSYAK--KTLKNIYLTTSSASGNVIFQNLTFEHSPQ
Pm    38:KANIGTVTPQVLVINSNISASSLTKVNMGANKTLIGSFQN--RTLENIHLRATAQSQNIILQNLIFKHSAN
Ec71  38:KSAVSGSAKTIVVLGLSLKASALTKVVFGSNKTIVGSFGGYANVLTNIHLRAESNSSNVIFQNLVFKHDVA
EcEr  38:KSAVSGSAKTIVVLGSSLKTSALTKVVFGSNKTIVGSFGG-ANVLTNIHLRAESNSSNVIFQNLVFKHDVA

         *  * *** **  *  ** **    **  *      *** * *  *** **** *  **** * * * **
Bs   141:INGNNDIQLYLDSGINYWIDHVTFSGHSYSASGSDLDKLLYVGKSADYITISNSKFANHKYGLILGYP-DD
Pm   107:IKANDDIQVYLNYGSKYWIDHCSFVGHSWSTTDGSEDKLLYIGEKADYATISNCFFGSHKYGLIFGHPADD
Ec71 109:IKDNDDIQLYLNYGKGYWVDHCSWPGHTWSDNDGSLDKLIYIGEKADYITISNCLFSNHKYGCIFGHPADD
EcEr 108:IKDNDDIQLYLNYGKGYWVDHCSWPGHTWSDNDGSLDKLIYIGEKADYITISNCLFSNHKYGCIFGHPADD

              *** *   *   *  ** ********** *                 *   ** * ** *
Bs   211:SQHQYDGYPHMTIANNYFENLYVRGPGLMRYGYFHVKNNY-SNNFNQAITIATKAKIYSEYNYFGKGSEKG
Pm   178:NNAAFNGYPRLTLCHNRFDNMEVRAPGLMRYGYFHVYNNY-INKFHLGFTLAQNANILSESNYFGEGSQNN
Ec71 180:NNSAYNGYPRLTICHNYYENIQVRAPGLMRYGYFHVFNQPTSINSTWPLQLRRNANLISERNVFGTGAENK
EcEr 179:NNSAYNGYPRLTICHNYYENIQVRAPGLMRYGYFHVFNNY-VNKFQLAFTVAQNANVISERNVFGSGAEKK

         *  **** *
Bs   281:GILDDKGTG-YFKDTGSYPSLNKQTSPLTSWNPGSNYSYRVQTPQYTKDFVTKYAGSQSTTLVFGY
Pm   248:GMLDDKGSG-TFTDTNSVPPITNQKSPKAQWTATSNYAYTLKTAAQAKDFTQKNAGAQAAALVFGS
Ec71 251:GMVDDKGNGSTLRIMAVHR-LRWRANRLRRNGRRHLTIHTV-----------------------
EcEr 249:GMVDDKGNGSTFTDNGSSPAAVASKSPAAKWTASSNYSYSLMTTAAAQSWVVSNAGAQNSALKFPS
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Fig. 2. Alignment of amino acid sequences of the *B. subtilis* (Bs), *P. marginalis* (Pm), *E. carotovora*, subsp. *carotovora* 71 (Ec71), and *E. carotovora* Er (EcEr) PNL proteins. Identical residues are marked with asterisks.

between the presumed promoter and the initiation codon, in which 9 of the 12 bases in the consensus binding site are conserved, might play a role in *ppr* expression.

A 14-base sequence (nt 1221–1234) 2 bp downstream from the stop codon of the CDS is followed by an inverted repeat at nt 1247–1260. These sequences appear to act as a transcription termination signal.

Although it is not known whether the PPase-R of *B. subtilis* is glycosylated, five potential N-glycosylation sites were identified at asparagine residues at amino acid positions 30, 102, 132, 249, and 315.

PNL activity of the product expressed from pUC9-2 in *E. coli* DH5α was assayed using the culture supernatant and the cell-free extract. The enzyme activity was detected in the cytosol. The thermal and pH stabilities of the *E. coli* enzyme were similar to those of the *B. subtilis* PPase-R. The maximum activities of the enzymes were also found at the same pH and temperature conditions. The first six amino acid residues at the N-terminus of the *E. coli* enzyme and the *B. subtilis* PPase-R were also identical. Detection of PNL activity of the *E. coli* enzyme and similality of physicochemical characteristics of the cloned and native PPase-R, confirm that the gene sequenced encodes PPase-R from *B. subtilis* IFO 3134. All the data presented in this paragraph are not shown and the details will be described elsewhere.

So far, seven *PNL* genes (three from *Aspergillus niger* [19–21], two from *E. carotovora* [22,23], one from *Glomerella cingulata* [24] and one from *P. marginalis* [25]) have been cloned and sequenced. Comparison of the amino acid sequence of PPase-R with those of the above seven PNLs revealed low homologies with the four eukaryotic genes (*Aspergillus* and *Glomerella*) but higher homologies with the three other prokaryotic genes (Fig. 2; 52% identity in a 294-amino-acid overlap for *P. marginalis*, 48% identity throughout the length of the *E. carotovora* Er, and 47% identity in a 268-amino-acid overlap for *E. carotovora* subsp. *carotovora* 71). A signal sequence was found in only the PPase-R protein. The amino acid sequence derived from the nucleotide sequence contained two consensus regions (W - DH and R - P - - R - G - - - - YNN) among pectinolytic enzymes including pectate lyase and PNL [26–30]. These regions seem to be a substrate binding site or a catalytic site of the enzyme. Furthermore, the

amino acid sequence of PPase-R showed a 25% identity in a 173-amino-acid overlap with a pectate lyase from *B. subtilis* [31], and the two above-mentioned consensus regions were conserved.

Regulation of other bacterial genes encoding PNL have been studied [11,12]. Recent studies seem to indicate that a RecA protein, which is involved in cleavage of LexA (the repressor of many genes in *E. coli*), is necessary for the production of several bacterial PNLs. The consensus sequence for an SOS box is found upstream of the *PNL* gene of the *P. marginalis* N6301 strain, suggesting that the gene expression is regulated directly by RecA [25]. In *E. carotovora* subsp. *carotovora* 71, transcription of the *PNL* gene is regulated by the Rdg protein which is also controlled by RecA in an unknown manner [11]. On the other hand, PNL activity was detected in the *E. coli* DH5α transformant (a *recA* gene-deficient strain) harboring the *ppr* gene. Furthermore, no sequence resembling a LexA binding site was found upstream of the structural gene. These results suggest that expression of the *ppr* gene is controlled not by the RecA protein but by a mechanism that is quite different from the regulation of other *PNL* genes.

B. subtilis IFO 3134 produces PPase-R under high-phosphate conditions. In contrast, the synthesis of alkaline phosphatase and phosphodiesterase in *B. subtilis* are repressed by phosphate: the expression of these genes are controlled by four kinds of regulators (Pho P, R, S, and T) [14,15]. In the expression of *ppr* under high-phosphate conditions, these regulators may have different functions or some unknown proteins may act as regulators. *B. subtilis* IFO 3134 produces another protopectinase (protopectinase-C), which catalyzes solubilization of sugar-beet protopectin by splitting L-arabinan [32,33]. Production of the enzyme is also enhanced by addition of phosphate at a high concentration to the culture medium (data not shown). The observations that this gene and *ppr* expressions are induced by phosphate are interesting because phosphate is most often limiting in soil or leaves, where some of the life cycle of *B. subtilis* may occur. The structural gene of protopectinase-C has already been sequenced (data not shown). Comparison of the nucleotide sequence of this gene with that of *ppr* isolated in this study may help to elucidate the mechanism by which the expression of these genes is stimulated by phosphate.

The genus *Bacillus* is a soil bacterium that has developed many regulatory mechanisms in order to adapt to environmental changes, and different classes of positive regulators, such as Sac V and Glt C, from *B. subtilis* have been identified. The production of many extracellular enzymes by *B. subtilis* is stimulated by binding of positive regulators to the regulatory regions of the genes encoding these enzymes [34]. In the case of the *ppr* gene, only one inverted repeat sequence (at nt 114–126 and nt 160–172) was found within 181 bp upstream of the ATG start codon. This region may be the target site of some regulatory proteins. Analysis of the regulatory region upstream of *ppr* is now being undertaken and will help to further characterize this gene.

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References

[1] Sakai, T. (1988) Methods Enzymol. 161, 335–350.
[2] Sakai, T., Sakamoto, T., Hallaert, J. and Vandamme, E.J. (1993) Adv. Appl. Microbiol. 39, 213–294.
[3] Sakai, T. and Okushima, M. (1978) Agric. Biol. Chem. 42, 2427–2429.
[4] Sakamoto, M., Shirane, Y., Naribayashi, I., Kimura, K., Morishita, N., Sakamoto, T. and Sakai, T. (1994) Eur. J. Biochem. 226, 285–291.
[5] Sakamoto, T., Hours, R.A. and Sakai, T. (1994) Biosci. Biotech. Biochem. 58, 353–358.
[6] Itoh, Y., Sugiura, J., Izaki, K. and Takahashi, H. (1982) Agric. Biol. Chem. 46, 199–205.
[7] Tsuyumu, S. and Chatterjee, A.K. (1984) Physiol. Plant Pathol. 24, 291–302.
[8] Kamimiya, S., Nishiya, T., Izaki, K. and Takahashi, H. (1974) Agric. Biol. Chem. 38, 1071–1078.
[9] Sone, H., Sugiura, J., Itoh, Y., Izaki, K. and Takahashi, H. (1988) Agric. Biol. Chem. 52, 3205–3207.
[10] Schlemmer, A.F., Ware, C.F. and Keen, N.T. (1987) J. Bacteriol. 169, 4493–4498.
[11] McEvoy, J.L., Murata, H. and Chatterjee, A.K. (1992) J. Bacteriol. 174, 5471–5474.
[12] Nikaidou, N., Kamio, Y. and Izaki, K. (1994) Biosci. Biotech. Biochem. 58, 2297–2298.
[13] Tommassen, J. and Lugtenberg, B. (1982) Ann. Microbiol. 133, 243–249.
[14] Seki, T., Yoshikawa, H., Takahashi, H. and Saito, H. (1987) J. Bacteriol. 169, 2913–1916.
[15] Seki, T., Yoshikawa, H., Takahashi, H. and Saito, H. (1988) J. Bacteriol. 170, 5935–5938.
[16] Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) Molecular Cloning; A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
[17] Sanger, F., Nicklen, S. and Coulson, A.R. (1977) Proc. Natl. Acad. Sci. USA 74, 5463–5467.
[18] Reverchon, S., Huang, Y., Bourson, C. and Robert-Baudouy, J. (1989) Gene 85, 125–134.
[19] Gysler, C., Harmsen, J.A., Kester, H.C., Visser, J. and Heim, J. (1990) Gene 89, 101–108.
[20] Kusters-van Someren, M.A., Harmsen, J.A., Kester, H.C. and Visser, J. (1991) Curr. Genet. 20, 293–299.
[21] Kusters-van Someren, M.A., Flipphi, M., de Graaff, L., van den Broeck, H., Kester, H.C., Hinnen, A. and Visser, J. (1992) Mol. Gen. Genet. 234, 113–120.
[22] Chatterjee, A., McEvoy, J.L., Chambost, J.P., Blasco, F. and Chatterjee, A.K. (1991) J. Bacteriol. 173, 1765–1769.
[23] Ohnishi, H., Nishida, T., Yoshida, A., Kamio, Y. and Izaki, K. (1991) Biochem. Biophys. Res. Commun. 176, 321–327.
[24] Templeton, M.D., Sharrock, K.R., Bowen, J.K., Crowhurst, R.N. and Rikkerink, E.H. (1994) Gene 142, 141–146.
[25] Nikaidou, N., Kamio, Y. and Izaki, K. (1992) Biochem. Biophys. Res. Commun. 182, 14–19.
[26] Ito, K., Kobayashi, R., Nikaido, N. and Izaki, K. (1988) Agric. Biol. Chem. 52, 479–487.
[27] Keen, N.T. and Tamaki, S. (1986) J. Bacteriol. 168, 595–606.
[28] Tamaki, S., Gold, S., Robeson, M., Manulis, S. and Keen, N.T. (1988) J. Bacteriol. 170, 3468–3478.
[29] Gijsegem, F.V. (1989) Mol. Microbiol. 3, 1415–1424.
[30] Nikaidou, N., Kamio, Y. and Izaki, K. (1993) Biosci. Biotech. Biochem. 57, 957–960.
[31] Nasser, W., Awadé, A.C., Reverchon, S. and Robert-Baudouy, J. (1993) FEBS Lett. 335, 319–326.
[32] Sakai, T. and Sakamoto, T. (1990) Agric. Biol. Chem. 54, 879–889.
[33] Sakamoto, T., Yoshinaga, J., Shogaki, T. and Sakai, T. (1993) Biosci. Biotech. Biochem. 57, 1832–1837.
[34] Klier, A., Msadek, T. and Rapoport, G. (1992) Annu. Rev. Microbiol. 46, 429–459.