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Title

Heterologous expression of a lectin from *Pleurocybella porrigens* (PPL) in *Phanerochaete sordida* YK-624

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Abstract

Pleurocybella porrigens is a mushroom-forming fungus, which had been consumed as a traditional food in Japan. However, in 2004, 55 people got poisoned by eating the mushroom and 17 people among them died of acute encephalopathy. We have already reported the purification, characterization, and cDNA cloning of a lectin from the mushroom (PPL) which might have caused the poisoning. Here, we report the heterologous expression of recombinant PPL by basidiomycete *Phanerochaete sordida* YK-624.

The glyceraldehyde 3-phosphate dehydrogenase gene promoter was used to drive the expression of the PPL gene (*ppl*) in *P. sordida* YK-624. Furthermore, the signal peptide of lignin peroxidase which is an extracellular protein was used to secrete rPPL into extracellular region. Seven regenerated clones were cultured on kirk HNHC broth, and the presence of lectin activity in the culture broth was checked by agglutination assays. The results indicated that the culture broth of rPPL-6 clone showed the strongest hemagglutination activity, and it was therefore used for subsequent analysis. The heterologous expression of rPPL by *P. sordida* YK-624 was confirmed by SDS-PAGE, lectin activity by the hemagglutination assay, and mass of rPPL by MALDI-TOF respectively, indicating that the extracellular secretion of rPPL as active form was successful.

Highlights:

The heterologous expression of a lectin by basidiomycetes, *P. sordida* YK-624.

The glyceraldehyde 3-phosphate dehydrogenase gene promoter was used to drive the expression of the PPL gene.

The signal peptide of lignin peroxidase was used to secrete rPPL into extracellular region.

Keywords: *Pleurocybella porrigens*, *Phanerochaete sordida* YK-624, heterologous expression, lectin.

1. Introduction

A basidiomycete *Pleurocybella porrigens* (division : Basidiomycota, order : Agaricales) is a mushroom-forming fungus, which had been consumed as a traditional food in Japan. In 2004, however, 55 people got poisoned by eating the mushroom and 17 people among them died of acute encephalopathy. Since then, the Japanese government has been alerting Japanese people to take precautions against eating the mushroom. Ever since the food-poisoning incident, we have been trying to elucidate the molecular mechanism for the acute encephalopathy and have reported the isolation and characterization of a lectin and unusual amino acids from the mushroom, which might have been related to the accident (Kawaguchi et al., 2009, Suzuki et al., 2009, Wakimoto et al., 2010). There are also some papers concerning the mushroom reported by other researchers (Hasegawa et al., 2007, Sasaki et al., 2006, Takata et al., 2009). However, the molecular mechanism for the acute encephalopathy still remains unsolved. Thus, to elucidate the molecular mechanism for poisoning, a recombinant expression system of *ppl* gene is needed as it is possible that native *Pleurocybella porrigens* lectin (PPL) from the mushroom also contains trace amounts of unknown toxins too.

Lectins are proteins (or glycoproteins) unlike antibodies and enzymes they bind specifically and reversibly to carbohydrates, resulting in cell agglutination or precipitation of glycoconjugates (Goldstein et al., 1980). Mushroom lectins have attracted wide attention due to their biological activities (Horibe et al., 2010, Kawagishi, 1995, Kobayashi et al., 2004, Kobayashi et al., 2004, Kobayashi et al., 2005, Kobayashi et al., 2010, Suzuki et al., 2012). Some mushroom lectins have been expressed at very high yields in *Escherichia coli* (Lam and Ng, 2011, Shimokawa et al., 2012, Tateno and Goldstein, 2003, Tateno et al., 2004) with yields ranging from 1 to 4 mg/l. Furthermore, yeast expression systems such as *Saccharomyces cerevisiae* and *Pichia pastoris* have been used for recombinant lectins production (Singh et al., 2010, Walser et al., 2004) with yields ranging from 1 to 190 mg/l. We previously tried heterologous expression of rPPL in conventional *E. coli* and yeast *Kluyveromyces lactis* expression system but the expressed protein did not show lectin activity. Thus, an alternative expression systems using basidiomycetes might be able to overcome the problem was tried.

Recently, gene transformation systems for several species of white-rot basidiomycetous fungi have been developed for the overproduction of ligninolytic enzymes and facilitating structure-function studies of these enzymes by site-directed mutagenesis (Mayfield et al., 1994, Tsukamoto et al., 2003, Tsukihara et al., 2006). We have previously constructed a gene transformation system for *Phanerochaete sordida* YK-624 using glyceraldehyde-3-phosphate dehydrogenase gene (*gpd*) promoter for heterologous expression of enhanced green fluorescent protein (EGFP) and homologous expression of recombinant lignin peroxidase (LiP) (Sugiura et al., 2009). Furthermore, a novel gene (*bee2*) promoter was used to drive the expression of the manganese peroxidase gene (*mnp4*) in *P. sordida* YK-624 under ligninolytic condition (Sugiura et al., 2012).

The *bee2* promoter demonstrated to be a useful regulator for high expression of genes under ligninolytic condition. Thus, gene transformation systems of white-rot fungi are effective in protein expression. However, there are not many reports on heterologous expression of various genes in white-rot fungi.

In the present study, we tried heterologous expression of recombinant PPL (rPPL) by the basidiomycete *P. sordida* YK-624. The *gpd* promoter was used to drive the expression of the *ppl* in *P. sordida* YK-624. Furthermore, the signal peptide of lignin peroxidase (SPL) was used to secrete rPPL at extracellular region. Moreover, hemagglutination activity of rPPL was examined in detail.

2. Materials and Methods

2.1. Strains

P. sordida YK-624 (ATCC 90872) and uracil auxotrophic strain UV-64 were used in this study. The fungus strain YK-624, was isolated from a decayed wood and identified as *P. sordida* (Hirai et al., 1994). The uracil auxotrophic strain UV-64 derived from *P. sordida* YK-624 were generated using a combination of ultraviolet radiation and 5- fluoroorotate resistance (Yamagishi et al., 2007).

2.2. Materials and reagents

All sugars and glycoproteins for hemagglutination inhibition tests were from Nacalai Tesque (Tokyo, Japan), Wako Pure Chemicals (Osaka, Japan), Calbiochem (La Jolla, CA, USA), or Sigma (St. Louis, MO, USA). Sepharose 4B was from GE Healthcare (Uppsala, Sweden). Acid treated Sepharose 4B was prepared by the method of Errson et al.(Ersson et al., 1973)

2.3. Erythrocytes

Rabbit erythrocytes were prepared as described previously (Kawagishi et al., 1994, Kawagishi et al., 2001, Kawagishi et al., 2001, Suzuki et al 2009). Briefly, rabbit erythrocytes were washed three times with 10 mM phosphate buffered saline, pH7.4 (PBS). A 10% suspension of the erythrocytes in PBS (10.0 ml) was treated with Pronase E (4.0 mg) for 10 min at 45°C and then the erythrocytes were washed three times with PBS and suspended at a concentration of 4% in PBS.

2.4.Construction of PPL gene expression vector, cotransformation of UV-64, and screening of regenerated clones

rPPL was expressed using *gpd* promoter since *gpd* is expressed strongly and stably in many species and the promoter has been used for endogenous and exogenous gene expression experiments on many kinds of white-rot basidiomycetes (Alves et al., 2004, Hirano et al., 2000, Irie et al., 2001). Lignin peroxidase (LiP) is known as an extracellular heme protein peroxidases, and we have previously constructed a gene transformation system for *P. sordida* YK-624 using *gpd* promoter for

homologous expression of recombinant LiP (rLiP) (Sugiura et al., 2009). Since these results of homologous expression of rLiP have indicated that the extracellular secretion of this protein was successful, we chose the SPL to secrete rPPL in extracellular region.

The procedure for constructing the PPL gene expression plasmid, p*GPDpro-ppl*, is shown in Fig. 1. The plasmid p*PsGPD-EGFP*, which was generated in our previous study (Yamagishi et al., 2007), was used for the construction of the *ppl*-expression plasmid. The *ppl* expression vector was constructed by a series of PCR procedures using the primers listed in Table 1. Primers gpdproF1 and gpdproR1 were designed to amplify the full-length sequence of the *gpd* promoter to introduce a multi-cloning site (*Kpn* I-*Afl* II-*Bgl* II-*Xba* I) in the 3'-terminal sequence and a *Spe* I site in the 5'-terminal sequence of the *gpd* promoter, respectively. These primers were used to amplify the *gpd* promoter using TaKaRa Ex Taq (TaKaRa, Kyoto, Japan) (step 1). Primers gpdterF1 and gpdterR1 were designed to amplify the region including the *Nsi* I site in the *PsGPD* terminator and to add a multi-cloning site (*Kpn* I-*Afl* II-*Bgl* II-*Xba* I) in the 5'-terminal sequence of the *PsGPD* terminator. These primers were used to amplify the *PsGPD* terminator fragment (step 2). A mixture of the step 1 and 2 PCR products was used as templates in a third PCR using the primers gpdproF1 and gpdterR1 (step 3). The amplified DNA fragment and p*PsGPD-EGFP* were digested with *Spe* I and *Nsi* I (New England Biolabs, Beverly, MA, USA) (step 4), and the digested DNA fragment was inserted into the *Spe* I and *Nsi* I sites of p*PsGPD-EGFP*, yielding plasmid p*GPDpro* (step 5). p*GPDpro* was sequenced to verify the absence of PCR errors.

The *ppl* sequence (GenBank Accession AB449246) was codon-optimized for *Phanerochaete chrysosporium* (Fig. 2) and synthesized to add the genomic DNA fragment encoding the SPL in the 5'-terminal sequence of the *ppl* gene by GenScript (Piscataway, NJ, USA). Primers ylpAF1and pplR1 were designed to amplify the *SPL* and *ppl* gene, and to introduce *Kpn* I site just before the first methionine codon and to add an *Bgl* II site just after the stop codon, respectively. (step 6). The amplified DNA fragment was digested with *Kpn* I and *Bgl* II (New England Biolabs) and cloned into *Kpn* I-*Bgl* II digested p*GPDpro* (step 7), yielding plasmid p*GPDpro-ppl* (step 8). *pGPDpro-ppl* was sequenced to verify the absence of PCR errors.

UV-64 protoplasts were prepared and then transformed with p*PsURA5* and p*GPDpro-ppl* using standard techniques (Yamagishi et al., 2007). The co-transformed clones were selected by PCR, as described previously, with the following modifications: primers ylpAF1 and pplR1 were designed to amplify the *ppl* gene.

2.5. Screening based on hemagglutination activity

The screening procedure that we have developed is designed to conveniently detect the secreted rPPL as active form. The 15 transformants (rPPL-1 to 15) were grown on PDA plates (*d* = 9 cm) for 5 days at 30°C. Two disks (diameter 8 mm) punched from the growing edge of the mycelia were added to a 300 ml Erlenmeyer flask containing 100 ml of Kirk medium (1% glucose, 12 mM ammonium tartrate, (Tien et al., 1988). The culture was statically incubated at 30°C for 7

days. Each extracellular fluid was separated from the mycelia by filtrations with glass fiber filter paper GA-100 (Advantec, Tokyo, Japan) and membrane filters (pore size = 1, 0.45, and 0.2 μm; Advantec). The filtered cultures were concentrated by ultrafiltration (1 kDa; Advantec), and then lyophilized. The lyophilized sample was used for the hemagglutination assay and further purification of rPPL.

2.6. Purification of rPPL

In the present study, acid-treated Sepharose 4B was selected as the affinity support, as native PPL is inhibited by galactose and lactose, hence acid-treated Sepharose 4B was used in purification of native PPL. Purification of rPPL was basically carried out according to the method described in our report (Suzuki et al. 2009). The concentrated culture fluid described above was percolated slowly through an acid-treated Sepharose 4B column (5 × 50 cm) equilibrated with PBS, and the adsorbed lectin was eluted with 0.2 M lactose. The eluate was further applied to a PROTEIN KW-2002.5 (2.0 × 30 cm) equilibrated with PBS. The column was washed with the same buffer, and the eluant was dialyzed extensively against distilled water, ultrafiltered, and lyophilized.

2.7. Molecular mass of rPPL.

To confirm whether rPPL was purified homogeneity or not, the molecular mass was analyzed by SDS-PAGE and MALDI-TOF mass spectroscopy. Samples were heated in the presence or the absence of 2-mercaptoethanol for 10 min at 100°C and SDS-PAGE (12% polyacrylamide gel) was done by the Laemmli method (Laemmli, 1970), with Coomassie Brilliant Blue staining. The molecular masses on SDS-PAGE were estimated using recombinant ladder markers (10 to 100 kDa; XL-Ladder Low; APRO Life Science Institute, Tokushima, Japan). The MALDI-TOF mass spectrum was acquired on an AutoFlex (Bruker Daltonics, Billerica, MA, USA). Each sample was dissolved in 0.1% trifluoroacetic acid (TFA):acetonitrile (2:1 v/v) and mixed with the matrix solution (1:4 v/v). The mixture (1 μl) was put on a stainless steel target and crystallized at room temperature. The spectrum was measured in linear mode using 20-kV ion acceleration without post-acceleration. The spectrum was recorded at a detector voltage of 1.65 kV, and was expressed as the averaged results of at least 300 laser shots. A mass calibration procedure was employed prior to the analysis of a sample using protein calibration standards I (Bruker Daltonics).

2.8. *N*-terminal sequence analysis.

N-Terminal sequence analysis of rPPL was performed to confirm the sequence with that of native PPL. The *N*-terminal sequence of rPPL was determined by Edman degradation using a Shimadzu PPSQ-21A automated protein sequencer (Shimadzu, Kyoto, Japan) coupled to reversed phase separation of PTH-amino acids on a WAKOSIL-PTH (4·6 × 250 mm) column (Wako) at 1 ml/min with detection at 235 nm.

2.9. Hemagglutination and inhibition assay.

Due to conformational differences between native PPL and rPPL, it is possible that rPPL shows different sugar-binding specificity. Therefore, hemagglutination inhibition assay was done to examine the sugar-binding specificity of rPPL. The hemagglutination activity of rPPL was measured by a 2-fold serial dilution procedure using rabbit erythrocytes. The hemagglutination titer was defined as the reciprocal of the highest dilution exhibiting hemagglutination. Inhibition was expressed as the minimum concentration of each sugar or glycoprotein required for inhibition of hemagglutination of titer 4 of the lectin.

3. Results

3.1. Construction of a *ppl*-expression transformant and screening of rPPL-producing fungi by hemagglutination activity

The codon of *ppl* sequence (GenBank Accession AB449246) was optimized for *P. chrysosporium* (Fig. 2) and synthesized to add the genomic DNA fragment encoding SPL in the 5'-terminal sequence of the *ppl*. A plasmid for the expression of *ppl* was constructed from p*PsGPD-EGFP* (Yamagishi et al., 2007) by inserting the genomic DNA of the SPL and the cDNA of *ppl* in frame with the *gpd* promoter and *gpd* terminator (Fig. 3A). The expression plasmid, p*GPDpro-ppl*, was introduced into UV-64 using p*PsURA5* as the marker plasmid. The presence of the SPL–PPL fusion gene in each uracil prototrophic clone was confirmed by PCR using genomic DNA as the template (Fig. 3B). Fifteen regenerated clones were cultured in Erlenmeyer flasks containing Kirk culture for 7 days, and the culture liquid from each flask was used to confirm the hemagglutination activity. Most of the transformants displayed hemagglutination activity, and rPPL-6 (16 titer/mg protein) showed the strongest lectin activity among them (Fig. 3C), and it was therefore used for subsequent analysis.

3.2. Purification and molecular properties of rPPL

Acid-treated Sepharose 4B was chosen as the affinity support since the hemagglutination activity of native PPL was inhibited by galactose and lactose. Initially, the filtered cultures were applied to acid-treated Sepharose 4B. Almost all of the lectin activity of the culture fluid was adsorbed to the affinity column and eluted with 0.2 M lactose. The eluate was further purified by PROTEIN KW-2002.5 gel filtration chromatography, and a pure rPPL was obtained (Fig. 4A). In this protocol, the yield of rPPL is approximately 0.2 mg/liter culture. After purification procedure, the hemagglutination activity of rPPL was 128 titer/mg protein.

rPPL gave a single band with an apparent mass of 14 kDa on SDS-PAGE (Fig. 4A). The MALDI-TOF mass spectrum gave a main peak at *m/z* 14244 (Fig. 4B). Furthermore, *N*-terminal

amino acid sequence analysis of rPPL gave a sequence of 14 amino acids (SIPAGTYLIRNVES), which is same as that of native PPL.

3.3. Properties of rPPL as a lectin

Table 2 shows the inhibition of hemagglutination activity of rPPL by various monosaccharides, oligosaccharides, and glycoproteins. The sugar-binding specificity of rPPL was almost the same as that of native PPL. Among the monosaccharides tested, the most potent inhibitor was GalNAc; its MIC was 0.78 mM. Lactose, lacturose, galactose, and methyl β-galactoside were also inhibitory at higher concentrations, 12.5, 25, 25, and 25 mM, respectively, than GalNAc. Asialo-BSM exhibited the strongest inhibitory activity among the glycoproteins used, and PSM and BSM also inhibited agglutination at higher concentrations than asialo-BSM.

4. DISCUSSION

In the present study, we succeeded in heterologous expression of rPPL showing lectin activity by the basidiomycete *P. sordida* YK-624. Yield of the rPPL is approximately 0.2 mg/liter culture. The heterologous expression of rPPL using other expression systems such as *E. coli* and a yeast *K. lactis* were not successful due to difficulty in protein folding or post-translational modifications. In contrast, the current basidiomycete *P. sordida* YK-624 expression system was appropriate for proper expression of rPPL.

Many gene transformation systems in white-rot basidiomycetes including *P. chrysosporium*, *Schizophyllum commune*, *Trametes versicolor*, *T. hirsuta*, *Pleurotus ostreatus*, *Lentinula edodes*, and *Pycnoporus cinnabarinus* have been reported (Akileswaran et al., 1993, Alic et al., 1989, Alves et al., 2004, Bartholomew et al., 2001, Kim et al., 1999, Munoz-Rivas et al., 1986, Sato et al., 1998, Tsukamoto et al., 2003, Yanai et al., 1996). As easier method for the purification of recombinant protein, using a SPL to secrete rPPL in extracellular region provides an efficient method.

The results of the SDS-PAGE, MALDI-TOF mass and *N*-terminal amino acid sequence analysis of rPPL indicated that the molecular properties of rPPL were completely identical to those of native PPL. The results of the inhibition of hemagglutination activity of rPPL by various monosaccharides, oligosaccharides, and glycoproteins suggested that sugar-binding specificity of rPPL was similar to that of native PPL. The hemagglutination activity of rPPL (128 titer/mg protein) indicated that the production of active recombinant lectin was successful.

To our knowledge, this is the first report of the heterologous expression of a lectin by another basidiomycete. In addition, the utilization of SPL region successfully demonstrated the secretion of rPPL to extracellular region. Thus, this gene transformation system is more efficient, especially for the difficult-to-express fungal proteins. Because we used the *P. sordida* expression system, rPPL was never contaminated with other toxins derived from *P. porrigens*. We are now trying to elucidate the molecular mechanism for this acute encephalopathy using this rPPL.

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1 **Table 1** Oligonucleotides used as primers in this study.

Primer name	Sequence (5'-3')
gpdproF1	GGACTAGTCTTGCTCCCAGAGTTATTCAGATTC
gpdproR1	TCTAGAAGATCTCTTAAGGGTACCTAGAGGATGGATGTGAGGGG
gpdterF1	GGTACCCTTAAGAGATCTTCTAGAGCGTCGCGTCACACCCATTC
gpdterR1	CTACCTCCGAGCTCGCATTC
ylpAF1	GGTACCATGGCCTTCAAGCAG
pplR1	AGATCTTCAGACCGCCTCGAAG

2

1 **Table 2** Inhibition of rPPL-mediated hemagglutination by mono- and oligo-saccharides and
2 glycoproteins
3

Inhibitor[a]	MIC[b]	
	native PPL	rPPL
	(mM)	(mM)
N-Acetylgalactosamine	0.39	0.78
Lactose	12.5	12.5
Lacturose	12.5	25
Galactose	25	50
Methyl β-galactoside	25	25
Methyl α-galactoside	50	50
Lactitol	50	25
Fucose	50	50
	(μg/ml)	(μg/ml)
Asialo-BSM	0.49	0.39
PSM	1.95	1.56
BSM	3.9	3.9

4
5 [a] Glucose, mannose, L-fucose, arabinose, L-arabinose, ribose, glucosamine, galactosamine,
6 mannosamine, raffinose, *N*-acetylglucosamine, *N*-acetylmannosamine, *N*-acetylneuraminlactose,
7 methyl α-glucoside, methyl β-glucoside, methyl β-mannoside, melibiose, chitobiose, chitotriose,
8 chitotetraose, chitopentaose, xylose, galacturonic acid, glucono-1,5-lactone, saccharose, rhamnose,
9 ribulose, gluctosamine hydrochloride, galactosamine, hydrochloride, mannosamine hydrochloride,
10 methyl α-*N*-acetylglucosamine, methyl β-*N*-acetylglucosamine, 2-deoxyribose, 2-deoxyglucose,
11 *N*-acetylchitotriose, phenyl α-*N*-acetylglucosamine, and phenyl β-*N*-acetylglucosamine did not
12 inhibit at all at concentrations up 400 mM. *N*-acetylneuraminic acid and *N*-glycolylneuraminic acid
13 did not inhibit at concentrations up to 40 mM. Lactbionic acid, mannnan, albumin (bovine), albumin
14 (chicken egg), transferrin (human), α_1-acid glycoprotein (human), fetuin, and asialo-fetuin did not
15 inhibit at all at concentrations up to 1 mg/ml.
16 [b] Minimum inhibitory concentrations required for inhibition 4 hemagglutination doses of the lectin.
17
18
19
20
21

Figure Captions

Fig. 1 Procedure used for the construction of the *ppl* expression plasmid p*GPDpro-ppl*. The small horizontal arrows indicate the locations and directions of the primers used for PCR amplification of the target DNA fragments. Genes are indicated by shaded boxed arrows.

Fig. 2 Codon optimization of *P. porrigens* lectin for *P. chrysosporium*.
Codon optimization was performed by GenScript. The top and bottom rows show the original and optimized sequence, respectively. Altered codons are boxed in black. The *sequence* encoding the SPL *is boxed.*

Fig. 3 Cotransformation of UV-64 with the p*GPDpro-ppl* expression plasmid.
(A) Restriction map of the expression plasmid p*GPDpro-ppl*. (B) Detection of *ppl* linked with the *LSP* from 15 regenerated clones cotransformed with p*PsURA5* and p*GPDpro-ppl* by PCR. 100-bp ladder size marker was loaded in lane M. The clone numbers are indicated above the gel lanes. (C) Screening of rPPL-producing fungi by hemagglutination activity. Fifteen regenerated clones were cultured in Erlenmeyer flasks containing Kirk culture for 7 days, and the culture liquid from each flask was used to confirm the hemagglutination activity.

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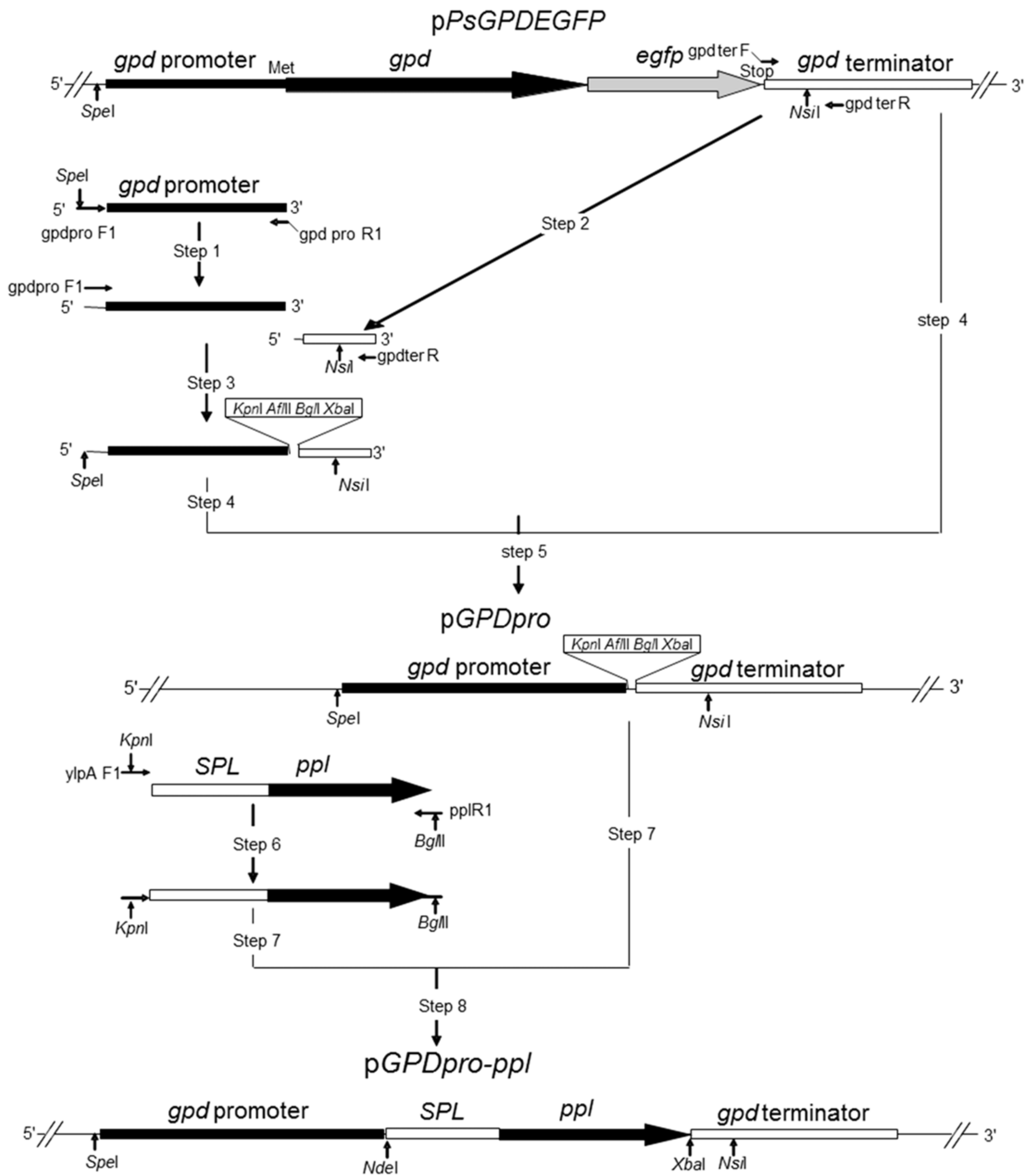
Fig. 4 The molecular structure of rPPL.
(A) SDS-PAGE of rPPL. Lane M, marker proteins; lane 1, rPPL, reduced with 2-mercaptoethanol. (B) MALDI-TOF mass spectrometry of rPPL.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gpd promoter
Met
gpd
egfp
gpd ter F
Stop
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3'
SpeI
NsiI
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SpeI
gpd promoter
gpdpro F1
gpd pro R1
Step 1
Step 2
Step 3
Step 4
step 4
step 5
gpdter R
KpnI AflII BglI XbaI
pGPDpro
gpd promoter
gpd terminator
KpnI
ylpA F1
SPL
ppl
pplR1
BglII
Step 6
Step 7
Step 8
pGPDpro-ppl
NdeI
XbaI

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Original 1      ATGGCCTTCAAGCAGCTCTTCGCAGCGATCTCCGTCGTTCTCTCGCTCTC

Optimized 51    GGCTGTGCAAGGTACATTTCTGCGCTGCCGGCGCACTCTGAACGGTGCTG
Original 51     GGCTGTGCAAGGTACATTTCTGCGCTGCCGGCGCACTCTGAACGGTGCTG

Optimized 101   ACGACGACATTCGCGGCTTGCAGCCGCCGTGGTCAAGGAGAAGCGCTCCA
Original 101    ACGACGACATTCGCGGCTTGCAGCCGCCGTGGTCAAGGAGAAGCGCTCCA

Optimized 151   TCCCTGCCGGGACCTACCTCATCCGCAACGTCGAATCCAACCTCTACCTC
Original 151    TCCCTGCCGGAACCTACCTTATTCGCAACGTGGAGTCTAACCTCTACCTC

Optimized 201   GACCTCCGCGGGTCCAACCCTGCCCCTGGCACCGACGCGATCGTCTGGGG
Original 201    GACCTCAGAGGTTCGAACCCGGCGCCTGGGACCGATGCCATCGTTTGGGG

Optimized 251   CCGCACCGGGAACAACAACCAGCGCTGGATCGTCACCACGCACTCCGACG
Original 251    AAGGACGGGAAACAACAATCAGAGGTGGATTGTCACGACCCATTCAGATG

Optimized 301   GCACCCGCACGCTCGAGACCGTCGGGATCAACTCGTCCGCGTTCATCGCC
Original 301    GCACCCGCACCCTCGAGACAGTCGGCATCAATTCCAGCGCCTTCATCGCG

Optimized 351   ACGATCCAACCTGGCGGGCGCGTCACCGGCCACCCCAACAACGAGACGCG
Original 351    ACCATCCAGCCGGGTGGAGGGTTACAGGCCACCCCAATAACGAAACGAG

Optimized 401   CCTCACCATCACGAACGTCAACCCTGGCGAATACAGCATCTCGGCGGGCG
Original 401    ACTAACGATCACGAATGTTAACCCCGGTGAATACAGTATTTCTGCTGGGG

Optimized 451   GGCTCCTCTGGCTCGCCAACACCCCGGTCGGCGGGACGGGCGAAGCGGTC
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Optimized 501   ACGCTCCAGGCGGCGGGCGGGGCGGCGCAGTCGCTCTGGGTCTTCGAGGC
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Optimized 551   GGTC
Original 551    CGTT
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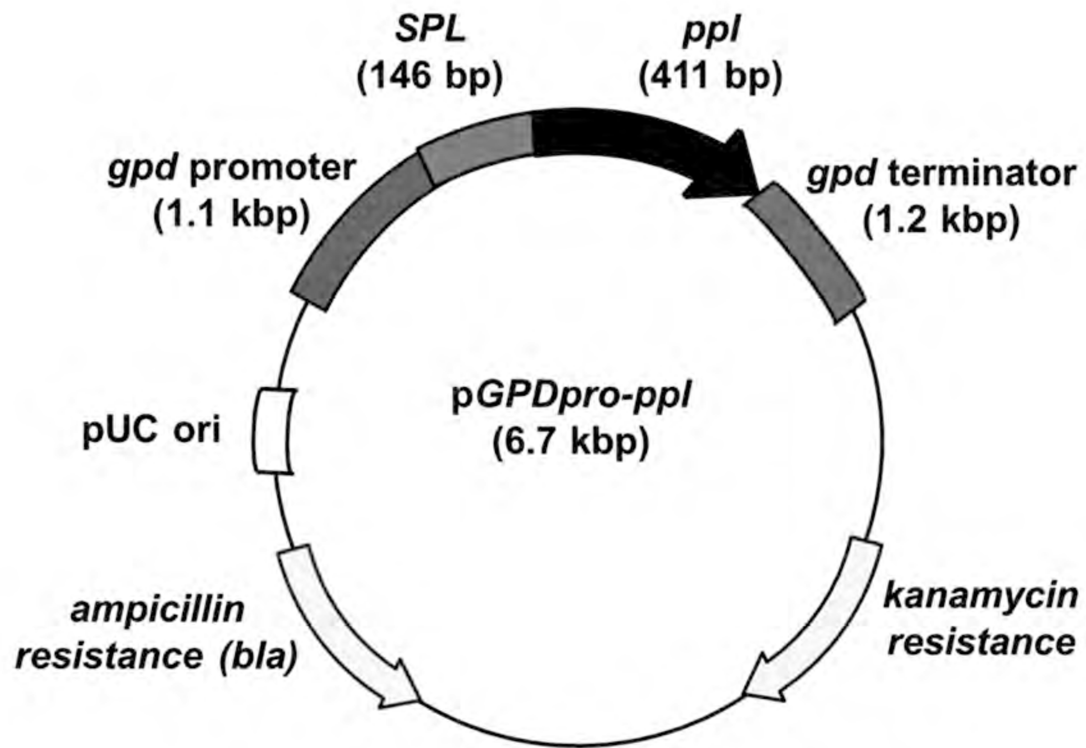
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ppl
(411 bp)
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(1.1 kbp)
gpd terminator
(1.2 kbp)
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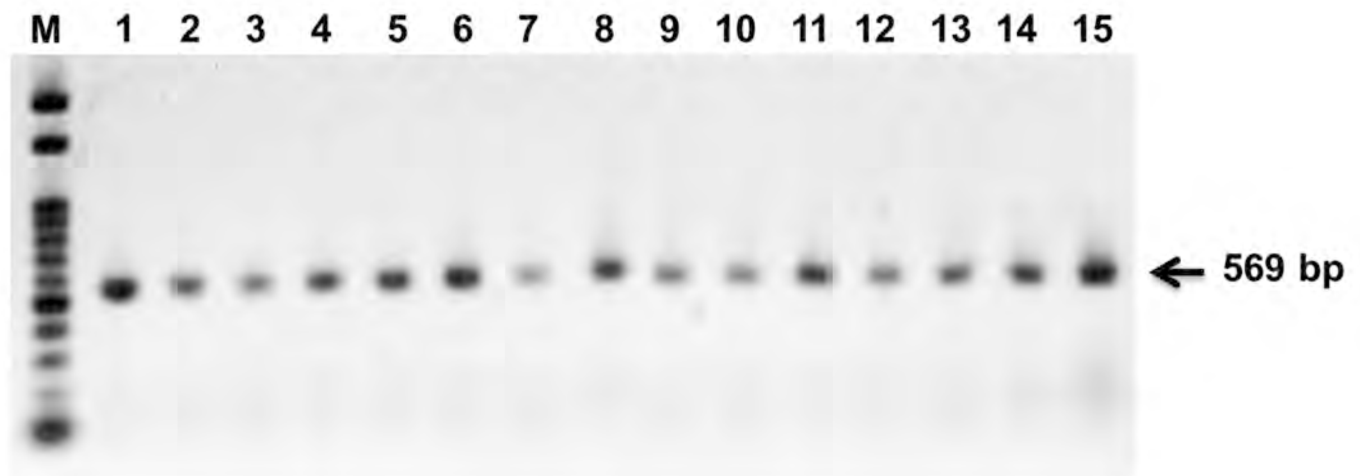
ampicillin
resistance (bla)
kanamycin
resistance


B)
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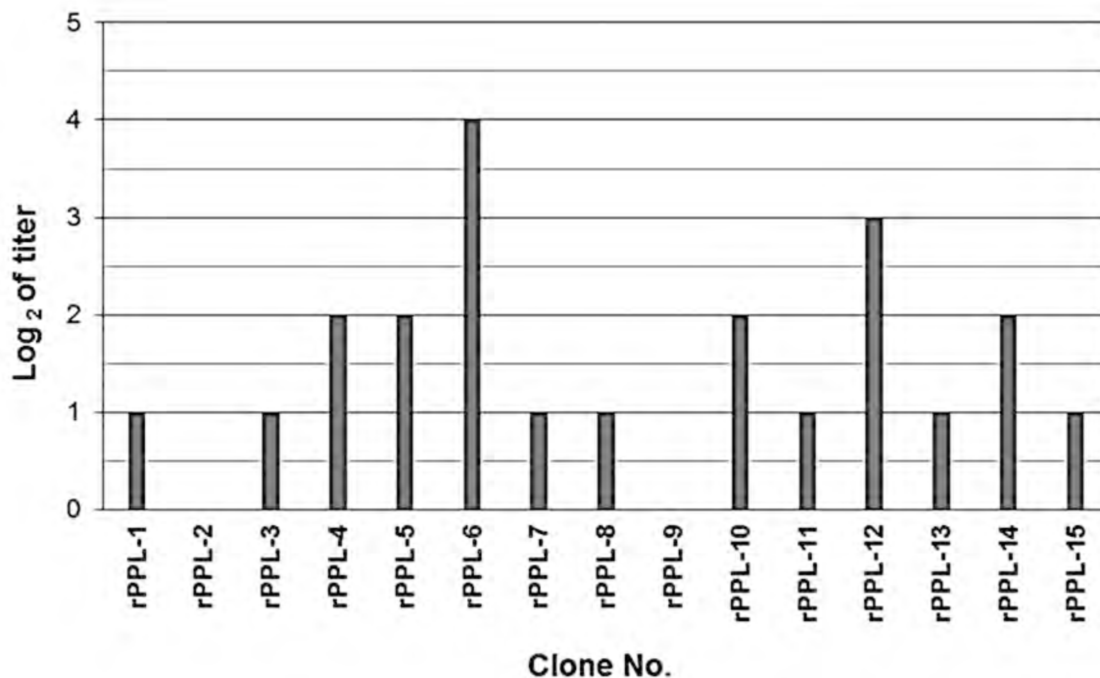
569 bp


C)
Log 2 of titer
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1
2
3
4
5
rPPL-1
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rPPL-3
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rPPL-6
rPPL-7
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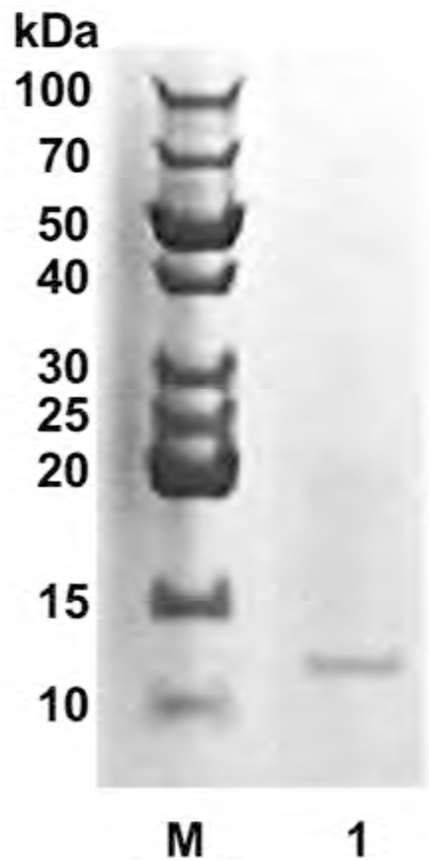
rPPL-15
Clone No.


A)
kDa
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70
50
40
30
25
20
15
10
M
1
B)
Intens.[a.u.]
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1000
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7187
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m/z