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Cloning and Analysis of the β-Lactamase Gene from ε-Poly-L-lysine–Producing Actinomycete *Streptomyces albulus* IFO14147

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***Streptomyces albulus* IFO14147 produces ε-poly-L-lysine, which exhibits antimicrobial activity. It is necessary for its molecular breeding to develop host-vector systems. We recently found a novel cryptic plasmid, pNO33, in this strain. As part of a search for a selectable marker gene for pNO33, we report here the isolation and analysis of the β-lactamase gene of this strain, which can grow on ampicillin-containing plates. It was shown that the β-lactamase production in *S. albulus* was induced by ampicillin. By introducing a genomic library of *S. albulus* into *Escherichia coli*, a 3.6-kbp fragment was identified as the region involved in ampicillin resistance. It contained three open reading frames, all of which are highly homologous to the β-lactamase (the *blaL* product) and its regulatory proteins (the *blaA* and *blaB* products) of *S. cacaoi*. The growth phenotypes and enzyme assaying of *E. coli* and *S. lividans* showed that the *blaL* homologue (*blaSa*) encodes a β-lactamase required for ampicillin resistance. The β-lactamse gene can be utilized as a selectable marker in a cloning vector of *S. albulus*. However, the β-lactamase activity was decreased in *E. coli* and repressed in *S. lividans* by the *blaA* and *blaB* homologues (*blaASa* and *blaBSa*). It appears as if the *blaASa* product is a repressor of *blaSa* instead of an activator as in *S. cacaoi*.**

Key words: ampicillin resistance, *bla* gene, β-lactamase, ε-poly-L-lysine, selectable marker, *Streptomyces albulus*.

Abbreviations: ε-PL, ε-poly-L-lysine; kbp, kilobase pair; ORF, open reading frame.

ε-Poly-L-lysine (ε-PL) is a microbiostatic L-lysine homopolymer that consists of 25–30 residues, with a linkage between the α-carboxyl group and ε-amino group, produced by the actinomycete *Streptomyces albulus* IFO14147 (*1*, *2*). ε-PL exhibits antimicrobial activity toward a wide spectrum of microbes including Gram-positive and -negative bacteria (*3*), as well as antiphage activity (*4*). Due to its safety and biodegradability, ε-PL has already entered the commercial market as a food preservative in Japan. The breeding of ε-PL-producing strains has been carried out by means of conventional mutation and selection methods. For instance, to improve the productivity of ε-PL, L-lysine analogue *S*-(2-aminoethyl)-L-cysteine plus glycine-resistant mutants were isolated from the parent strain after nitrosoguanidine treatment (*5*). It is necessary for the molecular breeding of ε-PL-overproducing strains to develop host-vector systems for *S. albulus*. We recently found a novel plasmid, pNO33, in *S. albulus* IFO14147 (*6*). This high-molecular-size plasmid (37-kb) is a cryptic plasmid, as none of its functions yet known, but it has characteristics desirable in a vector plasmid, such as a moderate number of copies and several unique restriction sites. It would be suitable for use as a cloning vector provided that a selectable marker gene resistant to antibiotics is introduced into it.

As a result of our search for a suitable marker, we found that *S. albulus* IFO14147 can grow on agar plates containing ampicillin, one of the β-lactam antibiotics. Many *Streptomyces* species, such as *S. badius*, *S. fradiae*, *S. cacaoi*, *S. clavuligerus*, and *S. lavendulae* (*7*, *8*), are reported to produce β-lactamase, which effectively hydrolyzes ampicillin. In some bacteria such as *Bacillus* and *Staphylococcus*, this production is regulated at the level of gene expression (*9*). Regulation of the β-lactamase gene (*bla*) has also been reported in *S. cacaoi* (*10*). It was shown that the *S. cacaoi* β-lactamase activity is induced by β-lactam compounds, and that the two β-lactamase genes (*blaL* and *blaU*) are under the control of the same regulatory system (*11*).

We report here the isolation and analysis of the β-lactamase gene of ε-PL-producing *S. albulus* IFO14147. Our results show that the β-lactamase production is induced by ampicillin and that the *blaL* homologue gene we isolated encodes a β-lactamase required for ampicillin resistance. The β-lactamase gene can be utilized as a selectable marker in a cloning vector of *S. albulus* IFO14147. In addition, the possible roles of the putative regulatory genes found in this strain in β-lactamase expression will be discussed.

MATERIALS AND METHODS

Strains and Plasmids—*S. albulus* IFO14147 was used as the DNA source for cloning of the *bla* gene. *S. lividans* strain TK21 (SLP1$^-$ SLP2$^-$) and plasmid pIJ702 (5.8 kbp) (supplied by D.A. Hopwood), carrying the thiostrepton resistance gene, were used to express the *bla* gene in *S. lividans*. *Escherichia coli* strain JM109 [*recA1* Δ(*lac*-

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proAB) *endA1 gyrA96 thi-1 hsdR17 relA1 supE44/*(*F' traD36 proAB*$^{+}$ *lacI*^{q} *ZΔM15*)] and plasmid pKF3 (2.2 kbp) (Takara Shuzo), carrying the chrolamphenicol resistance gene, were used for cloning and expression of the *bla* gene in *E. coli*.

Culture Media—*S. albulus* IFO14147 was grown in Luria-Bertani (LB) (*12*) or ISP.2 (1% malt extract, 0.4% yeast extract and 0.4% glucose). The *E. coli* and *S. lividans* recombinant cells were grown in LB containing chloramphenicol (100 μg/ml) (Wako Pure Chemicals) and ISP.2 containing thiostrepton (50 μg/ml) (Sigma), respectively. To examine the induction of the *bla* gene, ampicillin (Meiji Seika) was added to each medium. If necessary, 2% agar was added to solidify the medium.

DNA Manipulation and Transformation—Genomic DNA from *S. albulus* was isolated as described by Takagi *et al.* (*6*). Protoplast preparation and transformation of *S. lividans* TK21 were carried out by the methods of Hopwood *et al.* (*13*). Protoplasts were prepared from cultures grown in YEME plus 0.5% glycine (*13*). Protoplast cells transformed with recombinant plasmids were placed on R2YE agar plates and then incubated for 18 h at 30°C before overlaying with 2.5 ml soft nutrient agar containing thiostrepton (500 μg/ml) (*13*). The thiostrepton-resistant colonies were selected after a further 3 d incubation. Other conventional DNA techniques were used as described previously (*12*). The enzymes used for DNA manipulations were obtained from Takara Shuzo and were used under the conditions recommended by the supplier.

Cloning of the β-Lactamase Gene—Genomic DNA was prepared from *S. albulus* IFO14147 and then partially digested with *Sau*3AI. The *Sau*3AI fragments larger than 5-kbp were ligated into the unique *Bam*HI site of the pKF3 vector. *E. coli* JM109 competent cells were transformed with the ligated DNA, and over 10,000 independent chloramphenicol-resistant colonies were then replica-plated onto LB agar plates containing ampicillin (50 μg/ml). Two ampicillin-resistant colonies were isolated, the ampicillin resistance being of the plasmid-dependent phenotype. Two plasmids, pKFbla2.0 and pKFbla3.6, had 2.0-kbp and 3.6-kbp fragments, respectively, which overlap 2.0-kbp inserts, as judged on DNA sequence analysis. The nucleotide sequence of the cloned DNA fragment was confirmed with a model 377 DNA sequencer (PE Biosystems) by means of the dideoxy chain termination method.

Construction of Expression Plasmids in S. lividans—For expression of the β-lactamase gene of *S. albulus* IFO14140 in *S. lividans*, plasmids pKFbla2.0 (4.2 kbp) and pKFbla3.6 (5.8 kbp) were linearized with *Bgl*II, and then ligated with *Bgl*II-digested pIJ702 to construct pIJbla2.0 (10.0 kbp) and pIJbla3.6 (11.6 kbp), respectively.

Assaying of β-Lactamase Activity—β-Lactamase activity was measured using the chromogenic substrate nitrocefin (supplied by Y. Ishii) (*14*). For the actinomycete *albulus* and *S. lividans* strains, the culture supernatants were used as enzyme sources. The cell-free extracts of *E. coli* strains were prepared by sonic oscillation under cooling, and the supernatants obtained on centrifugation at 10,000 ×*g* for 20 min were used as enzyme sources. The initial rate of the increase in absorbance at 482 nm of the reaction mixture (final volume, 1 ml) comprising 50 mM phosphate buffer (pH 7.0), 0.1 mM nitrocefin, and the enzyme solution was measured at 30°C, and then that obtained for a solution containing all the materials except nitrocefin (blank) was subtracted. One unit of activity was defined as the amount of enzyme required to hydrolyze 1 μmol of nitrocefin/min at 30°C using an extinction coefficient for nitrocefin of 15,000 M^{-1} cm^{-1}. Protein concentrations were determined with a Bio-Rad protein assay kit. Bovine serum albumin was used as the standard protein.

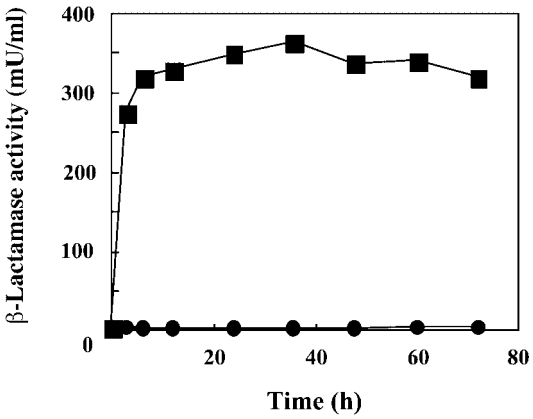

Fig. 1. **Time course of β-lactamase production in *S. albulus* IFO14147.** *S. albulus* IFO14147 was cultivated in LB medium. After 12 h of growth, ampicillin (100 μg/ml final) was added to one of the tubes (solid squares) and no ampicillin was added to the other tube (solid circles). The β-lactamase activities were assayed in the culture medium at the times indicated. The data shown are the means for four independent experiments. The variations in the values were below 10%.

Data Deposition—The GenBank™ accession numbers for the cloned 3.6-kbp DNA fragment including the *bla* gene of *S. albulus* IFO14147, *S. cacaoi blaL*, and its regulatory genes (*blaA* and *blaB*) are AB112742, D90201, and X63780, respectively.

RESULTS

β-Lactamase Production in S. albulus IFO14147 Is Induced by Ampicillin—*S. albulus* IFO14147 showed greater ampicillin resistance than *S. lividans* TK24, which is a β-lactamase–negative strain (data not shown). To examine the mechanism of ampicillin resistance, *S. albulus* IFO14147 was cultivated in LB medium in the presence or absence of ampicillin. Fig. 1 shows the course of β-lactamase production in the culture medium. The activity increased rapidly after the addition of 100 μg/ml ampicillin and reached the maximum level at 36 h after addition. However, no detectable activity was observed in the absence of amipicillin, presumably due to repression of enzyme synthesis. These results indicate that *S. albulus* IFO14147 produces an extracellular β-lactamase and that the production is induced by ampicillin, in a manner similar to its production by *S. cacaoi* (*10*, *15*).

S. alublus IFO14147 Has a β-Lactamase Gene That Is Homologous to That of S. cacaoi—As described under "MATERIALS AND METHODS," a genomic library of *S. albulus* IFO14147 was constructed in a high-copy-number

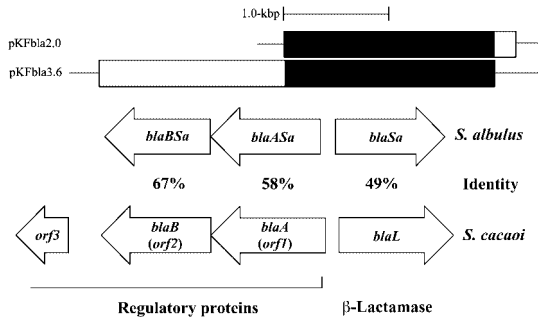

Fig. 2. **Schematic organization of the cloned 3.6-kbp DNA fragment in pKFbla2.0 and pKFbla3.6.** The two plasmids had an overlapping 2.0-kbp *Sau*3AI insert (black box). Three ORFs were revealed on DNA sequencing of the fragment. The predicted sizes and transcriptional orientations of the ORFs (the *blaSa*, *blaASa*, and *blaBSa* products) are indicated by open arrows. Amino acid identity analysis of each ORF was performed with the *S. cacaoi* β-lactamase (*blaL* product) and regulatory proteins (*blaA* and *blaB* products).

plasmid, pKF3. From two dependent ampicillin-resistant transformants, two plasmids (pKFbla2.0 and pKFbla3.6) were isolated and discovered to have an overlapping 2.0-kbp fragment insert (Fig. 2). Computer-assisted frame analysis (*16*) of the sequenced region revealed three possible open reading frames (ORFs) (positions 957 to 55, 1859 to 954, and 2001 to 2924) encoding 300, 301, and 307 amino acids, respectively. The DNA fragment had a G+C content of 66.8%, which is almost the same as that of the total DNA (69 to 78 mol%) isolated from streptomycetes (*17*).

On comparison of the amino acid sequence of each ORF to entries in protein databases (SwissProt, PIR, and PRF), the protein sequences within the overall region were found to be clearly homologous to those of the β-lactamase and regulatory proteins of *S. cacaoi* (*18*, *19*). The sequences of the three ORFs, encoding 300, 301, and 307 amino acids, exhibited 67%, 58%, and 49% identity to the *blaB*, *blaA*, and *blaL* gene products of *S. cacaoi*, respectively. *S. cacaoi* possesses two different unlinked β-lactamase genes: *blaL* and *blaU*, the products of which are 49% identical (*20*). Two adjacent genes, *blaA* and *blaB*, located just upstream of *blaL*, were required not only for induction but also for basal expression of *blaL* (*15*). Therefore, the three putative genes isolated from *S. albulus* IFO14147 were designated as *blaBSa*, *blaASa*, and *blaSa*, respectively (Fig. 2). As shown in Fig. 1, the β-lactamase of *S. albulus* IFO14147 is excreted into the growth medium. It is unlikely that cell lysis is caused by the production of active β-lactamase, because a putative signal sequence is present in the amino terminal region of *blaSa*, as is evident from the hydropathy plot (data not shown).

The stop codon of the *blaASa* product overlaps with the putative start codon of the *blaBSa* product (^{954}ATGA^{957}), suggesting translational coupling. It is likely that the *blaASa* and *blaBSa* genes are transcribed as a polycistronic mRNA. The start codons for individual ORFs were preceded by possible ribosome-binding sites (^{969}GGTGGT^{964}, ^{1871}AGAATG^{1866}, and ^{1989}GGAGGT^{1994}). Strohl (*17*) suggested that the majority of streptomycete promoters fall into two basic groups: (i) those with sequences similar to *E. coli* Eσ^{70}-like promoters; and (ii) those with a wide diversity of sequences. We found canonical sequences of *E. coli*-like promoters upstream of the *blaSa* and *blaASa* products (^{1906}TTGGAC^{1911} and ^{1931}GAACAT^{1936}, ^{1999}TTCGGA^{1994} and ^{1977}TCAAAG^{1972}, respectively).

The blaSa Gene Encodes a β-Lactamase—To further examine the function of the *blaL* homologue in *S. albulus* IFO14147 (*blaSa*), we examined the growth of the *E. coli* transformants on LB agar plates containing ampicillin (Fig. 3A). The *E. coli* cells carrying pKFbla2.0, which bears only *blaSa*, were capable of growing on ampicillin-containing plates, whereas the vector-harboring cells were sensitive to ampicillin. Table 1 summarizes the β-lactamase activities in *E. coli* cell extracts on cultivation in LB medium. A high level of activity was detected in the cells carrying pKFbla2.0, regardless of whether or not ampicillin had been added to the medium. This indicates that the *blaSa* gene encodes a β-lactamase required for ampicillin resistance. Although the crude extracts obtained from sonicated cells were assayed without further fractionation, the β-lactamases produced presumably accumulated in the periplasmic space of *E. coli*.

Similar results were obtained for the transformed *S. lividans* cells. The *S. lividans* cells carrying pIJbla2.0,

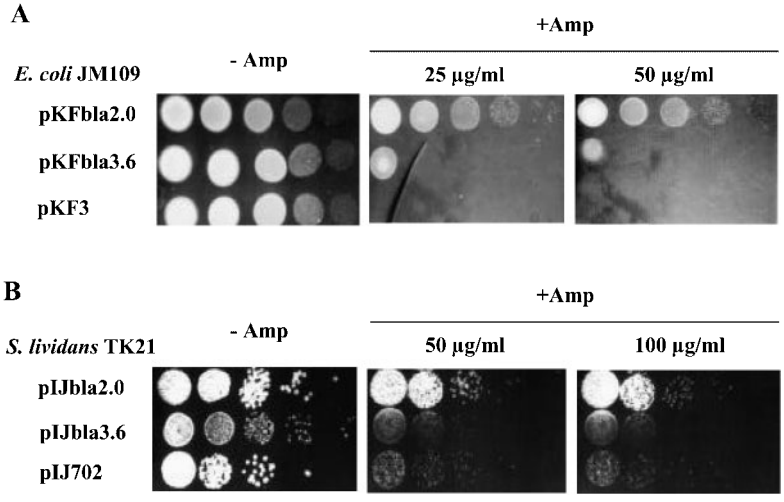

Fig. 3. **The growth phenotypes on ampicillin-containing medium of *E. coli* and *S. lividans* cells carrying the indicated plasmid.** Approximately 10^8 cells of each strain and serial dilutions of 10^{-1} to 10^{-4} (from left to right) were spotted onto LB plates for *E. coli* (A) and ISP.2 plates for *S. lividans* (B) in the absence (–Amp) and presence of ampicillin (+Amp). The plates were incubated at 37°C for 1 d.

Table 1. **β-Lactamase activities of the *E. coli* and *S. lividans* strains.**

Strain	Plasmid	Insert	β-Lactamase activity (mU/mg)	
			+ ampicillin	– ampicillin
E. coli JM109	pKFbla2.0	*blaSa*	1,400	1,200
	pKFbla3.6	*blaSa, blaASa, blaBSa*	140	110
	pKF3 (vector)		NT	0
S. lividans TK21	pIJbla2.0	*blaSa*	104	98
	pIJbla3.6	*blaSa, blaASa, blaBSa*	NT	0
	pIJ702 (vector)		NT	0

Assays were performed using cell-free extracts of *E. coli* strains and culture supernatants of *S. lividans* strains as enzyme sources. The data shown are the means for three independent experiments. The variations in the values were below 5%. NT, not tested.

which has only *blaSa*, acquired an ampicillin-resistant phenotype (Fig. 3B). Table 1 also shows the β-lactamase activity in the culture medium when *S. lividans* cells were grown in ISP.2 medium. The cells carrying pIJbla2.0 were found to produce β-lactamase independent of ampicillin, which is similar to the pattern observed for *E. coli*. We also infer that the β-lactamase signal sequence of *S. albulus* is functional in *S. lividans*. These results indicate that the *blaSa* gene, which encodes a β-lactamase, is expressed in both *E. coli* and *S. lividans* under its own promoter.

The blaA and blaB Homologues (blaASa and blaBSa) Reduced or Repressed β-Lactamase Activity—On the other hand, *E. coli* cells carrying pKFbla3.6, which has the whole fragment including *blaSa*, *blaASa*, and *blaBSa*, showed significantly lower ampicillin resistance than that of pKFbla2.0 (Fig. 3A). It is noteworthy that the cells carrying pKFbla3.6 exhibited greatly reduced β-lactamase activity (approximately 10% of that of the cells carrying pKFbla2.0) regardless of the presence or absence of ampicillin (Table 1). These results suggest that the putative regulatory genes *blaASa* and *blaBSa* lowered the β-lactamase activity in *E. coli*.

It is probable that the gene expression system, at the level of transcriptional activation or repression, is significantly different between bacteria such as *E. coli* and actinomycetes such as *Streptomyces* species. Interestingly, *S. lividans* cells carrying pIJbla3.6, which has the *blaSa*, *blaASa*, and *blaBSa* genes, showed no growth at all on ampicillin-containing plates, similar to the vector-harboring cells (Fig. 3B). Furthermore, no β-lactamase activity was detected in cells carrying pIJbla3.6 (Table 1). These results suggest that *blaSa* expression in *S. lividans* is fully repressed by the putative regulatory genes *blaASa* and *blaBSa*.

DISCUSSION

We found that a *blaSa* gene homologous to the *S. cacaoi blaL* encodes a β-lactamase, which is required for the ampicillin resistance of *S. albulus* IFO14147. Sequence analysis of the cloned fragment showed that two possible ORFs are located just upstream of *blaSa*. The protein sequences were also homologous to *S. cacaoi blaA*- and *blaB*-encoded proteins. In *S. cacaoi*, the *blaA* and *blaB* genes are reportedly required not only for induction, but also for basal expression of *blaL* (*15*). However, the growth phenotypes and enzyme assaying of *E. coli* and *S. lividans* showed that the *blaA* and *blaB* homologues in *S. albulus* IFO14147 (*blaASa* and *blaBSa*) greatly reduced or fully repressed β-lactamase activity, suggesting that *blaASa* or *blaBSa* is a repressor of *blaSa*, if not both.

In Gram-positive bacteria such as *B. licheniformis*, β-lactamase expression can be induced by the addition of penicillin (*21*). In contrast, most *Streptomyces* strains constitutively secrete β-lactamase into the culture medium (*22–25*). However, it is well known that *S. cacaoi* possesses two different β-lactamase genes, *blaL* and *blaU*, both of which are induced by β-lactam compounds (*10*). To our knowledge, the present study is the second to demonstrate induction of a β-lactamase gene in *Streptomyces* species. It has been shown that the *blaA*-encoded protein, which interacts with the promoter region (*26*), is a transcriptional activator belonging to the LysR family (*15*). Goethals *et al*. (*27*) previously proposed that LysR-like regulatory proteins, including the *blaA* product, bind to T-N_{11}-A nucleotide sequences centered on an inverted repeat. Such a motif was also discovered upstream of the *blaSa* gene (^{1873}T–^{1885}A). In *B. licheniformis*, inducible synthesis of β-lactamase is controlled by two genes, *blaI* and *blaR*. The *blaI* gene encodes a repressor (*28*) whose expression is autoregulated. Therefore, it is possible that the *blaASa* product in *S. albulus* IFO14147 is a repressor of *blaSa*, like *blaI* in *B. licheniformis*, instead of an activator as in *S. cacaoi*.

The biochemical study showed that the *blaB* product of *S. cacaoi* is an internal membrane-bound protein attached to the internal face of the cytoplasmic membrane, but does not act as a penicillin-binding protein. The exact role of the *blaB*-encoded protein remains to be elucidated (*29*). In *B. licheniformis*, the *blaR* gene is transcribed together with *blaI* from the *blaI* promoter (*30*). The *blaR* product, which is a membrane-bound penicillin-binding protein, functions as a penicillin sensory transducer (*31*, *32*). Although the function of the *blaBSa* gene in *S. albulus* IFO14147 remains unknown, it would be of great interest to isolate a transcriptional activator or a signal transducer for β-lactamase production in this strain.

To further such study, we need to examine the roles of the *blaASa* and *blaBSa* genes in β-lactamase induction by ampicillin in *S. albulus* cells. However, a method of transformation for this strain has not been developed yet. This is probably because the efficiency of regeneration from protoplast cells is extremely low (Hoshino, Y., *et al*., unpublished observations). Several experiments to introduce plasmid DNA into *S.albulus* cells are currently in

progress, as is the case for other *Streptomyces* species (*13*, *33*).

The metabolic pathway, and the enzymes related to ε-PL synthesis and degradation have not yet been clarified. Kito *et al.* recently reported that ε-PL-degrading enzymes were found in both *S. albulus* and an ε-PL-tolerant bacterium, *Sphingobacterium*, and that the latter enzyme was a cation-activated aminopeptidase, which catalyzes exotype degradation of ε-PL and thereby releases L-lysine (*34*). Taking into account that pNO33 has been found in only ε-PL–producing *S. albulus* IFO14147, it is probable that the plasmid encodes the gene(s) in ε-PL biosynthesis or resistance. Host-vector systems for this strain are essential not only for the study of biosynthesis and regulation of the unique homopolymer ε-PL, but also for the molecular breeding of ε-PL overproducers for industrial use. For its utilization as a cloning vector for engineered plasmid pNO33 containing the β-lactamase gene, we will soon attempt to isolate an ampicillin-sensitive mutant containing the *bla* gene mutation (β-lactamase-deficient) as a host strain. An ampicillin-inducible promoter upstream of the *blaSa* gene would allow us to construct an inducible expression vector for this strain.

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REFERENCES

1. Shima, S. and Sakai, H. (1977) Polylysine produced by *Streptomyces*. *Agric. Biol. Chem.* **41**, 1807–1809
2. Shima, S. and Sakai, H. (1981) Poly-L-lysine produced by *Streptomyces*, II. Taxonomy and fermentation studies. *Agric. Biol. Chem.* **45**, 2497–2502
3. Shima, S., matsuoka, H., Iwamoto, H., and Sakai, H. (1984) Antimicrobial action of ε-poly-L-lysine. *J. Antibiot.* **37**, 1449–1455
4. Shima, S., Matsuoka, H., and Sakai, H. (1982) Inactivation of bacteriophages by ε-poly-L-lysine produced *Streptomyces*. *Agric. Biol. Chem.* **46**, 1917–1919
5. Hiraki, J., Hatakeyama, M., Morita, H., and Izumi, Y. (1998) Improved ε-poly-L-lysine production of an *S*-(2-aminoethyl)-L-cysteine resistant mutant of *Streptomyces albulus*. *Seibutsukogaku* **76**, 487–493
6. Takagi, H., Hoshino, Y., Nakamori, S., and Inouye, S. (2000) Isolation and sequence analysis of plasmid pNO33 in the ε-poly-L-lysine-producing actinomycete *Streptomyces albulus* IFO14147. *J. Biosci. Bioeng.* **89**, 94–96
7. Jaurin, B., Forsman. M., and Häggström, B. (1988) β-Lactamase genes of *Streptomyces badius*, *Streptomyces cacaoi* and *Streptomyces fradiae*: cloning and expression in *Streptomyces lividans*. *Biochim. Biophys. Acta* **949**, 288–296
8. Sendouda, A., Urabe, H., and Ogawara, H. (1993) Cloning, nucleotide sequence and expression of a β-lactamase gene from *Streptomyces lavendulae*. *FEMS Microbiol. Lett.* **112**, 343–348
9. Bennett, P.M. and Chopra, I. (1993) Molecular basis of β-lactamase induction in bacteria. *Antimicrob. Agents Chemother.* **37**, 153–158
10. Forsman, M. Lindgren, L., Häggström, B., and Jaurin, B. (1989) Transcriptional induction of *Streptomyces cacaoi* β-lactamase by a β-lactam compound. *Mol. Microbiol.* **3**, 1425–1432
11. Magdalena, J., Gérard, C., Joris, B., and Forsman, M. (1997) The two β-lactamase genes of *Streptomyces cacaoi*, *blaL* and *blaU*, are under the control of the same regulatory system. *Mol. Gen. Genet.* **255**, 187–193
12. Sambrook, J. and Russell, D.W. (2001) *Molecular Cloning: a Laboratory Manual*, 3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York
13. Hopwood, D.A., Bibb, M.J., Chater, K.F., Kieser, T., Bruton, C.J., Kieser, H.M., Lydiate, D.J., Smith, C.P., Ward, J.M., and Schrempf, H. (1985) *Genetic Manipulation of Streptomyces: A Laboratory Manual*, John Innes Foundation, Norwich, United Kingdom
14. O'Callaghan, C.H., Morris, A., Kirby, S., and Shingler, A. (1972) A novel method for detection of β-lactamase by using a chromogenic cephalosporin substrate. *Antimicrob. Agents Chemother.* **1**, 283–288
15. Lenzini, M.V., Magdalena, J., Fraipont, C., Joris, B., Matagne, A., and Dusart, J. (1992) Induction of a *Streptomyces cacaoi* β-lactamase gene cloned in *S. lividans*. *Mol. Gen. Genet.* **235**, 41–48
16. Ishikawa, J. and Hotta, K. (1999) FramePlot: a new implementation of the Frame analysis for predicting protein-coding regions in bacterial DNA with a high G+C content. *FEMS Microbiol. Lett.* **174**, 251–253
17. Strohl, W.R. (1992) Complilation and analysis of DNA sequences associated with apparent streptomycete promoters. *Nucleic Acids Res.* **20**, 961–974
18. Lenzini, M.V., Nojima, S., Dusart, J., Ogawara, H., Dehottay, P., Frére, J.M., and Ghuysen, J.M. (1987) Cloning in *Streptomyces lividans* of a gene encoding extracellular beta-lactamase from *Streptomyces cacaoi*. *J. Gen. Microbiol.* **133**, 2915–2920
19. Lenzini, M.V., Ishihara, H., Dusart, J., Ogawara, H., Joris, B., Van Beeumen, J., Frére, J.M., and Ghuysen, J.M. (1988) Nucleotide sequence of the gene encoding the active site serine beta-lactamase from *Streptomyces cacaoi*. *FEMS Microbiol. Lett.* **49**, 371–376
20. Magdalena, J., Forsman, M., Lenzini, M.V., Brans, A., and Dusart, J. (1992) Two different β-lactamase genes are present in *Streptomyces cacaoi*. *FEMS Microbiol. Lett.* **99**, 101–106
21. Zhu, Y., Engelbert, S., Joris, B., Ghuysen, J.M., Kobayashi, T., and Lampen, J.O. (1992) structure, function and fate of the BlaR signal transducer involved in induction of β-lactamase in *Bacillus licheniformis*. *J. Bacteriol.* **174**, 6171–6178
22. Ogawara, H. (1981) Antibiotic resistance in pathogenic and producing bacteria, with special reference to β-lactam antibiotics. *Microbiol. Rev.* **45**, 591–619
23. Ogawara, H. (1996) Structure and evolution of β-lactamase genes from *Streptomyces*. *Actinomycetes* **10**, 104–111
24. Deák, E., Szabó, I., Kálmáczhelyi, A., Gál, Z., Barabás, G., and Penyige, A. (1998) Membrane-bound and extracellular β-lactamase production with developmental regulation in *Streptomyces griseus* NRRL B-2682. *Microbiol.* **144**, 2169–217
25. Forsman, L., Häggström, B., M. Lindgren, and Jaurin, B. (1990) Molecular analysis of β-lactamases from four species of *Streptomyces*: comparison of amino acid sequences with those of other β-lactamases. *J. Gen. Microbiol.* **136**, 589–598
26. Urabe, H. and Ogawara, H. (1992) Nucleotide sequence and transcriptional analysis of activator-regulator proteins for β-lactamase in *Streptomyces cacaoi*. *J. Bacteriol.* **174**, 2834–2842
27. Goethals, K., Van Montagu, M., and Holsters, M. (1992) Conserved motifs in a divergent *nod* box of *Azorhizobium caulinodans* ORS571 reveal a common structure in promoters regulated by LysR-type proteins. *Proc. Natl Acad. Sci. USA* **89**, 1646–1650
28. Himeno, T., Imanaka, T., and Aiba, S. (1986) Nucleotide sequence of the penicillinase repressor gene *penI* of *Bacillus licheniformis* and regulation of *penP* and *penI* by the repressor. *J. Bacteriol.* **168**, 1128–1132
29. Magdalena, J., Joris, B., Van Beeumen, J., Brasseur, R., and Dusart, J. (1995) Regulation of the β-lactamase BlaL of *Streptomyces cacaoi*: the product of the *blaB* regulatory gene is an internal membrane-bound protein. *Biochem. J.* **311**, 155–160
30. Kobayashi, T., Zhu, Y.F., Nicholls, N.J., and Lampen, J.O. (1987) A second regulatory gene, *bla*R1, encoding a potential penicillin-binding protein required for induction of β-lactamase in *Bacillus licheniformis*. *J. Bacteriol.* **169**, 3873–3878

31. Joris, B., Ledent, P., Kobayashi, T., lampen, J.O., and Ghuysen, J.M. (1990) Expression in *Escherichia coli* of the carboxyterminal domain of the BLAR sensory-transducer protein of *Bacillus licheniformis* as a water-soluble Mr 26000 penicillin-binding protein. *FEMS Microbiol.* **70**, 107–114
32. Zhu, Y.F., Curran, I.H.A., Joris, B., Ghuysen, J.M., and Lampen, J.O. (1990) Identification of *blaR*, the signal transducer for β-lactamase production in *Bacillus licheniformis*, as a penicillin-binding protein with strong homology to the OXA-2 β-lactamase (classD) of *Salmonella typhimurium*. *J. Bacteriol.* **172**, 1137–1141
33. Mazy-Servais, C., Baczkowski, D., and Dusart, J. (1997) Electroporation of intact cells of *Streptomyces parvulus* and *Streptomyces vinaceus*. *FEMS Microbiol. Lett.* **151**, 135–138
34. Kito, M., Onji, Y., Yoshida, T., and Nagasawa, T. (2002) Occurrence of ε-poly-L-lysine-degrading enzyme in ε-poly-L-lysine-tolerant *Sphingobacterium multivorum* OJ10: purification and characterization. *FEMS Microbiol. Lett.* **207**, 147–151