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Cloning and Sequencing of a Gene Involved in the Biosynthesis of Exopolysaccharide in *Zoogloea Ramigera* 115SLR

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Abstract: To identity the genes responsible for the biosynthesis of exopolysaccharide, recombinant plasmids pUEX10 and pLEX10 were constructed from plasmid pLEX3 which was isolated from the recombinant cosmid library of *Zoogloea ramigera* 115. The complete nucleotide sequence of the 1.7 kb genomic DNA insert in plasmid pUEX10 was determined. Its analysis identified two open reading frames (ORF3 & ORF4) which could encode two proteins. The amino acid sequence derived from ORF3 showed the homology with gumC protein in *Xanthomonas campestris* as well as exoP protein in *Rhizobium meliloti.* The partial amino acid sequence of ORF4 showed the homology with polysaccharide export protein in *Thermotoga maritima. Z. ramigera* 115SLR and *Z. ramigera* 115SLR/pLEX10 showed the similar pattern for EPS production. Yield of exopolysaccharides produced by *Z. ramigera* 115SLR and *Z. ramigera* 115SLR/pLEX10 was 0.26% (w/v) and 0.16% (w/v), respectively.

Key Words: Gene cloning, Exopolysaccharide, *Zoogloea ramigera* 115SLR

INTRODUCTION

Microbial exopolysaccharides have many commercial applications such as paper coating, drug delivery, metal recoveries, cosmetic products, food products, and separations[4]. The great diversity exhibited by exopolysaccharides can be attributed to differences in their chemical composition. Generally, functional properties of exopolysaccharides are largely influenced by their molecular weight, chemical composition, linkage-type, and functional side chains[13].

Zoogloea ramigera 115 is a gram-negative, floc-forming bacterium that produces a cell-bound exopolysaccharide (capsule-like matrix) with a strong affinity to several heavy metal ions[2,5,11,16] and unique rheological properties[21]. There have been several structural investigations of exopolysaccharides (EPS) produced from *Z. ramigera* 115[8,17]. Compositional analysis of EPS produced from *Z. ramigera* 115 and *Z. ramigera* 115SLR showed the presence of acetate and succinate, and indicated no difference in the content of glucose, galactose, acetate, succinate and pyruvate[9,24].

Considerable progress has been made recently in the study of genetic systems regulating synthesis of microbial exopolysaccharide. Genetic studies on *Xanthomonas campestris* have produced mutants with polymers altered in acetylation, pyruvylation, and carbohydrate composition. These polymers showed different functional properties. Most of the gene products in a cluster of 12 genes are involved in xanthan

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production, including the seven transferase activities[8,22].

The main effort for the genetic manipulation of exopolysaccharide produced by *Z. ramigera* 115SLR was previously focused on plasmid pLEX3 with a 15 kb chromosomal DNA[12]. The plasmid pLEX3 exhibits a homology with the pyruvyltransferase gene of *X. campestris*. In addition, the EPS negative mutant strain can be complemented by the plasmid pLEX3[4]. In particular, a middle region of the clone is also able to complement the slime-forming property of *Z. ramigera* 115SLR strain[10]. Thus, it was concluded that plasmid pLEX3 contained a significant proportion of genes required for the biosynthesis of EPS in *Z. ramigera* 115[4,10]. In addition, the genetic loci required for the production of EPS from *Z. ramigera* 115SLR was isolated[3,13,14]. The manipulation of EPS has been reported through genetic modification using a pyruvyltransferase gene[14].

In order to produce a novel EPS through metabolic engineering, mutant strains were required to be isolated. First of all, a mutant *Z. ramigera* 115SL as a slime forming strain was derived from *Z. ramigera* 115 by nitrosoguanidine mutagenesis[4]. Futhermore, to facilitate the gene manipulations in this microorganism, a spontaneous rifampicin resistant strain, *Z. ramigera* 115 SLR, was derived from *Z. ramigera* 115SL[4]. For complementing the slime-forming property of *Z. ramigera* 115SLR, various subclones were constructed from plasmid pLEX3[9]. As the tools for the gene transfer in *Z. ramigera* 115SLR, conjugation and electroporation has been successfully used[4,10].

Bacterial polysaccharides represent the largest potentials for development of novel and improved products with the successful application of new genetic engineering technologies[4]. In order to carry out the genetic modification of EPS, it is necessary to isolate and characterize the genes involved in the biosynthesis of microbial exopolysaccharides. This paper reports on the sequencing and characterization of a gene involved in the EPS biosynthesis of *Z. ramigera* 115SLR.

Table 1. Bacterial strains and plasmids used

Strain or plasmid	Relevant characteristics	Source or Reference
E. coli		
DH5α	F^- Φ 80d *lacZ*△M15 △(*lac*ZYA-*arg*F)U169 *end*A1 *rec*A1*hsd*R17 (r_k^- m_k^+)*deo*R*thi*-1*sup*E44 λ^- gyrA96*rel*A1	Gibco BRL
JM101	*sup*E △(*lac-pro*AB) F'*tra*D36 *pro*AB$^+$*lac*I^q*lacZ*△M15	Gibco BRL
S17-1	F^-*rec*A *hsd*R [RP4:: Ap^S,Tc^S, Km^S]	[19]
Z. ramigera 115		
SLR	R_f^R, derivatives of *Z. ramigera* 115	[4]
Plasmid		
pLAFR3	Tc^R, cosmid vector	[20]
pLEX10	Tc^R, pLEX5 derivative, 5.2 kb	This work
pUEX10	*ori*pUC, Amp^R, pLEX5 derivative, 5.2 kb	This work
pUC18	*ori*pUC, Amp^R	USB
M13mp18	Vectror for DNA sequencing	Gibco BRL

R_f^R, rifampicin resistance; Amp^R, ampicillin resistance; Tc^R, tetracycline resistance; USB: United States Biochemical

MATERIALS AND METHODS

Bacterial Strains and Plasmids

Z. ramigera 115SLR was grown in TSB (BBL, Cockeysville, USA) containing rifampicin (50 μg/ml) at 29℃ with rotary shaking at 150 rpm. For the recombinant DNA procedures, *E. coli* DH5α was routinely grown in an LB medium[18]. The pLAFR3 and pUC18 vectors were all obtained from Professor A. J. Sinskey (MIT, USA). The M13 cloning vector was purchased from Pharmacia Biotech. (Piscataway, USA). The genotypes of the strains and plasmids used in this work are described in Table 1.

DNA Manipulation and Subcloning

Routine DNA manipulation, including plasmid purification and *E. coli* transformations were performed as described by Sambrook *et al*[18]. The plasmid DNA was isolated from overnight cultures of *E. coli* by the alkaline lysis method[18]. The restriction and modifying enzymes were purchased from Promega (Madison, USA). The restriction enzyme digestions and ligations were performed according to the manufacturers' instructions. The restriction fragments were electrophoresed in 1.0% agarose gels and isolated using a QIAGEN DNA extraction kit (QIAGEN Inc., Chatsworth, USA).

Recombinant plasmids pUEX10 and pLEX10 prepared from plasmid pLEX3BM were used as a template for restriction mapping and DNA sequencing[9].

Nucleotide Sequencing

The nucleotide sequence of the 1.7 kb DNA fragment that located in upstream region of plasmid pUEX10 was determined using standard procedures. The 3.3 kb *Hin*dIII/*Sal*I DNA fragment was isolated from pUEX10 and subcloned into an M13mp18 cloning vector. After the double digestion of the clone with *Sac*I and *Bam*HI, the subclone was deleted using an Erase-a-Base deletion kit (Promega, Madison, USA), ligated, and then transformed into *E. coli* JM101 competent cells. The sequencing of each DNA fragment was performed by the dideoxy-mediated chain termination method using a Sequenase™ kit (USB, Cleveland, USA) and [α-^{35}S] dATP (Amersham, Arlington Heights, USA).

Production of EPS

Z. ramigera 115SLR, and *Z. ramigera* 115SLR/pLEX10 strains were grown in TSB broth including rifampicin (50 μg/ml) and/or tetracycline (10 μg/ml) at 29℃ for 20 hr. Each seed culture (0.5%) was transferred to the defined medium including glucose as a carbon source and incubated at 29℃ for 5~6 days. The EPS contents produced by two strains were determined. The cell-bound EPS from *Z. ramigera* 115SLR/pLEX 10 was extracted by treating the culture broth with 5 volumes of hot water (50℃) for 16 hr. The whole culture producing slime EPS was diluted with 2 volumes of hot water. After the removal of the cells by centrifugation (10,000 rpm for 20 min, GSA rotor, Sorval RC5B) the EPS was precipitated with 2 volumes of isopropanol at -20℃ for 6 hr and then recovered by centrifugation. The EPS pellets were dissolved with water and then recovered by isopropanol precipitation. Concentration of exopolysaccharides from culture broths and EPS solutions was determined by the phenol/sulfuric acid assay[4].

Protein determination

To determine the cell growth from *Z. ramigera* 115SLR and *Z. ramigera* 115SLR/pLEX10, total protein concentration in culture broths was determined using Bio-Rad protein assay (Bio-Rad Laboratories, California, USA). Cellular protein was released by boiling in 0.2 N NaOH. Bovine serum albumin was used as the standard protein.

Nucleotide Sequence Accession Number

The nucleotide sequence of the genes that involved in the biosynthesis of exopolysaccharide in *Z. ramigera* 115SLR was deposited in the NCBI/GenBank Library under the accession num-

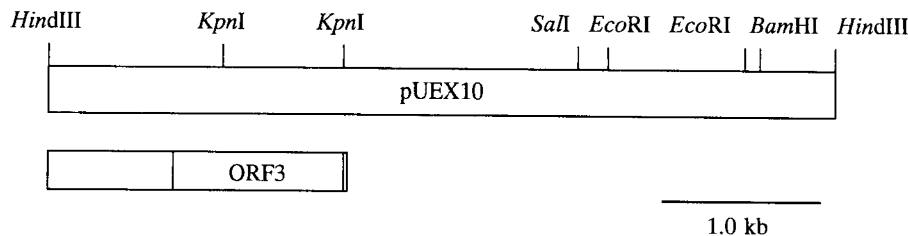

Fig. 1. Restriction map determined from the insert DNA of plasmid pUEX10. ORF3 means open reading frame 3; *Hin*dIII/*Kpn*I fragment (1.7 kb) indicates the sequenced region.

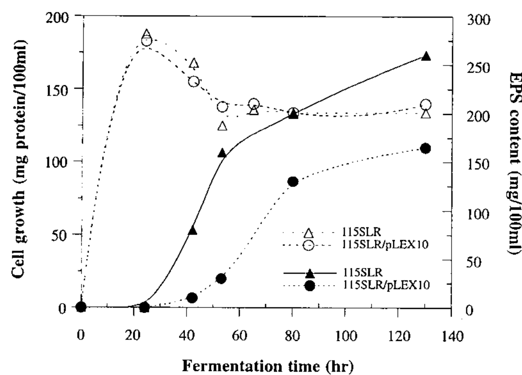

Fig. 2. Production of exopolysaccharides according to the growth of *Z. ramigera* 115SLR and *Z. ramigera* 115SLR/pLEX10 strains. EPS content (▲, ●); Cell growth (△, ○)

ber (AF236053).

RESULTS

Construction of Recombinant Plasmids

The 5.0 kb *Sma*I fragment derived from plasmid pLEX3 was modified with *Hin*dIII linker. Plasmids pUEX10 and pLEX10 including 5.0 kb *Hin*dIII fragment were constructed with pUC19 and pLAFR3 vectors, respectively. The restriction map of 5.0 kb DNA fragment of plasmid pUEX 10 was determined using various restriction enzymes (Fig. 1). *Sal*I, *Bam*HI and *Apa*I were identified as a single restriction site. DNA fragment also included *Kpn*I and *Eco*RI as double restriction sites, respectively.

The plasmid pLEX10 was transformed into *Z. ramigera* 115SLR. The transconjugants harboring plasmid pLEX10 were isolated by their appearance with capsular EPS and then used for the EPS production.

Production and Isolation of EPS

Z. ramigera 115SLR, and *Z. ramigera* 115SLR/pLEX10 were grown in defined media, and then the production of EPS was determined during fermentation period. Fig. 2 shows the pattern of EPS production and cell growth from *Z. ramigera* 115SLR and *Z. ramigera* 115SLR/pLEX 10 strains. As shown in Fig. 2, the *Z. ramigera* 115SLR strain produced the EPS significantly after fermentation for 40 hr. The *Z. ramigera* 115SLR harboring plasmid pLEX10 showed the EPS production after fermentation for 50 hr. After 50 hr, cells in both culture reached at the stationary phase indicating the constant cell growth. Yield of EPS produced by *Z. ramigera* 115SLR/pLEX10 was lower than that of *Z. ramigera* 115SLR. *Z. ramigera* 115SLR and *Z. ramigera* 115SLR/pLEX10 produced EPS of 0.26% and 0.16% after 130 hr, respectively. In particular, *Z. ramigera* 115SLR showed very viscous culture broth compared with that of *Z. ramigera* 115SLR/pLEX10.

Nucleotide Sequencing and Analysis

The nucleotide sequence of the 1.7 kb DNA fragment of pUEX10 was determined. The entire 1.7 kb DNA was analyzed for potential protein coding regions using the program "CODON PREFERENCE" and the results are shown in Fig 3. Two ORFs encoded by the same strand were found. One complete open reading frame (ORF3)

```
     ORF 4 ---->
1    CCCGGGCCGTTTCCCGCTCGACGCCAGCCGCAATCTGCTTGACGTGCTGGCACTGGCCGG
       P  G  R  F  P  L  D  A  S  R  N  L  L  D  V  L  A  L  A  G
61   CGGCGTCAATTCCGAAGGCGGCGACCAGGTGACGGTGCTGCGCACCGCAGACGGCAAGAC
       G  V  N  S  E  G  G  D  Q  V  T  V  L  R  T  A  D  G  K  T
121  CACCAAGCAAGTGATCGATATTACCGATGTCTTCAGTGGTGGCGACCAGACGGCGAGCCT
       T  K  Q  V  I  D  I  T  D  V  F  S  G  G  D  Q  T  A  S  L
181  GGAACTGAGCGGCAACGACGTGGTGTATATCGAACGCGCCCCGCTTCTACATCTACGGTG
       E  L  S  G  N  D  V  V  Y  I  E  R  A  P  L  L  H  L  R  *
241  AAGTACAGCGTCCGGGCATCTTCCGCATTGAACGTTCGATGACGGTGTTGCAGGCGCTGG

301  CGGCCGGCGGTGGCCTCACCCAGCGCGGCACCGAGCGCGGCCTGCGTATCAAGCGTCGTG
361  ACGCTACCGGCAAGATCGAGATCATCGAAGCGAAATACGATGACCTGGTTCGCGCCAACG
421  ATGTGCTGTACGTGCGCGAGAGCCTGTTCTAAGGCCGATGTTGCCTGACCTTCACCGAGA

         ORF3 --->
481  AAGATCACCATGAATTTCACTCATTTGTTTCTTGCCCTGCGCGCCCGCTACAAGATTGTT
               M  N  F  T  H  L  F  L  A  L  R  A  R  Y  K  I  V
541  TTACTGATCCTGGCAGTCACGGTGGCAGCTGCGCTGGCCATCACCGCCATGATGCCCAAG
      L  L  I  L  A  V  T  V  A  A  A  L  A  I  T  A  M  M  P  K
601  GTCTACAAGGCCAGCACTTCGCTGGTGCTCAATACCAAGGGCGTCGATCCCATCACCGGG
      V  Y  K  A  S  T  S  L  V  L  N  T  K  G  V  D  P  I  T  G
661  GTGACGTTGCCAGTGCAACTGATGACCGGCTATGTTGCCACCCAGGCCGACATCATCCGC
      V  T  L  P  V  Q  L  M  T  G  Y  V  A  T  Q  A  D  I  I  R
721  AGCAAGAGCGTGGCGCTCAAGGCCGTGGAAAACCTGCGCCTGGCGGAAAGCGCTGCCGTG
      S  K  S  V  A  L  K  A  V  E  N  L  R  L  A  E  S  A  A  V
781  CAGGAGCAGTTCCGCGAAGCGCGCGGTGGCCAGGGTTCGATCAAGGAATGGCTGGCAGGT
      Q  E  Q  F  R  E  A  R  G  G  Q  G  S  I  K  E  W  L  A  G
841  CTGCTGCTGGCCAAGGTGGACGTGGAGCCGTCGCGCGACAGCAGCGTGCTGACCATTAAC
      L  L  L  A  K  V  D  V  E  P  S  R  D  S  S  V  L  T  I  N
901  TTCCGTGGCAACGATCCGCAGTTTGTTGCAGCGGTGGCCAATGCCTTCGCGCAGGGGTAC
      F  R  G  N  D  P  Q  F  V  A  A  V  A  N  A  F  A  Q  G  Y
961  CTTCGATTTCACGGTGCAGCTCAAGACCGATCCGGCACTGCAGGCATCGGGCTTATCAAC
      L  R  F  H  G  A  A  Q  D  R  S  G  T  A  G  I  G  L  I  N
1021 ACCCAGATCAAGTTGTTGCGCGAACAGTACGAACTGGCGCAGAGCCGGTTGTCGAAGTAC
      T  Q  I  K  L  L  R  E  Q  Y  E  L  A  Q  S  R  L  S  K  Y
1081 CAGAAAGAAAACAACATCTACAGCGCCGACAACCGCGTCGATGTCGAGACCGCGCGCCTG
      Q  K  E  N  N  I  Y  S  A  D  N  R  V  D  V  E  T  A  R  L
1141 AACGAGCTGTCGAGCCAACTGGTGCAGGTGCAGGGCCAACTGATGGAAGCGGAATCGCGT
      N  E  L  S  S  Q  L  V  Q  V  Q  G  Q  L  M  E  A  E  S  R
1201 TCGCGTCAGGCCACCGGCAATGCAGGCGCCTCGCCAGACGTTCTCAACAACGGCCTGATC
      S  R  Q  A  T  G  N  A  G  A  S  P  D  V  L  N  N  G  L  I
1261 CAGAGCCTGAAATCGCAGCTGGCCACGGCAGAAGCACGGTTTGCCGATACCTCCCAGCGT
      Q  S  L  K  S  Q  L  A  T  A  E  A  R  F  A  D  T  S  Q  R
1321 CTGGCCTCGAATCACCCGCAATACATCTCGGCCAAGTCCGAAGTGGACAAGCTGCGTTCG
      L  A  S  N  H  P  Q  Y  I  S  A  K  S  E  V  D  K  L  R  S
1381 AACCTGGACGAGCAGATCCGCATCGCCTCGACCGGCGTGGCCAGTAGCAGCAATATCTAC
      N  L  D  E  Q  I  R  I  A  S  T  G  V  A  S  S  S  N  I  Y
1441 CGGCAGCGTGAAAACGAACTGCGCTCGGCCCTGTCGGCCCAAAAAGCCCGCGTGCTGGAA
      R  Q  R  E  N  E  L  R  S  A  L  S  A  Q  K  A  R  V  L  E
1501 TTGAACGGCGCACGCGACGAGTTCGTAGTTCTCAGCAACGAAGTGGACAATGCCCGCCGC
      L  N  G  A  R  D  E  F  V  V  L  S  N  E  V  D  N  A  R  R
1561 TCGTACGAAAGCGCGATGCAGCGTTATAACCAGACCAACCTCGAAGCCAGGCCCGCCAGT
      S  Y  E  S  A  M  Q  R  Y  N  Q  T  N  L  E  A  R  P  A  S
1621 CCGATATCGTGCTGTCCACGGCCGAAGTGCCGGGCAGCCCGACGTCGCCGCGCCTGATCG
      P  I  S  C  C  P  R  P  K  C  R  A  A  R  R  R  R  A  *
1681 TCAACCTGGCGCTGGCCATCGTTATCGGTACC
```

Fig. 3. Nucleotide and deduced amino sequences of 1.7 kb genomic fragment of *Z. ramigera* 115SLR. The nucleotide sequence of one strand of the DNA fragment is presented in the 5' to 3' direction. The two identified ORFs are indicated by the deduced amino acid sequence written below the nucleotide sequence. The arrows indicate the direction of the transcription. The putative Shine-Dalgarno ribosomal binding sequence (SD) is underlined.

and the possibly the C-terminus of another (ORF 4) were identified. ORF3 began with a characteristic ATG start codon. ORF3 sequence started at position 490, contained 1187 bp, and had a coding potential for a 50.0 kDa protein. ORF3 were not proceeded by a site similar to an *E. coli* consensus ribosomal binding (Shine-Dalgarno) sequence.

To identify the homology of the amino acid sequence, the amino acid sequences derived from ORF3 or ORF4 were analyzed by the BLAST search program. The similarity and identity of amino acid sequences were compared with those of other known proteins. The amino acid sequences derived from ORF4 showed the homologous region with polysaccharide export protein in *Thermotoga maritima* (26% identities and 42% positives). ORF3 protein was found to share a homology with protein responsible for biosynthesis of xanthan gum in *X. campestris.* The amino acid sequences derived from ORF3 showed the homology with gumC protein in *X. campestris* with 44% similarity and 22% identities. Therefore, it is concluded that the gene products from ORF3 and ORF4 are proteins involved in EPS biosynthesis of *Z. ramigera* 115SLR.

Localization and Characterization of ORF3

An *in vivo* gene expression was previously performed in order to confirm the DNA sequence and gene products from ORFs present in plasmid pLEX10. An *in vivo* gene expression confirmed the presence of three ORFs in the 5.0 Kb DNA fragment and showed three proteins with 50 kDa, 30 kDa, and 14 kDa[9].

By the DNA sequencing of 1.7 kb DNA fragment, the location of ORF3 in plasmid pUEX10 was determined. As shown in Fig. 1, ORF3 was located in the upstream region (1.7 kb) of plasmid pUEX10. The molecular weight of a protein expected from the ORF3 was about 50 kDa. On the other hand, ORF4 was found as an incomplete ORF. The hydrophobicity profile of the amino acid sequence of ORF3 is presented in Fig. 4, and the predicted transmembrane do-

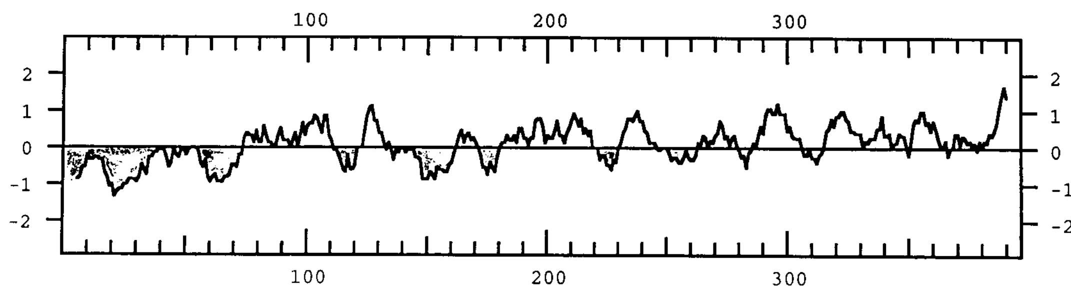

Fig. 4. Hydrophobicity plot of ORF3 protein. The possible integral membrane segments are lightly shaded.

mains are mainly located in N-terminal region.

DISCUSSION

Exopolysaccharides produced by microorganisms often function as bioactive agents and/or functional biopolymers[13]. The manipulation of structure/function of exopolysaccharides is possible through genetic engineering. In order to isolate and characterize genes involved in EPS biosynthesis of *Z. ramigera* 115SLR, a 1.7 kb DNA fragment located in the upstream region of 5.0 kb DNA fragment in plasmid pUEX10 was isolated and sequenced. In previous studies, the recombinant plasmid pUEX11 with a 3.3 kb *Hin*dIII DNA fragment, downstream region of 5.0 kb DNA fragment, played an essential role in the complementation of the slime-forming property of mutant strain. The nucleotide sequence of a 3.3 kb DNA fragment showed the presence of open reading frames (ORFs); two complete ORF1 and OFR2 encoding 30 kDa and 14 kDa proteins, respectively[9].

Considering the DNA sequencing of the entire 5.0 kb DNA fragment in plasmid pUEX10, three complete ORFs and one incomplete ORF are located in this region. Their clustered organization is such that they probably represent an operon. It indicates that 5.0 kb DNA fragment is part of cluster genes involved in EPS biosynthesis of *Z. ramigera* 115SLR. In addition, from an *in vivo* gene expression, the plasmid pTEX10 with 5.0 kb DNA fragment showed three proteins with 50 kDa, 30 kDa and 14 kDa[9]. The upstream region (1.7 kb) in plasmid pTEX10 was enough in size to include an open reading frame encoding 50 kDa protein. It was confirmed that ORF3 encoding 50 kDa protein was located in the 1.7 kb DNA fragment.

In comparison of amino acid sequences analyzed by BLAST search program, a gene product of ORF3 showed the homology with gumC protein in *X. campestris* as well as exoP protein that may be involved in polymerization or the export of the completed polymer in *R. meliloti*[1,6,15]. The hydrophobicity profile of ORF3 protein indicated that the protein is probably anchored at the amino terminus to the cytoplasmic membrane. A gene product of ORF4 showed the homology with exopolysaccharide export protein in *T. maritima.*

Generally, various enzymes played an important roles for the biosynthesis of microbial exopolysaccharide. For example, the water-soluble monosaccharide components as specific substrates, have to be converted into high energy molecules; they may also need some modification; they must be passed through the lipophilic cell membrane and assembled into polysaccharide chains. Finally, the chains are released to form a capsule or slime at the cell surface[23]. Although at present we have no biochemical evidence for the gene product of ORF3 as an enzyme required for the synthesis of exopolysaccharide in *Z. ramigera* 115SLR, it is considered that the gene product of ORF3 is involved in the final step of these functions. The upstream and downstream regions of a 5.0 kb

DNA fragment will be an essential part to isolate other genes involved in EPS biosynthesis of *Z. ramigera* 115SLR.

Z. ramigera 115SLR showed higher EPS production than that of *Z. ramigera* 115SLR/pLEX10 strain. It may be due to the inhibitory effect of tetracycline used as a selection marker of plasmid pLEX10. In the synthesis of exopolysaccharides by micro-organisms, nutrient imbalance in the presence of large amounts of utilizable carbohydrate leads to increased polysaccharide production; particularly favourable are high carbon: nitrogen, carbon: phosphorus, or carbon: sulphur ratios[23]. Therefore, to enhance the production of EPS, it may be required the preparation of the optimum composition of defined medium. The morphological difference of EPSs produced from *Z. ramigera* 115SLR and *Z. ramigera* 115SLR/pLEX10 allow us to encourage the research for understanding its function in microbial cells. Capsular EPS produced by *Z. ramigera* 115SLR/pLEX10 is considered for the various application such as metal binding and floc-formation. The functional properties of slime EPS, negatively charged biopolymer, allows to be utilized as an ingredients for food system as well as biomedical application.

In conclusion, an 1.7 kb DNA fragment isolated from 5.0 kb DNA fragment in plasmid pUEX10 was sequenced and characterized. The DNA sequence showed one complete open reading frame (ORF3) and an incomplete ORF. The gene product of ORF3 showed the homology of gumC in *X. campestris* and exoP in *R. melitoti.* It was confirmed that the gene product of ORF3 was involved in the biosynthesis of EPS in *Z. ramigera* 115SLR.

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=국문초록=

Zoogloea Ramigera 115SLR로부터 다당류 생합성에 관여하는 유전자의 분리 및 염기서열 결정

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Zoogloea ramigera 115SLR로부터 다당류 생합성에 관여하는 유전자를 분리하기 위해서 균주의 genomic DNA로부터 제조된 gene bank로부터 plasmid pLEX3이 얻어졌다. 이로부터 재조합된 5.0 kb DNA fragment를 포함하는 plasmid pLEX10은 다당류의 형태를 변환시키는 유전자를 포함하고 있으며, 이중에서 upstream 영역에 해당하는 1.7 kb DNA fragment가 분리되었다. 1.7 kb DNA 염기서열의 결과로부터 단백질을 인지할 수 있는 2개의 ORF가 존재하였으며, 50 kDa 단백질을 인지할 수 있는 ORF3은 *X. campestris*의 다당류 생합성 유전자들인 gumC와 *R. meliloti*의 exoP와 아미노산의 동질성을 나타내었다. ORF4는 N-terminal 영역이 결여된 단백질을 인지하며, *Thermotoga maritima*의 다당류 export에 관여하는 단백질과 동질성을 보였다. *Z. ramigera* 115SLR and *Z. ramigera* 115SLR/pLEX10은 각각 slime 또는 capsule 형태의 다당류를 생합성하며 이들로부터 생합성된 다당류양은 각각 0.26% (w/v) and 0.16% (w/v)였다.

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