Differential Expression of *Zymomonas mobilis* Sucrase Genes (*sacB* and *sacC*) in *Escherichia coli* and Sucrase Mutants of *Zymomonas mobilis*

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**ABSTRACT**

The *sacB* and *sacC* genes encoding levan sucrase and extracellular sucrase respectively were independently subcloned in pBluescript (high copy number) and in *Z. mobilis*-E. coli shuttle vector, pZA22 (low copy number). The expression of these genes were compared under identical background of *E. coli* and *Z. mobilis* host. The level of *sacB* gene expression in *E. coli* was almost ten fold less than the expression of *sacC* gene, irrespective of the growth medium or the host strain. In *Z. mobilis* the expression of *sacB* and *sacC* genes was shown to be subject to carbon source dependent regulation. The transcript of *sacB* and *sacC* was three fold higher in cells grown on sucrose than in cells grown on glucose/fructose. Northern blot analysis revealed that the transcript levels of *sacC* was approximately 2-3 times higher than that of *sacB*. These results suggested that the expression of *sacC* gene was more pronounced than *sacB*.

**Key words:** Zymomonas mobilis, Escherichia coli, Expression, Levansucrase, Extracellular sucrase

**INTRODUCTION**

*Zymomonas mobilis*, a gram negative bacterium, has been shown to produce ethanol at a rate three to four fold, and at a higher final yield compared to the traditionally used yeast strains (Rogers et al., 1982). However, this organism uses only a narrow range of substrates, which is limited to glucose, fructose and sucrose (Swings and DeLey, 1977). However, when grown on media containing sucrose as the carbon source, the ethanol yield is considerably reduced because of the by products formed such as levan, fructo-oligomers and sorbitol (Viikari, 1984). When *Z. mobilis* is grown on sucrose medium, extracellular sucrase hydrolyzing enzymes such as intracellular sucrase (SacA or InvA), extracellular levansucrase (SacB or Inv C) and extracellular sucrase (SacC or InvB), are produced. The SacA enzyme is a monomer with a molecular weight of 58 kDa. The sacA gene from *Z. mobilis* has been cloned, sequenced and characterized (Gunasekaran et al., 1990a). The deduced amino acid sequence of *sacA* gene product showed strong homology with the intracellular sucrase of *Bacillus subtilis* and yeast invertases. The intracellular sucrase SacA from *Z. mobilis* was purified to homogeneity from a recombinant *E. coli* strain containing the *sacA* gene under an expression system. The protein was monomer with a molecular mass of 58kDa. The

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sucrase activity was maximal at 25°C and thermal stability of the purified protein was low and maximal activity was at pH 6.5 Therefore, SacA alone is unable to support the growth of Z. mobilis on sucrose (Ait-Abdelkader et al., 2000).

Levansucrase, a beta-fructo-furanosidase, is an extracellular enzyme that is responsible for both sucrose hydrolysis and levan formation. Levansucrase has been purified from Z. mobilis cells (Lyness and Doelle, 1983) and from culture broth (Mortatte et al., 1983). It is a monomer with a molecular weight of 51 kDa in Z. mobilis ZM4 (Preziosi et al., 1990b). The SacB enzyme purified from culture filtrate of Z. mobilis Z6C has a molecular weight of 56 kDa (Yanase et al., 1992). The gene encoding (sacB) levansucrase and (sacC) in Z. mobilis NRRL B.806 and ATCC10988 has been cloned and sequenced (Gunasekaran et al., 1995). Recently, the over expressed levansucrase (SacB) was purified from the recombinant E. coli by a simple method and characterized (Sangiliyandi and Gunasekaran, 1998). A levansucrase (SacB) was purified to electrophoretic homogeneity from a recombinant E. coli. The 55 kDa enzyme hydrolyzed β-fructosides but not α-glucosides and catalysed levan formation from sucrose as well as raffinose. The optimum temperature for polymerase activity was at 30°C and hydrolase activity at 50°C. However, the polymerase activity was selectively abolished by para-chloromercuribenzoate (Sangiliyandi et al., 1999a) and also by in-vitro mutagenesis (Sangiliyandi et al.,1999b). Recently, a levansucrase mutant was isolated by in-vitro mutagenesis using hydroxyamine. By mutation in the protien, the levanforming activity was abolished but the sucrase activity was retained in the mutant strain. This uncoupling of the sucrase activity from the levan-forming activity suggests a change in the protein conformation due to mutation and elevated temperature could have led to the loss of activity (Sangiliyandi and Gunasekaran, 2001).

An extracellular sucrase (Sac C or InvB) has a high specific activity for sucrose hydrolysis. Nearly this enzyme contributes 60% of the extracellular sucrose activity. However, it catalyses neither fructose polymerization into levan nor degradation of polyfructose such as levan or inulin. Thus this enzyme differs from the B. subtilis SacC, which shows levanase activity in addition to sucrose hydrolysis (Kannan et al., 1995). The purified active enzyme from Z. mobilis strain CP4 is a monomer with a molecular weight of 46-47 kDa (O Mullan et al., 1992). The sacC gene was cloned and expressed in E. coli (Kannan et al., 1995). The overexpressed extracellular sucrase of Z. mobilis was purified from a recombinant E. coli by repeated cycles of freezing and thawing, followed by hydroxyapatite column chromatography. The purified enzyme had a Mw of 46kDa, as determined by SDS-PAGE. Its km value for sucrose was 86mM and had an optimal pH and temperature at 5.0 and 36°C respectively (Sangiliyandi and Gunasekaran, 2000). Here we show the differential expression of levansucrase and extracellular sucrase activity of Z. mobilis sucrose genes in E. coli and Z. mobilis hosts.

MATERIALS AND METHODS

Bacterial strains and plasmids
Escherichia coli JM109 (RecA1, endA1, gyrA96 thi, hsdR17, SupE, relA1(lac proAB) [F' traD36 proAB, lacIq, ΔM15] BL21(DE3) F’ hsdS gal (r m ) lon ompT. XL1 Blue EndA1 hsd17 (r m ) supE44 thi-1 λ-gyrA96 recA1 Δlac (F' proAB lacIq ΔM15 Tn10). Plasmid pBluescript KS+(Stratagene, USA). Z. mobilis wild type (NRRL B-806). The recombinant plasmid pLSS41 carrying 5 kb chromosomal DNA fragment coding for sacB and sacC of Z. mobilis. Plasmid pRK2013 was used as a helper for conjugations.

Growth conditions
Z. mobilis strains were grown in complete media RM containing (per liter) glucose 20 g, yeast extract 10 g, KH2PO4 2 g and pH adjusted to 6.0. Whenever necessary, different carbohydrate sources such as glucose (RMG) or fructose (RMF) or sucrose (RMS) was added to RM. The cultures were grown at 30°C at static condition. E. coli strains were grown at 37°C under agitation in LB or M63 medium.

Construction and screening of recombinant sucrase clones
A 1.7 kb DraI fragment containing an ORF encoding levansucrase (SacB) from the plasmid pLSS41 was purified by the method of Suzuki et al. (1991) and then ligated with EcoRV digested pBluescript KS+. The resultant plasmid pLS3 had only sacB gene located downstream of the T7
promoter in the pBluescript KS+. This recombinant plasmid was transformed into E. coli JM109. A 2.1 kb Pvu II fragment containing an ORF encoding levansucrase (SacB) from the plasmid pLS3 carrying sacB was purified and cloned into Eco RV site in the shuttle vector pZA22. The resultant plasmid was designated as pLSP13. A 2.1 kb Pvu II fragment containing an ORF encoding extracellular sucrase (SacC) from the plasmid pLS41 carrying sacC was purified and cloned into the Eco RV site of pBluescript KS+. The resultant plasmid was termed pZS63. A 2.1 kb Pvu II fragment containing an ORF encoding extracellular sucrase (SacC) from the plasmid pLS63 carrying sacC was purified and cloned into the Eco RV site of the shuttle vector pZA22. The resultant plasmid was termed pZSP62.

**Assay of levansucrase activity**
Sucrose hydrolyzing and levan forming activities were assayed as previously described (O’Mullan et al., 1992). Levan was estimated by hydrolyzing the levan in 0.1 N HCl at 100 °C for 1 h according to Viikari and Gisler, (1986) and expressed as fructose units. One unit of levansucrase was defined as the amount of enzyme required to produce 1 µmole of levan (as fructose units) in one minute. One unit of sucrase hydrolysing activity was defined as the amount of enzyme releasing 1 µmole of reducing sugar per minute. The protein concentration was determined by the method of Lowry et al. (1951).

**Recombinant DNA techniques**
Preparation of plasmid DNA was carried out by standard method (Sambrook et al., 1989). Purified plasmid was obtained using plasmid purification kit (Qiagen Inc., USA). The DNA fragments were separated as mentioned by Suzuki et al. (1991). All cloning experiments were carried out according to standard methods (Sambrook et al., 1989). Transformation was performed as described by Chung and Miller, (1988).

**Bacterial conjugations**
Bacterial conjugations were performed by triparental filter matings as previously described (Arvanitis et al., 2000). For counter selection against donor cells, 100mg/ml nalidixic acid was used for ATCC 10987. All transconjugants were tested for their plasmid content with plasmid isolation, back transformation, and restriction analysis.

**Dot Blot hybridization**
Northern Dot Blots containing up to 5µg of denatured RNA were prepared using 9x12cm Zeta Probe GT Nylon membranes and a Bio Dot SF Microfiltration apparatus (Bio-Rad 170-6543). Membranes were hybridized using Express Hyb Hybridization solution (Clontech), and DNA probes end radiolabeled by using (γ32P) dATP (3000Ci/mmol) or by direct incorporation using (α-32P) dCTP (3000Ci/mmole) Amersham redivue). Membranes were washed twice for 30min at 61°C in 1mM EDTA, 40mM NaHPO4, pH 7.2 and 5% according manufacturers instructions. RNA signal in the same sample using phosphorimager analysis and the data acquisition packages provided with instrument.

**Northern Blot hybridization**
Total RNA of Z. mobilis and E. coli was isolated as described previously (Ausubel et al., 1994). Samples (5µg) were electrophoresed on a 0.8% agarose-formaldehyde gel and transferred to a nylon membrane filter (Schleicher and Schuell). Pre-hybridization and hybridization was carried out in hybridization solution (50% formaldehyde, 5x Denhardt reagent, 50mM sodium phosphate (pH5.5), 120 mM NaCl, 0.1% SDS, 125g of sonicated salmon sperm DNA per ml) at 50°C. The gene specific probes were labeled with random-primed labeling kit (Boehringer Mannheim). Filters were washed twice at room temperature and twice at 65°C in 2X SSC-0.5% SDS and then washed once at room temperature in 0.1x SSC. These filters were exposed to X-ray films.

**RESULTS AND DISCUSSION**

**Subcloning of sucrase genes of Z. mobilis in E. coli**
In order to study the promoter strength of levansucrase and extracellular sucrase genes, the sacB gene and sacC gene were cloned and expressed in E. coli. Z. mobilis chromosomal DNA fragment (a 5.0 kb HindIII fragment) present in the plasmid pLSS41 possess ORF1 and ORF2 that encoded extracellular levansucrase (SacB) and extracellular sucrase (SacC) respectively.
(Gunasekaran et al., 1995). The levansucrase gene \textit{sacB} resides within the two \textit{DraI} restriction sites separated by 1.7 kb. The purified 1.7 kb fragment was cloned into \textit{EcoRV} site of pBluescript \textit{KS}’. The ligated mixture was used to transform \textit{E. coli} JM109. The transformants were selected on LB agar containing ampicillin (20 mg/l), IPTG (40 mg/l) and X-gal (20 mg/l) by Blue-white selection. All the white colonies were scored as recombinant clones. The recombinant clones were independently grown in M63 medium supplemented with glycerol (1% v/v), cell lysate was prepared and assayed for both sucrose hydrolyzing activity and levan forming activity. The cellular fractions of all recombinant clones exhibited sucrase activity and levan forming activity on standard plate screening. Plasmid was isolated from a recombinant clone and was designated as pLS3. The plasmid pLS3 was used to transform \textit{E. coli} JM109. All the transformants exhibited levansucrase positive phenotype i.e. sucrose hydrolyzing and levan forming activities, confirming the plasmid pLS3 carried \textit{DraI} fragment encoding levansucrase. The same selection procedure was followed for screening of pZS63 plasmids.

2.1 kb \textit{PvuII} fragment in pLS3 carrying \textit{sacB} was isolated and cloned into \textit{EcoRV} digested pZA22. The ligated mixture was used to transform \textit{E. coli} JM109. Transformants were selected on LB agar containing Cm (30 µg/ml) and replica plated on LB agar containing Tc (15µg/ml). The Cm-Tc cells were selected as recombinant clones and were analyzed for levansucrase activity. Plasmids from one of the levansucrase positive clone was isolated and designated as pLS13. For subcloning of \textit{sacC} gene, a 2.1 kb \textit{PvuII} fragment from pZS63 was isolated and ligated with \textit{EcoRV} linearized pZA22. The ligation mixture was used to transform \textit{E. coli} JM109. Transformants were selected on LB Cm (30 µg/ml) plates, replica plated on LB Tc plates and tested for sucrose hydrolysis activity as described earlier. One of the sucrose positive clones carried a recombinant plasmid, designated as pZSP62. The presence of the 2.1 kb insert in this plasmid was confirmed by restriction analysis. This plasmid was 8.8 kb in size and carried \textit{sacC} gene downstream to T7 promoter.

### Expression of Sucrase genes in \textit{E. coli}

The plasmids pLSP13 and pZSP62 (in pZA22, shuttle vector) were separately transferred into the \textit{E. coli} strains JM109 and XL1Blue in which the \textit{sacB} and \textit{sacC} genes use their own promoter for expression. Comparison of the recombinant strains grown in LB medium revealed that the \textit{E. coli} strain carrying \textit{sacB} gene exhibited sucrase activity of 0.24-0.27 U/mg while the strains carrying \textit{sacC} gene exhibited sucrase activity of 3.03-3.10 U/mg. Similar comparison of the strains grown in M63 medium showed that the sucrase activity of the strain carrying cloned \textit{sacB} gene was 0.60-0.63 U/mg while the strain carrying cloned \textit{sacC} gene expressed an activity of 7.04-7.10 U/mg. These results suggested that, the level of \textit{sacB} gene expression in \textit{E. coli} was almost ten fold less than the expression of \textit{sacC} gene, irrespective of the growth medium or the host strain (Fig. 1.)

The plasmids pLS3 and pZS63 (in \textit{pBSKS}+) were independently transferred into \textit{E. coli} strains and the expression of \textit{sacB} and \textit{sacC} genes were studied under the conditions of with and without IPTG. The cloned genes were expressed using T7 as well as the native promoter in \textit{E. coli}. Analysis of the LB grown cells revealed that \textit{E. coli} (pLS3) produced a low level of sucrase activity (0.56 – 0.60 U/mg) than the \textit{E. coli} (pZS63) (5.79-5.80 U/mg). Comparison of the cells grown in different media revealed that the M63 medium grown cells produced higher level of sucrase activity. In this medium, the \textit{sacB} gene carrying cells produced sucrase activity of 1.12-1.20 U/mg while the \textit{sacC} gene carrying strains exhibited sucrase activity of 9.15 –9.20 U/mg. These results suggested that the expression of \textit{sacB} was almost 10 fold lower than that of the \textit{sacC} gene, when it was expressed by its own promoter.

### Comparison of induced expression of \textit{sacB} and \textit{sacC} genes in \textit{E. coli} BL21

As we expected, the previous experiments showed that the expression of SacC was more than SacB by its own promoter. Then we examined the \textit{E. coli} (carrying plasmids for \textit{sacB} and \textit{sacC} in which the genes were cloned under T7 promoter) cultures were grown in M63 medium with glycerol or fructose till they reached 1 OD at A600. IPTG (400 µM) was added to the cells and harvested after 4 h of incubation at 37°C. Addition of IPTG to the growing cultures of recombinant \textit{E. coli} BL21 induced the expression of \textit{sacB} or \textit{sacC}.
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Isolation of sucrase negative mutant of *Z. mobilis*

A highly stable extracellular levansucrase, extracellular sucrase and intracellular sucrase mutant (SNM10) of *Z. mobilis* strain B-806 was isolated after NTG mutagenesis (50 µg/ml for 45 min at 30 °C) from the strain LS1A (Kannan et al., 1998). It was repeatedly sub cultured in glucose medium and plated on RM agar plates. The cellular extracts of the randomly selected colonies were subjected to zymogram analysis for the detection of sucrase activity. Some of the isolate did not produce the sucrase band corresponding to SacA, SacB and SacC and it was designated as SNM 1-10. Among 10 isolates, one of the isolate was taken for further studies. For confirming the stability of the SacA, SacB and SacC mutation, the strain (SNM10) was subcultured (3 times, 18 h growth period) in RM glucose medium, and then plated on RM agar plates. Randomly selected colonies were grown in RM glucose/fructose medium and the cellular fractions were analysed on native -PAGE and by zymogram staining (data not shown). None of the culture showed any reversion of the SacA, SacB and SacC mutation. As this strain was derived from the strain LS1A, the cellular fraction did not exhibit sucrase band corresponding to levansucrase (SacB) and intracellular sucrase (SacA). Therefore, the mutant LS1A was phenotypically defective for the synthesis of levansucrase and intracellular sucrase. The novel complete sucrase negative mutant SNM10 was phenotypically defective for the synthesis of all sucrases. We used this mutant (SNM10) for the expression of each plasmid encoded sucrase genes in *Z. mobilis*.

Expression of sucrase genes in *E. coli* and *Z. mobilis*

We examined the effect of plasmid encoded sucrase synthesis in *E. coli* and different sucrase mutants of *Z. mobilis*. The plasmid were transformed in to *Z. mobilis* as described in Material asnd Methods section. Table 2 shows the extracellular sucrase activities exhibited by extract of *E. coli* and *Z. mobilis*. Strain B-806 was expressing sucrase genes by chromosomal encoded but the mutants strains of *Z. mobilis* were expressing by plasmid encoded sucrase genes. Most of the extracellular sucrase activity (nearly 70%) produced by sacC gene and remaining activity was produced by sacB gene in *Z. mobilis*, as well as the same result was also observed in *E. coli* strains. Previous experiments have shown that the sacC gene carrying *E. coli* strain caused dramatic increase in the functional expression. The same effect was observed in *E. coli* and recombinant *Z. mobilis* strains.

Table 1 - Comparison of induced expression of sacB and sacC genes in *E. coli* BL21.

<table>
<thead>
<tr>
<th>E. coli BL21 strain carrying the plasmid</th>
<th>Name of the gene expression</th>
<th>Sucrase activity U/mg Un induced</th>
<th>Sucrase activity U/mg Induced</th>
</tr>
</thead>
<tbody>
<tr>
<td>pLS3</td>
<td>sacB</td>
<td>4.5</td>
<td>72.0</td>
</tr>
<tr>
<td>pLS13</td>
<td>sacB</td>
<td>1.9</td>
<td>19.0</td>
</tr>
<tr>
<td>pZS63</td>
<td>sacC</td>
<td>24.6</td>
<td>2672.0</td>
</tr>
<tr>
<td>pZSP62</td>
<td>sacC</td>
<td>12.8</td>
<td>1948.0</td>
</tr>
</tbody>
</table>
Table 2 - Expression of plasmid encoded sucrase genes in E. coli and sucrase mutants of Z. mobilis

<table>
<thead>
<tr>
<th>Strains</th>
<th>Basal level expression Without plasmid</th>
<th>Basal level expression plus SacB (pLSP13)</th>
<th>Basal level expression plus SacC (pZSP62)</th>
<th>Total (Basal level +SacB + SacC Levan forming activity+ Sucrose hydrolysing Activity (U/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-806</td>
<td>5.9</td>
<td>5.9 + 2.0</td>
<td>5.9 + 2.0 + 4.0</td>
<td>11.9</td>
</tr>
<tr>
<td>LS1</td>
<td>3.9</td>
<td>3.9 + 2.15</td>
<td>3.9 + 2.15 + 4.2</td>
<td>10.25</td>
</tr>
<tr>
<td>LS1A</td>
<td>3.8</td>
<td>3.8 + 2.0</td>
<td>3.8 + 2.0 + 4.1</td>
<td>9.9</td>
</tr>
<tr>
<td>SNM10</td>
<td>0.0</td>
<td>0 + 2.25</td>
<td>0 + 2.25 + 4.3</td>
<td>6.8</td>
</tr>
</tbody>
</table>

We successfully introduced sucrase genes for synthesis of sucrase in wild type and sucrase negative mutants by expressing the Z. mobilis genes encoding sacB and sacC separately. Under the control of the each gene promoter, the specific activities of these two enzymes ranges from 2.0 to 4.3 U/mg (Table 2). However, both the genes were expressed successfully in all strains. The level of expression of the enzymes by plasmids was considerably higher than the levels of expression observed in chromosomal encoded. The difference between the levels of expression may be a function of a plasmid copy number and of transcript coupled with and protein size and stability.

**Transcriptional analysis of sacB and sacC genes**

According to the previous experiments, the sacC gene was expressed more stronger than sacB gene, irrespective of the medium and strains. we wanted to examine the same effect of sacB and sacC gene in E. coli as well as Z. mobilis by transcriptional analysis. Hybridization of RNAs from Z. mobilis and E. coli (carrying sacB and sacC genes) cells, which were grown on glucose, fructose and sucrase was performed in order to determine whether enzyme induction was the result of transcriptional regulation of sucrase genes by carbon sources or the effect of promoter. The bands of 1.5 and 2.9 kb in size were also detected from cells grown on media containing glucose or fructose and sucrase and also E. coli cells grown minimal media. From these results, we concluded that sacB and sacC genes were expressed constitutively at the basal level (data not shown).

**Dot blot analysis**

In order to determine, if the high level expression of SacC was due to its strong promoter or was influenced by any carbon sources. Dot blot analysis of sucrases of mRNA levels in Z. mobilis cells grown on glucose, fructose and sucrase was performed. Surprisingly, the levels of sucrase mRNA of Z. mobilis cells grown on sucrase were found to be approximately three fold higher than the levels observed in cells grown on glucose and two fold higher than the levels observed in cells grown on fructose. Similar cases have been postulated in the gene expression of Z. mobilis and also depends on carbon source dependent regulation (Liu et al., 1992) It was of interest to determine if the increased level of transcript in sucrase grown cultures was also a general phenomenon for the expression of sucrase genes. Filters prepared with RNA samples were subjected to hybridization with sacB and sacC gene specific probes . Strong hybridization signals were detected for the RNA samples hybridized with sacC probes than sacB probes. Among the RNA samples from the cultures grown in different carbon sources, the maximum sucrase transcript was from the culture grown in sucrose (Figs. 2a and 2b). These experiments showed that the high amount of specific mRNA were present in sucrose grown culture than glucose and fructose grown cells.

**NORTHERN ANALYSIS**

To compare the expression of plasmid encoded sacB and sacC genes in Z. mobilis, and recombinant E. coli strains, the RNA samples from all cultures were subjected to northern hybridization analysis using specific probes for sacB and sacC. While hybridized with sacB gene probe, strong signals were detected for the RNA samples of Z. mobilis and E. coli (pLS3) but relatively faint signal was observed for RNA samples from E. coli (pLSP13). Quantitative analysis of multiple hybridization experiments consistently showed two fold higher level of
transcripts detected in Z. mobilis and E. coli (pLS3) than in E. coli (pLSP13). The sacB probe did not hybridize with sacC transcript from E. coli (pZS63) (Fig. 3a.). Similarly the sacC specific probe did not hybridize with the transcript from levansucrase clones i.e. E. coli (pLS3). The hybridization of sacC probe with the RNA isolated from Z. mobilis and E. coli was studied. The levels of sacC mRNA levels in E.coli (pZS63) was three fold higher than E.coli (pZSP62) and two folds higher than Z. mobilis (Fig. 3b). The SacC probe did not hybridize with transcript from E. coli (pLS3).

Comparison of the sacB and sacC transcripts, in the above strains revealed that the level of sacC transcript was approximately three fold higher than sacB transcript in Z. mobilis and also E. coli expressed sacB gene. Comparison of the transcript levels in the strains [ E. coli (pLS3) with (pZS63) and E. coli (pLSP13) with E. coli (pZSP62)] revealed that the sacB transcripts were much lower than the sacC transcript. These results suggested that the expression of sacB in either Z. mobilis or E. coli was much lower than the expression of sacC gene, probably due to inefficiency of sacB promoter. However, it is observed that extracellular sucrase (SacC) was unstable than extracellular levansucrase (SacB) (Sangiliyandi and Gunasekaran, 1998; Sangiliyandi et al., 1999a; Sangiliyandi et al., 1999b).

A high relative abundance of sacC specific transcripts might compensate for the instability of the Z. mobilis extracellular sucrase. Similar result was observed with the polycistronic mRNA of the Z. mobilis glf, zwf, edd, glk operon. Liu et al (1992) suggested that a high relative abundance of glk specific transcripts might compensate for the instability of the gluokinase. Eddy et al. (1989) reported that the ratio of gap message to pgk message was approximately 5:1 ratio. The differential expression of gap and pgk appears to be controlled at the mRNA level.

The pattern of codon usage in sacB gene was compared with two classes of genes known in Z. mobilis - highly expressed genes like pyruvate decarboxylase (pde), glucose-6-phosphate dehydrogenase (zwf) and poorly expressed genes like intracellular sucrase (SacA), acid phosphatase (PhoC) (Pond et al., 1989) and ligase (lig) (Gunasekaran et al., 1990a). The codon bias was calculated according to Shark and Conway (1992). It was found that the codons in sacB strongly correlated with codon usage and bias with poorly expressed genes. The three codons TGC, CTA and TAC were not used in sacB. The codon TGT only was used for cys in sacB. In sacB the preference of codons AAT, GAT, CAT and AAA were similar to low expressed genes. However, the use of codon CAG and TAT in sacB was similar to highly expressed genes. Thus, sacB could be classified as one of the low to intermediated expressed genes of Z. mobilis.

The relative abundance of the SacC enzyme in Z. mobilis might be due to high levels of sacC mRNA, a high rate of translation of the sacC message, or resistance of the sacC to proteolysis. Since most of the enzymes remain at high level at stationary phase, this factor alone can not be accounted for the high SacC activity. Therefore, the high level of extracellular sucrase results from an abundant sacC message, perhaps due to a combination of rapid transcription and significant mRNA stability. Stability of the sacC message might be imparted by the substantial stem-loop structures located both upstream and downstream of the sacC gene as suggested for several other Z. mobilis genes. In Northern blot analysis, increased expression of the SacC in cells grown on sucrose was proven to be the result of an increased transcription rate, compared with cells grown on glucose and fructose (Figs. 3a and 3b). Similar results were also observed in the gene expressions of Z. mobilis; carbon source dependent regulation (Liu et al., 1992). Considering the amount of transcripts, both the genes in the operon seemed to be expressed differently from each other, because of the existence of terminator sequence of sacB and sacC gene (Song et al., 1999). Similarly, Newbury et al. (1987) postulated that an intercistronic transcription terminator could act as a gene expression regulator in the analysis of a polycistronic malEFG operon of E. coli. Northern blot analysis suggested that the levels of sacC transcript were approximately three folds higher than sacB transcript in Z. mobilis as well as the E. coli (pZS63 or pZSP62). Thus, it is surprising that, the gene dosage can improve the hydrolysis of sucrose substrates during bioconversion to ethanol. Optimization of such a system by manipulating the growth conditions and genetically modifying the host metabolism as well as development of ethanol retrieval technologies for the growth medium, could lead to production of ethanol by this organisms at an industrial level.
Figure 1 - Expression of sacB and sacC genes in *E. coli*.
The cultures of *E. coli* were grown in LB and M63 media and sucrase activity was assayed after 24 hrs.

Figure 2 - Dot blot analysis of sucrase gene transcripts
*Z. mobilis* B-806 was grown to mid logarithmic phase in media glucose (1) or fructose (2) or sucrose (3) internal control (4) and total RNA was extracted. The sacB (A) and sacC (B) specific transcripts were identified using sacB and sacC genes probes respectively.
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Figure 3 - Northern blot analysis of sucrase genes transcripts

Z. mobilis B-806 was grown to mid logarithmic phase in fructose and E. coli strains were grown on M63 medium. A) Hybridization of sacB specific probes with total RNA from Z. mobilis (lane 1); E. coli pLS3 (lane 2); E. coli pLS3 (lane 3) and internal control (lane 4). B) Hybridization of sacC specific probes with total RNA from Z. mobilis (lane 1); E. coli pZSP62 (lane 2); E. coli pZS63 (lane 3) and Internal control (lane 4).

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REFERENCES


RESUMO

Os genes do sacB e do sacC que codificam o levansucrase e o sucrase extracellular respectivamente foram sub-clonados independentemente no pBluescript (número elevado de cópias) e no vetor ponte para Z. mobilis-E. coli, pZA22 (baixo número de cópias). A expressão destes genes foi comparada tanto em E. coli quanto em Z. mobilis. O nível da expressão do gene sacB em E. coli foi quase dez vezes menor do que a expressão do gene sacC, independente do meio de cultivo ou da linhagem hospedeira. Em Z. mobilis, a expressão dos genes sacB e sacC mostrou regulação dependente da fonte de carbono. Os níveis dos transcritos sacB e sacC estavam três vezes mais aumentados em células cultivadas em sacarose do que aquelas cultivadas em glicose/frutose. A análise por Northern blot revelou que o nível do transcripto sacC era 2-3 vezes maior do que aquele de sacB. Esses resultados sugerem que a expressão do gene sacC foi mais pronunciada do que sacB.


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