Effect of SinR Transcriptional Factor on the Expression of Bacilysin Biosynthetic Operon in *Bacillus subtilis*

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***Abstract*— In *Bacillus subtilis*, bacilysin is a nonribosomally synthesized dipeptide antibiotic composed of L-alanine and L-anticapsin. It is active against a wide range of bacteria and even *Candida albicans*. The biosynthesis of bacilysin depends on the *bacABCDEywfG* operon (*bac* operon) and the adjacent monocistronic gene *ywfH*. In our previous study, *lutR* mutation significantly decreased the maximum transcription level of the bacABCDEywfG operon at the onset of stationary phase to about 57% of wild-type level. In this study, we aimed to test the possible effect of global regulator SinR on the expression of the *bac* operon. For this *sinR* gene was disrupted in the transcriptional *bacA-lacZ* fusion bearing strain (OGU1) to generate "*sinR::cm bacA::lacZ::erm*" bearing strain (OGU1SR). Additionally, we also test whether *sinR* and *lutR* gene products affect the *bac* operon expression mutually due to the close regulatory interactions between LutR and SinR transcriptional factors . For this, *ΔsinR-ΔlutR* double mutant (*sinR*::cm *lutR::Tn10::spc bacA::lacZ::erm)* strain (OGU1SRLR) was constructed. Finally, all of that resulting strains and OGU1 were grown in Perry and Abraham (PA) medium at 37ºC and β–galactosidase activities were measured throughout the different stages of growth. β–galactosidase assay results indicated that in the absence of *sinR* gene product *bac* operon expression was severely effected. Since, disruption of *sinR* gene resulted with complete loss of transition state dependent induction of *bac* operon expression while almost the same *bacA*-expression profile as the single *sinR* mutant was detected in the *sinR-lutR* double mutant.**

***Keywords- Bacillus subtilis;* bacilysin*; bacABCDEywfG; bac* Operon**

I. INTRODUCTION

Antibiotics have a crucial role in keeping the public healthy. Understanding the genetic and molecular basis of the regulation of antibiotic biosynthesis is of great interest for providing new strategies for targeted genetic engineering of antibiotic producing strains.

Bacilysin is a simple dipeptide antibiotic which is composed of an L-alanine residue at the N-terminus and an unusual amino acid, L-anticapsin, at the C-terminus [1] Antimicrobial activity of bacilysin depends on the anticapsin moiety [2]. It is active against wide range of bacteria and even *Candida albicans.*

*B*acilysin biosynthesis is regulated either positively or negatively. Global regulatory proteins CodY and AbrB negatively regulate the *bac* operon, on the other hand global regulatory proteins Spo0A and ComA positively regulate the *bac* operon expression [3]. In addition to master regulatory genes, bacilysin biosynthesis in *Bacillus subtilis* was also demonstrated to be under the control of GntR type transcriptional regulator LutR which was identified by transposon mutagenesis technique [4].

SinR is a global transcriptional regulator that is required for sporulation, competence, motility, exoprotease production and biofilm formation in *B. subtilis.* In our previous study, it was shown that LutR is a transcriptional factor that has a function in the regulation of many pathways and there is a close overlap among the targets of LutR and SinR regulatory proteins [5]. In this study, we aimed to test the possible effect of global regulator SinR on the expression of the *bac* operon as well as to test whether *sinR* and *lutR* gene products affect the *bac* operon expression mutually by using *lacZ* fusion analysis.

The rest of this paper is organized as follows. In Section II we described the materials and methods used in this study. In Section III we described and evaluated the main results obtained in this study.

II. MATERIALS AND METHODS

A. Bacterial strains, media and culture conditions

The strains of *B. subtilis* used in this study are listed in Table 1. All these strains were the derivatives of *B. subtilis* 168.

TABLE 1. BACTERIAL STRAINS AND THEIR GENOTYPE

Strain	Genotype	Source
B.subtilis PY79	Wild type, BSP cured prototrophic derivative of *B.subtilis* 168	P.Youngman
OGU1	*bacA::LacZ::erm*	İ.Öğülür
SinR mutant *B. subtilis*	*ΔsinR::cm*	AkosT. Kovacs
OGU1LR	*lutR::Tn10::spc bacA::lacZ::erm*	T. E. Köroğlu
OGU1SR	*ΔsinR::cm bacA::LacZ::erm*	In this study
OGU1SRLR	*lutR::Tn10::spcΔsinR::cm bacA::lacZ::erm*	In this study

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B. subtilis strains were normally grown in Luria Bertani (LB) medium at 37°C. The Perry and Abraham (PA) medium was used for bacilysin production. Antibiotic concentrations employed in this study for direct selection were as follows: erythromycin (1 µg/ml), lincomycin (25µg/ml), spectinomycin (100 µg/ml), chloramphenicol (5 µg/ml) and ampicillin (100 µg/ml).

B. Insertional inactivation of regulatory genes

For construction of OGU1SR (*sinR::cm bacA:.lacZ::erm*) strain , genomic DNA of *sinR* disrupted *B. subtilis* strain that harbors *ΔsinR::cm* mutation was isolated and transformed into the competent cells of *bacA-lacZ* fusion bearing strain OGU1. *ΔsinR-ΔlutR* double mutant strain OGU1SRLR was constructed by transforming competent cells of OGU1LR (*lutR::Tn10::spc bacA::lacZ::erm*) strain with chromosomal DNA from OGU1SR (*sinR::cm bacA:.lacZ::erm*) strain.

C. β–Galactosidase Assay

β-Galactosidase assay was carried out as described by Miller (1972). The specific activitiy is expressed as [A_{420}-1.75 X A_{550} per Minute per OD_{595}]X1000.

III. RESULTS AND DISCUSSION

To analyse the effect of *sinR* and *lutR* insertional inactivations on the *bac* operon expression, transcriptional *lacZ* fusion analysis is performed. For that purpose, we first constructed the congenic derivatives of the transcriptional *bacA-lacZ* fusion bearing OGU1 with the mutation of the interested genes *sinR* and *lutR*. The resulting strains OGU1SR, OGU1SRLR, OGU1LR and OGU1 as a control, were cultured in PA medium at 37°C and sampled in every 1 h for the β-galactosidase assay. As shown in Figure 1, expression of the *bac* operon was persistent during the exponential growth phase. However, it induced in the course of transition between exponential and stationary phases and reached to its top level upon entry into stationary phase. As shown previously, *lutR* mutation significantly reduced the maximum transcription level of the *bac* operon (Figure 1). On the other hand, the deletion of the *sinR* gene severely affected the *bacA-lacZ* expression and resulted with the complete loss of transition state dependent induction of the *bac* operon expression. However, almost the same *bacA-* expression profile as the single *sinR* mutant was detected in the *sinR-lutR* double mutant.

IV. CONCLUSION

Results of this study indicate that both SinR and LutR regulatory proteins are required for the maximum *bac* operon expression but SinR activity is essential for the transition state induction of the *bac* operon expression.

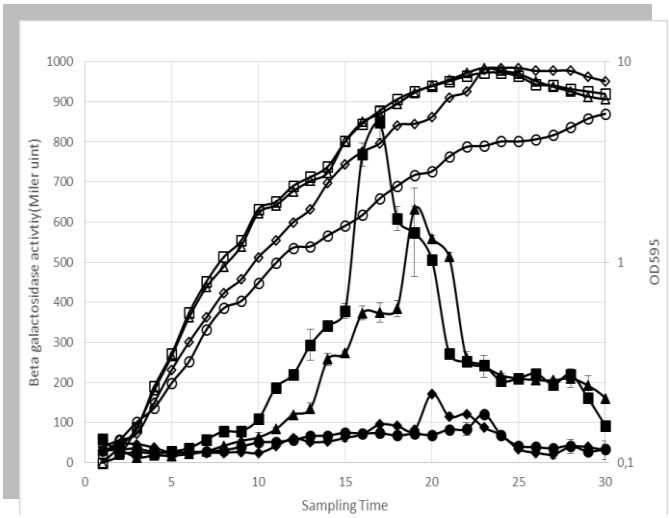

Figure 1. Growth curves and β-galactosidase activity of *bacA::lacZ* fusion OGU1 (white squares and black squares) and its derivatives *lutR* mutant (white triangles and black triangles), *sinR* mutant (white circles and black circles) and *lutR-sinR* mutant (white diomands and black diomands) in PA medium. Error bars represent the standard deviation of three independent experiments .

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