DETECTION OF NOVEL SMALL RNAs FROM BACILLUS SUBTILIS BY DNA MICROARRAY

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INTRODUCTION: Small RNAs are the regulatory molecules that act post-transcriptionally to regulate the expression of target mRNA molecules. In B. subtilis only a few small RNA molecules have been described in the recent past (2, 3). Here we report ~20 novel small RNA molecules identified by DNA microarray approach from B. subtilis 168.

METHOD: Small RNAs were predicted from B. subtilis 168 genome using Intergenic Sequence Inspector (ISI) program (1) (available online free at www.biochpharma.univ-rennes1.fr/). Intergenic (IG) sequences from B. subtilis 168 genome were compared with the genomes of other closely related Gram positive bacteria using Standalone BLAST and potential small RNA encoding IG regions were selected on the basis of sequence homology (>80ntd) and GC% (>35%). Out of ~100 candidates predicted, 42 were explored using DNA microarray, which was developed by spotting PCR products in quadruplicates from selected IG regions using Piezoarray (M/s Perkin Elmer, USA). Total RNA isolated from B. subtilis 168 were compared with the genomes of other closely related Gram positive bacteria using Standalone BLAST and potential small RNA encoding IG regions were selected on the basis of sequence homology (>80ntd) and GC% (>35%). Out of ~100 candidates predicted, 42 were explored using DNA microarray, which was developed by spotting PCR products in quadruplicates from selected IG regions using Piezoarray (M/s Perkin Elmer, USA). Total RNA isolated from B. subtilis 168 was reverse transcribed and labeled with TSA labeling kit (Micromax, USA). Labeled cDNA was hybridized to the DNA probes on microarray slides for 16hrs at 60°C. After processing for washing and detection, microarray slides were scanned in DNA Microarray Scanner (Perkin Elmer, USA) and Spot intensities were analyzed with Scan array Express.

RESULTS: Small RNA expression in B. subtilis was studied by DNA microarray at exponential and post-exponential phase in a rich medium (Luria Bertani broth). Microarray image was analyzed for expression of selected 42 IGRs (Intergenic regions). IGRs were classified in three groups depending upon normalized intensity of all four spots of a single IGR, viz., IGRs showing intensity >10,000, between 5000-10000 and < 5000. IGRs showing more than 10000 intensity were considered the most likely candidates for small RNAs. Out of 42 IGRs, two IGRs from exponential phase and 20 IGRs from post-exponential phase RNA samples
showed intensity more than 10,000. Five IGRs from exponential and 3 from post exponential phase showed intensity between 5000-10000.

**DISCUSSION:** We hereby for the first time report ~20 novel small RNA molecules expressed in the post-exponential phase of *B. subtilis*. Post exponential phase of *B. subtilis* is marked by quorum sensing and quorum sensing dependents processes like surfactin production, competence development and initiation of sporulation.

![Expression profile of small RNAs of B. subtilis across microarray during post-exponential phase](image.png)

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**REFERENCES:**