Deciphering the Role of the sinR Gene Homolog in Halobacillus BBL2006

J. Martinez and G. Kirchner
Department of Biology, Indiana University Southeast, New Albany, IN 47150

ABSTRACT

The sinR gene and its protein are responsible for the regulation of biofilm formation in Bacillus subtilis. Bacterial biofilms are important ecologically and medically, so the study of biofilm formation in the environmental isolate Halobacillus BBL2006 could contribute significantly to the knowledge of these bacterial systems. We have recently sequenced and annotated the whole genome of Halobacillus BBL2006, and identified and isolated a homolog to the sinR gene. We plan to mutate (knock out) the sinR gene in the Halobacillus bacteria to determine the effect upon biofilm formation in this organism.

INTRODUCTION

The moderate halophiles are a fascinating group of salt tolerant bacteria that can respond to increasing or decreasing salt concentrations in the environment. The gram positive, spore forming bacteria of the genus Halobacillus are representative of the moderate halophiles. Due to their tolerance of varying salt concentrations, moderate halophiles are promising candidates for the bioremediation of salt contaminated water. In addition, these bacteria can form biofilms, complex communities of bacterial cells, which may enhance their ability to bind metal contaminants. As part of our research we have characterized their ability to bind metal contaminants. As part of our research we have characterized 

METHODS

- Locating the sinR gene homolog of Halobacillus BBL2006
  We used the protein Basic Local Alignment Search Tool (BLASTp) from the National Center for Biotechnology Information (NCBI) to identify the SinR protein
  in Halobacillus halophilus which is homologous to that of the SinR protein of Bacillus subtilis.
  
- Next, we used the nucleotide BLAST (BLASTn) to locate the sinR gene homolog from our whole genome sequence of Halobacillus BBL2006 using the nucleotide sequence of H. halophilus.
  
- Cloning Strategy
  * Clone sinR PCR product into pGEM T-easy vector which is a T vector designed to clone PCR product amplified with Taq polymerase
  * The pGEM sinR DNA is ligated and transformed into competent E. coli cells
  * The cloned plasmid is extracted and digested with BglII restriction enzyme which will allow for the cutting open of the sinR gene where the chloramphenicol acetyltransferase (CAT) gene will be inserted using matching BglII ends
  * Use of our original primers designed for sinR PCR will amplify a large quantity of this sinRcat gene construct
  * This amplified DNA will then be used to transform Halobacillus BBL2006, which should result in the strain having a defective sinR gene due to insertional inactivation
  * Transformed cells will grow on LB agar plates supplemented with chloramphenicol antibiotic.
  * The result that we expect is long-chain biofilm-like growth in Halobacillus BBL2006
  * Primers were ordered from Integrated DNA Technologies
  * PCR was performed using New England Biolabs (NEB) OneTaq Quick-Load 2x Master mix with standard buffer according to manufacturer protocol with an annealing temperature of 47°C
  * Restriction enzyme digests and DNA ligations were performed with NEB restriction enzymes or T4 DNA ligase according to manufacturer protocols

RESULTS

The sinR gene homolog sequence was successfully located in Halobacillus BBL2006 using the BLAST (NCBI). We have recovered two products of the sinR homolog with extra DNA upstream and downstream from the gene. The extra DNA on either side of the gene allows for additional homology which will increase the uptake of the cloned DNA containing the inactivated sinR gene into the Halobacillus BBL2006 bacteria. The uptake and incorporation of the sinR DNA into the chromosome of Halobacillus will allow for the insertional inactivation of the sinR gene and the ability to grow on media containing the antibiotic chloramphenicol.

DISCUSSION

The formation of a Halobacillus BBL2006 mutant which would grow as a filamentous form, necessary to assemble into a biofilm community, may be of significant value in bioremediation of wastewater containing high salt concentrations. Currently our research is close to completion of a plasmid construct which would accomplish the inactivation of the gene of Halobacillus BBL2006 which is homologous to the master regulator of biofilm formation in Bacillus subtilis. The sinR gene encodes for the transcriptional regulator protein SinR which controls formation of biofilms in B. subtilis.

The Halobacillus sinR mutant will be screened for filamentous growth and used in experiments to determine its ability to remove copper contamination from wastewater at various salt concentrations.

REFERENCES


ACKNOWLEDGEMENTS

We would like to thank the Indiana University Southeast Research and Grants Committee and the McCullough Fund for Molecular Genetics and Biochemistry for their generous support of our research.