Genetic analysis of two mutants altered with respect to formation of polyhydroxyalkanoic acid and identification of putative RNA helicase, nuclease and gas vesicle genes in {\it Bacillus megaterium\/}

Ning Li, *University of Massachusetts - Amherst*

Abstract

To study polyhydroxyalkanoate (PHA) accumulation and PHA regulation in Bacillus megaterium, transposon mutagenesis with Tn917-LTV1 was carried out and seventy two mutants that produce more or less PHA than normal were isolated and partially characterized. The chromosomal regions of B. megaterium flanking the Tn917-LTV1 insertions were cloned and sequenced from two PHA over-producing and six leaky mutants. The results showed that Tn917-LTV1 was less than ideal for generating a mutant bank due to the unavoidable occurrence of sibling transposants. Furthermore, the sequence data revealed that chromosomal deletions mediated by Tn917-LTV1 insertions were common. An alternative method was developed using a direct plating procedure and it was shown to circumvent these two problems.^ One PHA leaky mutant and one overproducing mutant were studied in more detail. The PHA leaky mutant, T4, had significantly reduced levels of PHA accumulation in all media tested and could not form spores. Chromosomal DNA, contiguous with the transposon was cloned and sequenced. Analysis of the sequence data showed Tn917-LTV1 inserted 24-bp upstream of an operon encoding a putative RNA helicase (deaD) gene and a nuclease (nucP) gene. A chromosomal target repeat 5$\sp\prime$-TATTT-3$\sp\prime$ was found on both sides of the insertion which indicated that no deletion was

involved in the Tn917-LTV1 transposition. A plasmid carrying the intact RNA helicase operon was constructed and transformed into mutant T4. The transformant recovered the ability to form spores and accumulate wild-type levels of PHA in minimal media. Deletions in either DeaD or NucP did not complement the T4 mutant to the phenotype of VT1660. The results show that both DeaD and NucP are involved in PHA accumulation and are required for spore formation. The DeaD protein of B. megaterium is the first protein in the DEAD-box helicase family that is not essential for the organism. The fact that the growth rate of mutant T4 and VT1660 were similar in both LB and minimal media indicates that this DeaD protein may target specific mRNA, such as those of pha and spo genes.^ B001S, a PHA over-producing mutant, unlike its parental strain VT1660, produced large quantities of PHA in rich media. The chromosomal DNA flanking the transposon was cloned from both sides of the insertion. The 8.4 kilobase pairs of chromosomal sequence from left end (IR-L side) of Tn917-LTV1 coded for sixteen open reading frames (ORFs). Ten putative products of the sixteen ORFs shared sequence homology with known gas vesicle proteins (Gvp). The 8.4-kb fragment and its deletion derivatives were cloned into pBluescriptIISK and the plasmids were transformed into E. coli. Gas vesicles were formed and observed by phase contrast microscope, differential interference contrast microscopy and electron microscopy. The deletion analysis and sequence comparison with known gas vesicle proteins suggested 14 out of the 16 ORFs formed the gvp operon of B. megaterium VT1660. The deletion analysis showed that 11 genes are the maximum required for the gas vesicle formation in E. coli. The E. coli cells containing gas vesicles showed increased buoyancy. This is the first time that a functional organelle has been transferred to E. coli. ^

Subject Area

Biology, Molecular|Biology, Genetics|Biology, Microbiology

Recommended Citation

Ning Li, "Genetic analysis of two mutants altered with respect to formation of polyhydroxyalkanoic acid and identification of putative RNA helicase, nuclease and gas vesicle genes in {\it Bacillus megaterium\/}" (January 1, 1998). *Doctoral Dissertations Available from Proquest.* Paper AAI9823750.
http://scholarworks.umass.edu/dissertations/AAI9823750