CLONING AND ANALYSIS OF ALANINE RACEMASE (ALR) GENE AND ITS ENCODED ENZYME FROM BACILLUS THURINGIENSIS SUBSP. AIZAWAI SP41

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ABSTRACT

The alanine racemase (alr) gene was cloned from Bacillus thuringiensis subsp. aizawai strain SP41. This gene has an open reading frame of 1170 bp long including stop codons, and an encoded enzyme with 389 amino acid residues with molecular mass of 43740 Da. The gene was 89.5% and 95.9% homologous to Bacillus cereus group at nucleotide and amino acid levels, respectively. In order to inactivate the alr gene on chromosomal DNA of SP41, plasmid pSETAlr1 with mutated alr was constructed by deleting a 344 bp fragment within the middle region of the gene. The gene fragment was subcloned into thermosensitive pSET5s, which could not replicate at 42°C, to help in integration of mutated Δalr gene in chromosomal DNA of SP41. This plasmid was transformed into SP41 in order to inactivate alr gene by double crossing-over homologous recombination. After the cultivation temperature was raised to 42°C, no transformant with mutated alr gene was obtained from replicating of 4000 colonies. Another plasmid was constructed, which was pSETAlr1T by cutting 216 and 243 bp from 5' and 3' end of alr gene, in order to inactivate alr gene in SP41 chromosome via single crossing-over. From replicating 24000 colonies of transformants obtained after raising temperature to 42°C, clone with mutated alr gene was still not obtained. Analysis of plasmids in SP41 transformant showed that mutation at primer annealing sites for repA gene amplification was detected. Construction of pTSAlr1T which contained only orl-ts from pSET5s was performed and transformed into SP41. However, mutation at primer annealing site of repA was still detected. Non-methylated DNA from E. coli JM110 (dcm-, dam-) was then used in transformation of SP41 instead of DNA from E. coli DH5α. No mutation was detected in SP41 transformants transformed by isolated DNA from E. coli JM110. No alr mutant was obtained from replication of 4500 SP41 (pTSAlr1T) transformants after temperature shifting to 42°C. All transformants grew well in medium without D-alanine which suggested that alr gene in the chromosome still functioned. Studies on the effect of multicopy of alr gene on growth and Alr activity were performed, compared to SP41 wild type and SP41 harboring truncated alr gene. It was found that cell growth (OD_{600}) of SP41 (pTSAlr1) during stationary and sporulation phase was 1 OD lower than that of wild type and cell harboring truncated gene. Specific activity of Alr at 24 hour in SP41 (pTSAlr1) was only 0.002 U/mg compared to 0.044U/mg and 0.034U/mg in wild type and SP41 (pTSAlr1T), respectively, when transformants were cultured in NB supplemented with tetracycline. All strains grew and produced comparable level of Alr specific activity in NB without tetracycline at all stages of growth.

KEY WORDS: BACILLUS THURINGIENSIS SUBSP. AIZAWAI / ALANINE RACEMASE

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