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IDENTIFICATION OF GENES SPECIFYING PROLINE BIOSYNTHESIS IN MARINE BACTERIUM ALTEROMONAS HALOPLANKTIS

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SUMMARY

A gene bank of total DNA of a marine bacterium, *Alteromonas haloplanktis*, was constructed on pBR322. Two hybrid plasmids pUS2010 and pUS2011 carrying inserts of 8.2 and 5.7 kb, respectively, were isolated that complemented the *pro*BA deletion in *E. coli* CSH26. Restriction map of the inserts showed that both plasmids in common carried a 5.7 kb fragment. This restriction fragment thus contains both the genes involved in proline biosynthesis in *A. haloplanktis* and could be expressed in *E. coli*.

INTRODUCTION

Marine bacteria are characteristic for their requirement of NaCl for growth (MacLeod 1965; MacLeod 1968; Reichelt and Baumann 1974). Intracellular accumulation of K^+ of up to 0.3 M occurs in some marine bacteria when growing under the osmotic conditions of normal seawater (Thomas and MacLeod 1974). In some species, several lines of evidence indicate that certain amino acids like glutamate, proline and gamma amino butyrate, are accumulated in the cytosol in direct response to increase in NaCl concentration of growth medium (Makemson and Hastings 1979: Koujima et al. 1978). This response presumably serves to establish an osmotic balance across the cytoplasmic membrane. It is already reported in the past that osmotic stress results in large increases in the intracellular level of proline in a variety of bacteria (Measures 1975).

This work is directed to identify the biosynthetic genes for solutes which are suspected to be accumulated, in response to osmotic stress of NaCl, in marine organisms. A typical marine bacterium, *Alteromonas haloplanktis* 214 accumulates solutes by a Na^+-dependent transport system (Niven and MacLeod 1980). Since it has already been shown that genes for biosynthetic pathways for various solutes including genes for Na^+-dependent transport of *A. haloplanktis* 214 could be expressed in *E. coli* (MacLeod and MacLeod 1986), in the present studies *A. haloplanktis* was chosen to identify genetic determinants for biosynthesis of proline, a suspected osmoprotectant.

In this paper I report the evidence of identifying a fragment from A. haloplanktis chromosomal DNA which harbors proA and proB-genes specifying two initial steps in proline biosynthetic pathway. As in terrestrial strains both these genes appear to be closely associated on the genome of marine bacterium.

MATERIALS AND METHODS

The marine bacterium A. haloplanktis 214, variant 3, ATCC 19855 was obtained from American Type Culture Collection, MD, and grown on medium suggested by Droniuk et al (1987). E. coli CSH26 Δ(lac-proBA) thi ara (Miller 1972) and E. coli AC80 thr leu met hsdR hsdM (Chakrabarty et al. 1978) were obtained from the U.S.EPA culture collection and were grown on Luria-Bertani medium (Maniatis et al. 1982). M63 medium (Cohen and Rickenberg 1956) was used to grow the auxotroph, CSH26. Chromosomal DNA of A. haloplanktis was obtained by a modification of procedure of Meade et al. (1982) using 40 ml of the overnight grown culture. Plasmid DNA from E. coli was prepared based on the methods used earlier by Guerry et al (1973). DNA manipulation experiments were carried out as adapted by Sangodkar et al (1988).

For construction of genomic library, DNA of A. haloplanktis was partially digested with Sau3AI, under carefully controlled conditions so as to yield fragments of approximately 5 to 20 kb in size. The plasmid vector pBR322 was digested with BamHI and the linearized plasmid was then dephosphorylated with calf intestinal alkaline phosphatase. The Sau3AI fragments of A. haloplanktis DNA (10 μg) were mixed with BamHI digest of pBR322 (2 μg) in total volume of 25 μl and T4 ligase (1 unit) was added. E. coli AC80 was transformed with ligated DNA and the transformants scored for ampicillin (Ap) resistance as well as for sensitivity to tetracycline (Tc). A gene bank of about 10,000 clones carrying recombinant DNA was stored at 70°C in Hogness freezing medium (Franklin 1985). Plasmid DNA was prepared from the clone bank after amplification.

RESULTS AND DISCUSSION

Plasmid preparation of gene bank obtained from E. coli AC80 was used to transform E. coli CSH26, an auxotrophic mutant which grew on M63 medium only when supplemented with thiamine (10 μg/ml) and proline (50 μg/ml). Selective medium was prepared by omitting proline. Ampicillin (100 ug/ml) was included in the media used to select prototrophic transformants to ensure that only those cells which contained a plasmid grew on the medium.

A total of 6,340 Ap-resistant transformants containing the hybrid plasmid, after replica plating on M63 agar supplemented with thiamine, resulted in 70 clones which complemented the proBA mutation of CSH26. Each of the transformants were examined for the presence of hybrid plasmid pBR322 by isolating the plasmid DNA and digesting with HindIII. The digested preparations were run on 1% agarose gel electrophoresis. Two populations of hybrid plasmids were detected based on their sizes. The sizes of

inserts in the BamHI site of pBR322 were computed after considering the restriction map of the vector. One group of the hybrid plasmids carried an insert of 8.2 kb and was designated as pUS2010, where as the other, designated as pUS2011, harbored a smaller insert of 5.7 kb. Large scale plasmid DNA preparations were made after growth of the representative clones carrying respective hybrid plasmid. Purified preparations of each were then used to transform the E. coli CSH26 strain. In each case the transformants of the expected prototrophic conditions occurred, establishing that complementation of the mutations was due to the presence of appropriate plasmid from the gene bank. MacLeod et al (1985) had already described the preparation of a gene bank of A. haloplanktis DNA and had shown that various biosynthetic genes in the gene bank could be expressed in E. coli (MacLeod and MacLeod 1986). The results described in this paper present yet another evidence that the genes from marine bacterium, A. haloplanktis can be expressed in E. coli.

Figure 1A shows the fragments generated by PstI, PstI + HindIII and EcoRI + HindIII digestions of the hybrid plasmids pUS2010 and pUS2011. Complete restriction maps of both the plasmids were established, based on the digestions with other enzymes like BamHI, PvuI, PvuII, EcoRV, SalI, SpeI and Sau3AI (Fig 1B). It can be noted that both the plasmids share a common region of 5.7 kb. Plasmid pUS2010 has an additional 2.5 kb fragment. Since the gene bank is constructed on the restriction sites within the tetracycline resistant gene (tet) it is obvious to suggest that the expression could be due to the functioning of tet-promoter. However, this implication can be ruled out since both plasmids pUS2010 and pUS2011 have the common 5.7 kb portion of the insert in opposite orientations (Fig. 1B). The expression of the genes of A. haloplanktis in E. coli CSH26 therefore appear to be due to the recognition of A. haloplanktis promoter sequences by E. coli RNA polymerase. Further, in E. coli both proA and proB map close to each other, at about 6 min on the chromosome (Bachmann and Low 1980). Both these genes from E. coli have been sequenced and shown to form an operon with the direction of transcription from proB to proA (Deutch et al. 1984). Similar order of transcription is also confirmed to be functional in Salmonella tiphimurium (Mahan and Csonka 1983). Although the organization of these genes in Alteromonas is not known at this point in time, complementation of both deletion mutations, proA and proB, of E. coli CSH26 by a fragment as small as 5.7 kb suggests that these genes are fairly clustered within a 5.7 kb region on A. haloplanktis genome. Since this fragment is now available, it would be of interest to know whether the organization of genes is identical to that of enterobacteria. The phylogenetic similarities of A. haloplanktis to enterobacteria are already discussed in literature (de Smedt et al. 1980; MacLeod et al. 1985). The usefulness of this fragment in osmoprotection studies could be assured after understanding the mode of expression of the proline biosynthetic genes.

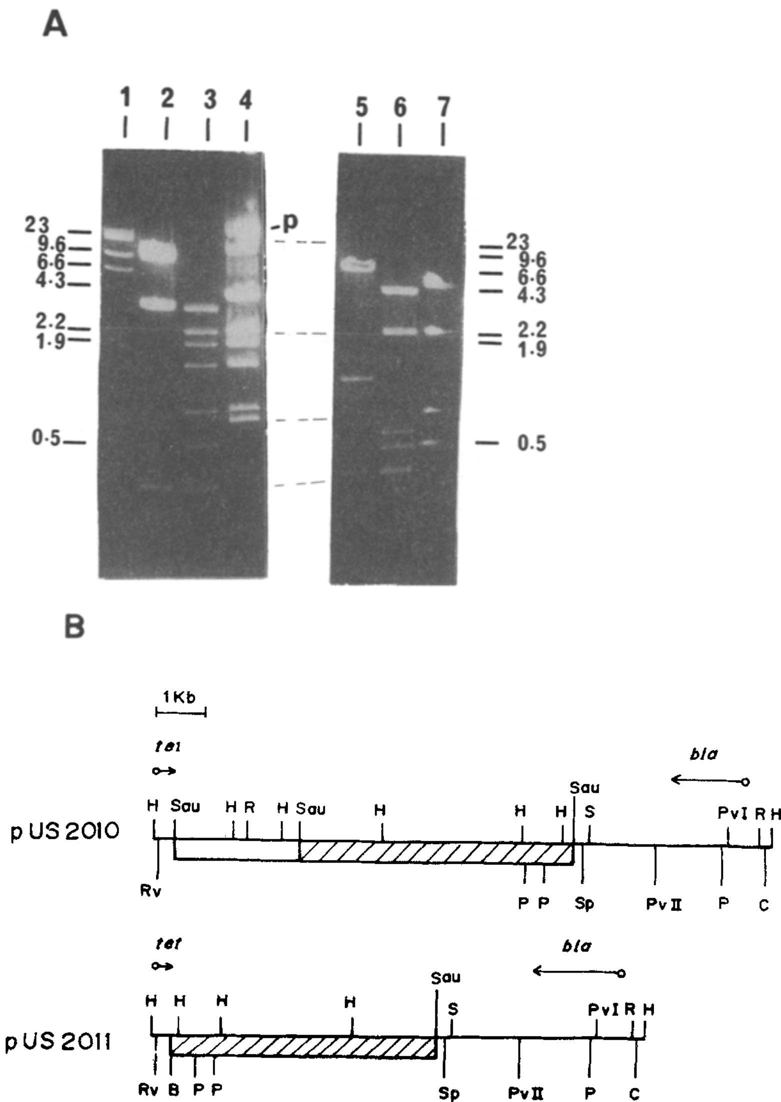

Fig 1 A,B. Agarose gel electrophoresis of restriction endonuclease digests of hybrid plasmids carrying proA and proB genes (A) Lane 1, lambda DNA digested with HindIII; Lanes 2 and 5 PstI digests of plasmids pUS2010 and pUS2011, respectively; lanes 3 and 6, PstI + HindIII digests of pUS2010 and pUS2011; lanes 4 and 7, EcoRI + HindIII digests of pUS2010 and pUS2011, respectively; p, incompletely digested plasmid band. Restriction maps of pUS2010 and pUS2011 (B). The segment from the vector pBR322 is shown by single line. Hatched boxes represent the common fragments, in both plasmids, encoding proA and proB genes. Arrows represent the direction of transcription. B, BamHI; H, HindIII; P, PstI; PvI, PvuI; PvII, PvuII; R, EcoRI; Rv, EcoRV; S, SalI; Sp, SpeI; Sau, Sau3AI; tet, promoter of tetracycline resistance gene; bla, β-lactamase gene.

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