EXPRESSION OF THE THREONINE OPERON FROM ESCHERICHIA COLI IN BREVIBACTERIUM FLAVUM AND CORYNEBACTERIUM GLUTAMICUM

M. Pátek, O. Navrátil, J. Hochmannová, J. Nešvera, J. Hubáček
Institute of Microbiology, Czechoslovak Academy of Sciences, Vídeňská 1083, CS-142 20 Praha 4, Czechoslovakia

SUMMARY
The threonine operon from Escherichia coli was cloned in plasmid pBR322, subcloned into the shuttle vector pCEM300 and the resulting recombinant plasmid was transferred into Brevibacterium flavum and Corynebacterium glutamicum. The expression of E. coli threonine genes in these coryneform bacteria was demonstrated by complementing the thrA and thrB mutations and by assaying homoserine dehydrogenase activity.

INTRODUCTION
Gram-positive bacteria Brevibacterium flavum and Corynebacterium glutamicum belong to the coryneform group widely used in industrial production of amino acids (Hirose and Shibai, 1980). Several genes from these bacteria involved in amino acid biosynthesis (e.g. thrB, Mateos et al., 1987; trp genes, Sano and Matsui, 1987) were cloned in Escherichia coli and identified according to complementation of the relevant mutations. Conversely, genes conferring resistance to chloramphenicol, tetracycline (Ozaki et al., 1984) and kanamycin (Santa-maria et al., 1984) in E. coli were expressed in corynebacteria and brevibacteria when used as genetic markers in the constructed shuttle vectors for coryneform bacteria. Recently, the activity of E. coli promoters trp, lacUV5 and tac (Morinaga et al., 1987) as well as λ phage promoters of the P\textsubscript{rib}-cI857 repressor operator system (Tsuchiya and Morinaga, 1988) in coryneform bacteria was demonstrated. These findings, and also resemblance of some potential promoter and Shine-Dalgarno sequences of coryneform bacteria and E. coli (e.g. trp operon, Sano and Matsui, 1987; hom-thrA\textsubscript{2}, thrB and thrC genes, Mateos et al., 1988) indicate that the transcription as well as the translation systems of these bacteria are similar in both their structure and function.

We report here on the expression of the E. coli threonine operon (genes thrA\textsubscript{1} + thrA\textsubscript{2}, thrB and thrC) in B. flavum and in C. glutamicum.

MATERIALS AND METHODS
Bacterial strains. Escherichia coli K12 strains used in this work were: HfrH-90 (Hubáček and Weiserová, 1980), SK1590 (Kush-
The following strains of coryneform bacteria were used: Brevibacterium flavum ATCC 21127 (thr^ and thrA^ mutant), B. flavum ATCC 14067, B. flavum ATCC 14067 thrB (Bučík et al., 1984) and Corynebacterium glutamicum 9366-H-454 (Plachý, 1970).

Growth media and enzymes. E. coli was cultured in LB medium or in minimal medium M9 (Maniatis et al., 1982). Growth media for B. flavum and C. glutamicum were complete medium CY (Pátek et al., 1988) and minimal medium MM (Kaneko and Sakaguchi, 1979). For selection of transformants and for checking kanamycin resistance (Km^r) of cells, kanamycin was added at 120 μg/ml to the DM3 regeneration medium (Chang and Cohen, 1979) and at 10 or 20 μg/ml to the other media. Strains of E. coli were cultivated at 37°C, strains of B. flavum and C. glutamicum at 30°C. Restriction endonucleases, T4-DNA ligase and alkaline phosphatase were purchased from Boehringer and used according to the supplier’s instructions.

DNA isolation and analysis. Chromosomal DNA from E. coli was prepared according to Marmur (1961). Fragments obtained by digesting this DNA with endonuclease HindIII were separated in 0.7% agarose gel from which the fraction from 7 to 10 kilobase pairs (kb) in size (containing the 8.6 kb fragment carrying the whole threonine operon according to Miwa et al., 1983) was electroeluted and used for cloning experiments. DNA of plasmids harboured by E. coli was isolated using the method of Birnboim and Doly (1979) and CsCl-ethidium bromide gradient centrifugation. Plasmid DNA from B. flavum and C. glutamicum was extracted as described elsewhere (Pátek et al., 1989). Electrophoresis of DNA was carried out in agarose gels according to Maniatis et al. (1982).

Transformation. E. coli cells were transformed using the method of Kushner (1978), transformation of coryneform strains was done as described (Pátek et al., 1988, 1989).

Determination of plasmid copy number. The number of plasmid copies per E. coli or per B. flavum chromosome was estimated following the procedure described in another paper (Pátek et al., 1989). 4700 kb for E. coli and 2800 kb for B. flavum were the values of chromosome size used in the calculations.

Assay of homoserine dehydrogenase activity. Homoserine dehydrogenase activity was determined essentially according to the method of Patte et al. (1963). The protein concentration was estimated by the method of Lowry et al. (1951).

RESULTS AND DISCUSSION

Cloning of the threonine operon from E. coli. HindIII fragments present in the 7 - 10 kb fraction of digested E. coli HfrH-90 chromosomal DNA (2 μg) were ligated with the HindIII-cleaved and dephosphorylated plasmid pBR322 (1 μg). The resulting li-