GENE MANIPULATION IN RUMEN STREPTOCOCCI

K. Boďa, V. Kmeť, P. Javorský, K. Horský
Institute of Animal Physiology, Slovak Academy of Sciences, 040 11 Košice, Czechoslovakia

and

T.N. Shevchenko
Institute of Molecular Biology and Genetics 252 143 Kijev, USSR

Introduction

Streptococci, facultative anaerobic bacteria quantitatively abundant in the rumen of young ruminants, represent a suitable model for applications in genetics manipulations namely due to the well documented genetics of clinical isolates (Kmeť and Boďa, 1987). In the present study the genom of ruminal *S. bovis* strain was used as a source of genes for cloning experiments and *E. faecium* strain as a potential recipient strain for a transfer of recombinant plasmids. The alpha amylase gene of *S. bovis* was isolated by cloning with *B. subtilis* as a host (Javorský et al. 1988) and the gene of the lysine biosynthesis from *B. subtilis* was used to modify ruminal *streptococci* (Shevchenko et al., 1988).

Materials and Methods

The ruminal amylolytic strain *S. bovis* AO 24/85 (Kmet, 1985) served as the source of the cloned DNA and the *B. subtilis* amy E^- as a host. The shuttle vector pMX 39 (*Streptococcus* – *E. coli*) was used as a cloning vector. After partial digestion of *S. bovis* DNA with Sau 3A, fragments were ligated into Bam III site of pMX 39 and transformed into *B. subtilis* amy E^-. The positive *B. subtilis* clones were selected by means of unsoluble starch in the presence of erythromycine (10 $\mu g \cdot ml^{-1}$).

Recombinant plasmid pAT2, containing *B. subtilis* DNA fragment and *Streptococcus* pyogenes vector pPl 251 served as the source of lysine biosynthesis genes (Shevchenko et al., 1988) for rumen *streptococci*. The method of DNA transformation of protoplasts (Kmet et al., 1988) was used for transfer of the genes. The host strain *S. bovis* AO 24/85 was treated by the method of chemical mutagenesis using N-nitro-soguanidine in the presence of S-aminoethylcysteine (AEC) in the medium.

Results

The restriction analyses of recombinant plasmid pJK 108 isolated from *B. subtilis* alpha amylase positive clone showed that 2.8 kb fragment of *S. bovis* chromosomal DNA was inserted to the Bam HI site of pMX 39. The origin of the alpha amylase gene was confirmed by Southern blot analyses. The positive *B. subtilis* clone secreted alpha amylase activity approximately in the same quantity as did the donor *S. bovis*.

The recombinant plasmid pAT2 was transformed to the protoplasts of the rumen strain *E. faecium* AL6. The original strain, *E. faecium* possessed units of diaminopimelate – (DAP) decarboxylase activity per mg of protein whereas after the delivery of pAT2 the value was increased to the 18 units per mg protein. The pAT2 plasmid isolated from *S. faecium* strain was analysed by agarose electrophoresis. The results showed that DNA fragment containing the lysine genes was present in the recombinant plasmid without any damage. The method of chemical mutagenesis provided the mutants of *S. bovis* strain AO 24/85 resistent to AEC with a high DAP – decarboxylase activity ranging from 17 to 60 units$\cdot mg^{-1}$ protein.

Discussion

Molecular genetic studies of rumen microorganisms represents currently the most rapidly expanding area of rumen microbiology. Progress in this field is limited by the informations on the genom of important rumen bacteria. The study of separate genes from the selected ruminal micro organism could therefore accelerate the progress

in this field.

Our previous reports showed that *S. bovis* AO 24/85, as a colonisate preparation, stimulates alpha amylase activity of the epimural ruminal microflora in the lambs. The modification of this strain by increasing its amylase activity and its ability to produce lysine into the mdeium, respectively, could substantially spread up the spectrum of the effects of this preparation. The level of the DAP – decarboxylase, a last step in the lysine biosynthesis, showed that the introduction of plasmid pAT2 into do *E. faecium* increased their production approximately threefold.

The transfer of pAT2 into the *S. bovis* strain has not been documented as yet, since the frequency of the regeneration of the *S. bovis* protoplasts is very low, only 0.01%. The results obtained by chemical mutagenesis with combination of plasmids transfer indicate the potential possibilities to increase the DAP-decarboxylaze activity. (Key Words: Alpha Amylase, Lysine Biosynthesis, Rumen Streptococci)

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