Ann Zootech (1996) 45, Suppl, 356

Electroporation of G+ host plasmids into *Selenomonas ruminantium*

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There are now several systems by which new genetic material can be introduced into rumen bacteria as plasmids (Thomson et al, 1991, Curr Microbiol, 24, 49-54). The technique that has proven generally useful for inserting new DNA is that of electroporation. Combining this with plasmids originating from the bacterial species to be modified has led to at least two of the more successful processes reported (Cocconcelli et al, 1992, FEMS Microbiol Lett, 941, 203-208; Beard et al, 1995, Curr Microbiol, 30, 105-109).

All these experiments were done with *Butyrivibrio*, *Bacteroides* and *Ruminococcus* species. With *Selenomonas ruminantium* we have tried to introduce our constructs with ampicilin resistance from *E. coli* with the help of electroporation. Transformants were usually very unstable (Kopecny and Fliegerova, 1994, Proc Soc Nutr Physiol, 3, 150).

In this case plasmid pJW1 of *Selenomonas ruminantium* was cut with Pst1 and subcloned into pG+ host 4 in *E. coli* strain SURE. The vector was chosen because of some G+ features of rumen selenomonas (Flint, personal communication). The construct pJW1/pG+ host4 (Pst1) was isolated and orientation of pJW1 insert verified. For transfection was used apparatus TA 750 (Krüss, Germany). Electroporation buffer (EBc) was composed of 10 mM PIPES pH 7, 15% glycerol and 60 mM $CaCl_2$. Buffer was prepared anaerobically and autoclaved. Into the transfection chamber 1 ml of culture in EB and 10-50 µl (10-20 ng/µl) of plasmid was added. Treatments were done in range of 1000-15000 V/cm with 100-800 µs duration and 1-9 pulses. Temperature of the electroporation cell and cell culture was 4°C. After electroporation cells were cultivated for 18 hours in M8 medium at 33°C and than selected on M8-agar with erythromycin.

Advantage of these clones is the thermostability of the plasmid pJW1/pG+host up to 35°C.