Influence of temperature and nutrients on R+ plasmid conjugation transfer from an environmental *E. coli* strain

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The increase in the number of bacteria capable of transfer resistance plasmids, detected in aquatic systems, may be due not only to an increase in dumpings, but also to the fact that in these ecosystems processes of plasmid transfer actively occur. Mc Nichol *et al.* (1982) suggested that the latter may be the origin of the formation of plasmid pools.Toranzo *et al.* (1984) reported the possibility that the pathogen microorganims may participate in *in situ* conjugations processes in aquatic systems. Scanferlato *et al.* (1989) remarked the survival of G.E.M.s in these systems.

The incidence of these facts on public health requires that more detailed studies be done on those systems which are potentially suitable for plasmid transfer by conjugation. Because *in situ* experiments are subjet to numerous environmental factors, not always predictable nor controlable, *in vitro* experimental models are necessary, despite their multiple limitations.

The aim of our first experiments was, therefore, to determine the ambient factors which may limit plasmid transfer by conjugation in aquatic systems.

From the freshwater system isolations, we chose as donor a plasmid containing *E.coli* strain, R+ to ampicillin and gentamicin. As the recipient strain we used a non plasmid containing *E.coli* K12, J62, chromosomic resistant to nalidixic acid. As inoculum we used 10^8 u.f.c. of donor and 0,5 10^8 u.f.c. of recipient strain, in 5ml of mating medium. Transconjugants were selected and counted on Mc Conkey agar (OXOID) plates, suplemented with ampicillin (64 µg/ml) and nalidixic acid (32 µg/ml).

In order to determine the influence of the river temperature, mating experiments were carried out in T.S.B. broth (OXOID) during 2 hours, at 20°C (mean river temperature), and that the control 37°C. The transfer frequencies obtained both at 20°C and 37°C were of the order of 10^{-3} (n° of transconjugants /n° of initial donors). These results suggest that the river temperature is not a limiting factor for transfer by conjugation.

To determine the minimun nutritional requirements, mating experiments were done on T.S.B. and on a series of decreasing T.S.B. concentrations and finally in the absence of nutrients (autoclaved distilled water). The inocula were obtained in standard conditions and later washed 3 times with P.S.B.(pH 7,2). Our first results show a decrease in transfer frequency, parallel to the decrease in nutrient concentration, although the frequency values obtained never were below 10^{-6}. We must highlight that transfer frequency values are obtained even in the absence of nutrients.

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