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Introduction of a Catalase Gene into *Streptococcus pneumoniae*

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Streptococcus pneumoniae is a facultative anaerobe lacking catalase enzyme and requires exogeneous catalase added to culture media for aerobic growth.

We had introduced a catalase gene (*kat*) of *Listeria seeligeri* into *S. pneumoniae* and tried to see if the *kat* gene was stably maintained and expressed within pneumococcal host. The *L. seeligeri kat* gene along with its original promoter region was intergrated into pneumococcal chromosome using a non-replicating plasmid pAHA-LSt3. One of three resulting transformants was confirmed to contain the full sequence of the *kat* gene and designated as EHS2. In addition, the *kat* gene was subcloned in *Esterichia coli* in frame to the *lac* promoter of a shuttle vector pDL276 to yield pDL-Kat, which was subsequently used for the natural transformation of pneumococci. Four identical pneumococcal recombinants were selected after restriction analysis.

By performing RT-PCR, it was observed that the *kat* gene was transcribed within pneumococcal recombinant strains from its original promoter (EHS2) or from the *lac* promoter (pDL-Kat). In contrast to the *E. coli* recombinants, however, the pneumococcal kat^+ strains failed to reveal any catalase activities detectable by ferricyanide staining on non-denaturing PAGE. When the pDL-Kat DNA purified from pneumococci was allowed to transform *E. coli* again, many kat^+ recombinants were obtained, ruling out the possibility of the detective *kat* gene within pneumococci. The observation that the *kat* gene in pneumococci was unable to produce the functional catalase enzyme, which requires a heme group at its active site and a cofactor NADPH, confirms pneumococci have defects in heme prodution.