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Exocellular and membrane-bound transpeptidases in *Streptomyces*.

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Both the exocellular DD-carboxypeptidase-transpeptidase and the membrane-bound transpeptidase of *Streptomyces* R61 are susceptible to β-lactam antibiotics and exhibit similar specificity profiles for peptide acceptors. However, there are quantitative differences between the two enzyme fractions with respect to sensitivity to antibiotics and yield in transpeptidations. Triton X-100 (2 %, v/v) solubilizes most of the membrane components, but not the enzyme activity which remains associated with the pellet (50,000 g). Urea at a 2 M concentration is an effective mean for the resolution of the enzyme and releases at least 50 % of the activity in a "solubilized" form. After "solubilization" from the membrane, the enzyme performs both transpeptidase and DD-carboxypeptidase activities. This observation suggests that, *in situ*, the enzyme which acts solely as a transpeptidase must be deeply integrated in a lipid phase of the membrane to which water is not accessible. The exocellular DD-carboxypeptidase-transpeptidase is reversibly inhibited by benzylpenicillin. Not all the membrane-bound transpeptidase activity is reversibly inhibited by benzylpenicillin. About 30-40 % of the activity cannot be restored by washing and treatment with β-lactamase. Inhibition of the membrane-bound enzyme by penicillins and cephalosporins showed that it is the killing target of these antibiotics.

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DD-Carboxypeptidase and Transpeptidase Activities in Membranes of *Streptococcus faecalis* ATCC 9790.

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Isolated membranes of *Streptococcus faecalis* ATCC 9790 show a carboxypeptidase activity on peptides presenting the general structure L-R_3-R_2-R_1(OH). The enzyme has a strict requirement for D-Ala at the R_2 position. It has also a high specificity for the occurrence of a D-amino acid preferentially D-Ala at the R_1 position and a relatively long aliphatic side chain at the L-R_3 position. The carboxypeptidase activity is irreversibly inhibited by penicillin G. ID50 value is 10^{-6} M .

In addition to the DD-carboxypeptidase activity, these membranes exhibit an atypical transpeptidase activity. They catalyze the transfer of N^{α},N^{ε}-diacetyl-L-Lys from the dipeptide donor N^{α},N^{ε}-diacetyl-L-Lys-D-Ala to either [^{14}C]-D-Ala or [^{14}C]-Gly acceptors with the formation of N^{α},N^{ε}-L-Lys-[^{14}C]-D-Ala or N^{α},N^{ε}-L-Lys-[^{14}C]-Gly. This transpeptidase is sensitive to penicillin G. ID50 value is 10^{-4} M .