A method of enzyme preparation involving sonication as well as the parameters for optimum enzymatic synthesis of mycobacillin were standardised. It was found that mycobacillin synthetase activity which appeared to be present in the cytosol of vegetative cells, became associated with the membrane of the producer cells in the stationary phase with concomitant modification of the polypeptide chain. This conformational change induced by the membrane binding of the enzyme accounted for its substrate recognition capabilities as distinct from those of the soluble counterpart. It was further observed that mycobacillin synthetase which appears to form noncovalent bond lacks both racemase activity and pantothenic acid arm and needed 26 moles of ATP for the synthesis of one mole of mycobacillin.