DETERMINING THE STRUCTURAL ROLE THAT AUXILIARY PROTEINS HAVE ON THE ACTIVATION OF GLYCOSYLTRANSFERASES INVOLVED IN THE BIOSYNTHESIS OF MACROLIDE ANTIBIOTICS.

De Sá, L.A.¹; Dias, M.V.B.¹

¹Departamento de Microbiologia, Instituto de Ciências Biomédicas – Universidade de São Paulo- SP, Brazil

INTRODUCTION. Natural products represent a significant source of bioactive molecules which can be used for several therapeutic purposes. Among these natural products exist the antibiotics, such as erythromycin which is a macrolide. Macrolides are characterized by the presence of an aglycone with several glycosylations. Glycosyltransferases catalyze the attachment of sugars, although several of them are active only in the presence of an auxiliary protein. Understanding the structural role that auxiliary proteins have on glycosyltransferases might contribute to generate new derivatives of antibiotics through in vitro glycodiversification.

OBJECTIVES. The objective of this work is to clone glycosyltransferase genes and their respective auxiliary proteins desVII/desVIII and tyIM2/tyIM3 from the biosynthesis of methymycin and tylosin, respectively, overexpress, purify the complexes and posteriorly crystalize the proteins (individually and complexes) to determine their structures.

MATERIALS AND METHODS. Synthetic genes cloned in pUC57 were obtained. These genes were posteriorly subcloned in pET28a and pET15b. Each construct was expressed individually. Efforts to purify the protein complexes were carried out with His-trap affinity column.

RESULTS AND DISCUSSIONS. The glycosyltransferase genes desVII and tyIM2 have been successfully cloned in pET28a and their respective auxiliary pair, desVIII and tyIM3 in pET15b. They were transformed in BL21(DE3) cells, overexpressed and purified using his-tag affinity purification. Results were checked on SDS-PAGE gels. Further experiments are in progress aiming to co-transform and co-express the glycosyltransferase/auxiliary pair.

CONCLUSIONS. Further optimization of expression and purification is need. New constructs of these complexes are in progress aiming to obtain either monomeric enzymes or better yield and pure sample to carry out biophysics and structural studies.

Key words: macrolide, glycosyltransferase, molecular biology