

Institute of Technology and Life Sciences

National Research Institute

Falenty

**Title: The occurrence and characterization of the cellulolytic and xylanolytic
Actinomycetes in selected meadow soils of Poland**

Author: Mgr inż. Beata Zielińska-Polit

Supervisor: Prof. Dr hab. Stefan Russel

Meadow soils contain a high amount of organic substances, among which cellulose and xylan are predominant. The available literature provides relatively scarce information concerning the incidence of actinomycetes in grassland soils.

From the practical point of view, the ability of actinomycete to produce various kinds of enzymes, including cellulase and xylanase is of crucial importance. These enzymes, apart from their biological importance, have also some practical applications. At the moment cellulases and xylanases are used in the textile, paper and food industries, in the production of chemical raw materials and animal feeds.

Considering deficient knowledge of actinomycetes and at the same time their interesting bionomics, specific lifecycle and vital role in natural habitat, this in-depth research has been carried out into determining the abundance and species composition of cellulolytic and xylanolytic actinomycetes in several grassland soils of Poland towards the Ph. D. dissertation.

The test samples have been collected from the meadow soils of the Institute of Technology and Life Sciences National Research Institute in Falenty five times in the course of the growing season and once from the area operated by 17 Regional Chemical-Agricultural Stations across the country of Poland. In every layer of the soil profile there has been determined the total number of actinomycetes on the starch substrate, and the cellulolytic ones on saltagar, subsequently scrutinising the amount of cellulolytic microorganisms with use of tissue paper discs. The total amount of xylanolytic actinomycetes has been worked out using a substrate containing 1% of wheat bran as a source of xylan.

In order to better illustrate the activity of microorganisms and the distribution of the microbial population, soils' physicochemical properties such as pH, nitrogen and carbon have been taken into account. The concentration of investigated microorganisms has been expressed in colony forming units (cfu) per 1 gram of dry matter.

The results of the microbiological tests have then been subjected to statistical analysis, one and two-way analysis of variance and estimation of correlation coefficients. As a result of the microbiological studies, 27 different strains of actinomycetes have been isolated. Based on the 16S rRNA gene sequence analysis, their phylogenetic belonging has been identified mainly to the following genera: *Streptomyces*, *Actinomyces*, *Nocardia*, *Frankia* and *Micromonospora*.

Obtained isolates have been subjected to a detailed morphological characteristics, eg. growth, size and profile of the colony, presence or absence of aerial mycelium, capacity of production of pigment, intensity of the odour. Substrates and nutrient media used in the microbiological research include among others starch, non-nitric, Czapek medium, nutrient agar, Dubois with bran, glycerine, Water Agar with tissue paper, Dubois with salts for cellulolytic, and variable incubation conditions. The shape, arrangement and size of vegetative cells have been specified with use of techniques of light microscopy, Scanning Electron Microscopy and Fluorescence Laser Microscopy.

To determine the physiological and biochemical properties of strain actinomycetes API 50 CH tests have been used, and also the ability to assimilate various carbohydrates i.e. arabinose, starch, lactose, mannitol have been examined. In order to extract potentially most useful actinomycetes featuring cellulolytic and xylanolytic properties, colorimetric assays have been performed in cultures with added straw, xylan, wheat bran, tissue paper, Avicel cellulose and carboxymethyl cellulose.

The analysis of antagonistic properties of actinomycetes has been conducted with use of selected bacterial strains of the genus: *Bacillus subtilis*, *Sarcinia lutea*, *Micrococcus sp*, *Pseudomonas fluorescens*, *Escherichia coli*, *Serratia sp.*

The studies demonstrated that the grassland soils are a good source for procurement of new strains of cellulolytic and xylanolytic actinomycetes with high enzymatic potential.

Keywords: cellulolytic actinomycetes, xylanolytic actinomycetes, meadow soil