

Discoveries of new natural antibiotics are now relatively rare, therefore the construction of strains producing hybrid substances seems to be a very promising opportunity to gain new interesting biologically active compounds. This work is part of a larger project focused on the preparation of new biologically active substances derived from the antibiotic lincomycin. Lincomycin is composed of saccharide (MTL) and amino acid (propylhygric acid) moieties condensed by amide bond. Various modifications of amino acid moiety, especially of the side alkyl chain, are known to improve the antibiotic properties of final molecule. The bottleneck of biosynthesis of such modified compounds is the condensing enzyme NDL-synthetase, and especially its A-domain, which, similarly to nonribosomal peptide synthetases (NRPS), specifically recognizes and activates the amino acid precursor.

In this work a set of degenerate primers for PCR searching of NRPS A-domains was proposed and the conditions of PCR reaction were optimized. In the first step a collection approximately 800 isolates of soil actinomycetes will serve as a source of genetic information for search of interesting NRPS A-domains, applicable for the construction of hybrid biosynthetic clusters. The isolates of this collection have been also characterized taxonomically (16S rDNA sequencing) and the collection was sorted in order to eliminace the duplicate strains. Chromosomal DNAs were isolated from selected strains, which will serve as a template for search of NRPS A-domains and possibly in future also other interesting gene activities. The proposed set of degenerate primers on the other hand can also be used for searching on a wider scale, such as in soil metagenom.