Abstract

Hybrid antibiotics combine the structures or mechanisms of action of two or more known antibiotics and thus represent one way to improve the effectiveness of antibiotics. In our laboratory, the hybrid antibiotic CELIN was prepared *in vitro.* This antibiotic combines the structurally similar antibiotics lincomycin and celesticetin. CELIN has in its structure a propyl group attached to proline, which is naturally found in the antibiotic lincomycin, and salicylic acid, which is a natural component of the antimicrobial agent celesticetin. Since it is a combination of two naturally synthesized antibiotics, it should be possible to prepare a producer of this hybrid antibiotic. However, efforts to prepare a CELIN producer by mutagenesis of a lincomycin-producing strain of *Streptomycces lincolnensis* have not yet been successful. The aim of this thesis is to determine the key steps for the preparation of a CELIN producer. First, the ability of the *Streptomyces caelestis,* which naturally produces celesticetin, mutant to produce CELIN was verified. Next, in order to detect the interaction partner of the LmbF protein, which catalyzes the key biosynthetic step in lincomycin biosynthesis, the pulldown method was performed with *Streptomyces lincolnensis* bacterial lysate. *In vitro* reactions containing different combinations of LmbF, LmbG and CcbF proteins were also performed. The results of this thesis indicate that *Streptomyces caelestis*, without additional genetic modifications, is not a suitable organism for the production of CELIN, since the ability of the prepared mutant *Streptomyces caelestis* to produce a hybrid antibiotic was not detected. The analysis of the pulldown method indicate that the possible interaction partner of the LmbF protein could be the LmbG protein. From the results of the *in vitro* reactions, it can be concluded that the CcbF protein is probably able to catalyze the reaction leading to its natural product as well as the reaction leading to the analog of product of the enzyme LmbF. The results of this work therefore show another direction in the preparation of the CELIN producer by mutagenesis of *Streptomyces lincolnensis,* when to stop the production of lincomycin, it is also necessary to inactivate the gene encoding the enzyme for the subsequent step, *lmbG*, in the previously prepared *Streptomyces lincolnensis* mutant with the deleted *lmbF* gene and the inserted *ccbF* gene and the genes encoding the biosynthesis of the salicylate subunit.

Key words

lincomycin, celesticetin, hybrid antibiotics, biosynthesis