STUDY OF THE NEW MANUMYCIN-TYPE METABOLITE BIOSYNTHESIS – NOVEL FACTORS AFFECTING CARBON CHAIN LENGTHS

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Introduction. To the date, the family of manumycin metabolites consists of more than 30 compounds structurally characterized by the central 2-amino-4-hydroxycyclohex-2-enone ring (*m*-C_7N unit) to which, in *meta* fashion, two short carbon chains of polyketide origin are connected. In most congeners, the 'lower' chain is terminated by the 2-amino-3-hydroxycyclopent-2-enone moiety (C_5N unit).
The members of the family are known to have a broad range of antibiotic functions including antibacterial, anticoccidial, and antifungal activities. The compounds also display strong inhibitory activities against farnesyltransferase, IκB kinase β, interleukin-1β-converting enzymes, and neutral sphingomyelinase and are considered as drug candidates to treat cancers, inflammation, and Alzheimer disease.
In this presentation, we report the isolation and characterisation of the new member of manumycin antibiotics family – colabomycin E. The complete biosynthetic gene cluster involved in its biosynthesis is described and the novel genes responsible for the control of carbon chains are identified.

Methods. Molecular biology procedures and DNA manipulations were carried out according to standard protocols. The cosmid genomic library of *S. sp.1/5* was constructed by cloning the *Sau*3AI partially digested genomic DNA into pSupercos 1 vector. Ligation mixtures were further packaged with the Gigapack III Gold Packaging kit (Stratagene) and transfected into *E. coli* strain. Nucleotide sequencing reactions were performed with the ABI Prism BigDye terminator, version 3.1, cycle sequencing kit (Applied Biosystems, Warrington, United Kingdom) according to the manufacturer's instructions. Fragments were analyzed with an ABI Prism 3100 DNA sequencer (Applied Biosystems). To construct mutants, the PCR targeting system was used. Structure of the major compound, colabomycin E, as well as structures of accompanying congeners, were determined with the help of MS and NMR techniques. The immunomodulatory activities were assayed using the THP-1 human monocyte/macrophage cell line.

Results. The producer of a novel manumycin-type compound was isolated by a genetic screening of the collection of streptomycete strains isolated from various soil environments. The gene cluster encoding the complete biosynthetic route of the compound was identified and cloned in *Streptomyces coelicolor* M512 strain, where the production of colabomycin E was achieved. The complete nucleotide sequence of the gene cluster was determined and the genes responsible for the biosynthesis of the central *m*-C_7N unit, C_5N unit and both carbon chains were identified. The mutants with deleted genes for the lower tetraene chain and the upper pentaene chain were constructed and the missing genes were complemented by different combinations of PKS genes from the original strain and from the asukamycin producer *S. nodosus* ssp. *asukaensis*. A novel type of the chain length factor (CLF) controlling the length of the lower carbon chain was identified in both strains. Deletion of the gene coding for the protein of the DSBA/HCCI family resulted in the abolishment of the production of colabomycin E, with pentaene representing its upper chain, while the synthesis of tetraene-containing colabomycin A and other triene-containing congeners remained unaffected. This effect points to the role of the gene in the biosynthesis of the upper chain starter unit.

Similar to other manumycins, the colabomycin E shows anti-inflammatory effects on the TNFα-stimulated human macrophages, blocks release of of caspase 1-dependent interleukins and down-regulates the expression of numerous pro-inflammatory genes.

Conclusions. The new member of manumycin-type family of antibiotics was discovered, its biosynthetic pathway was characterised and novel types of short carbon-chain-length-controlling factors were identified.

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