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HYBRID ANTHRACYCLINES BY A GENETICALLY ENGINEERED *STREPTOMYCES GALILAEUS* MUTANT

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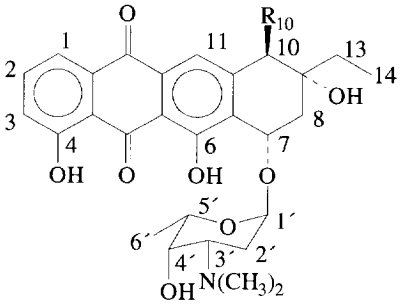
Abstract: A *Streptomyces galilaeus* mutant carrying genes transfered from *S. purpurascens* is reported to produce two hybrid anthracyclines. This study demonstrates the possibility of producing hybrid anthracyclines in a combinatorial way by genetic engineering of anthracycline producing strains.

Several anthracycline antibiotics such as adriamycin, aclarubicin and menogaril have found clinical use in the treatment of cancer. Still, the use of the most succesful cytostatics is restricted by severe side effects and resistance occuring towards these compounds. Therefore, work has to be done in order to develop drugs with improved efficacy and with reduced side effects. Novel analoques of anthracyclines are continuously being developed by screening of bacteria isolated from soil samples, by mutagenising producing strains, by chemically modifying biosynthetic compounds and by synthesising novel molecules. A new approach is the use of genetic engineering of antibiotic producing strains.[1]



site	1	2	3
1	7.80 dd	7.78 d	7.83 dd
2	7.68 t	7.70 t	7.69 t
3	7.29 dd	7.30 dd	7.30 dd
4-OH	12.00 bs	11.98 bs	12.10 bs
6-OH	12.68 bs	12.53 bs	12.67 bs
7	5.28 bs	5.11 t	5.25 bs
8a	2.53 dd	2.23 bs	2.41 d
8e	2.35 d	2.23 bs	2.23 d
9-OH	4.27 bs	-	4.26 bs
10	4.11 s	4.56 s	2.95 dd
11	7.67 s	7.83 s	7.56 s
13	1.65 m	1.73 m	1.69 m
14	1.10 t	1.10 t	1.06 t
15	3.70 s	-	-
1'	5.52 bs	5.46 bs	5.52 bs
2'	1.85 dd	1.80 dd	1.82 dd
3'	3.65 m	3.62 m	3.62 m
3'-NMe_2	2.26 s	2.22 s	2.23 s
4'	3.74 bs	3.72 bs	3.72 bs
5'	4.07 q	4.06 q	4.10 q
6'	1.39 d	1.49 d	1.39 d

b = broad, d = doublet, m = multiplet, q = quartet, s = singlet, t = triplet

Structure of mutant product **1** (R_{10} = $COOCH_3$) and hybrid molecules **2** and **3** (R_{10} = OH and H, respectively)

We have found *S.galilaeus* to be a favorable host for the development of hybrid anthracyclines because of two special features. Firstly, this strain produces glycosides of aklavinone, the prototype of anthracycline

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aglycones. As the aglycone part of the molecules produced in S. galilaeus is not modified by any activities, the transformation of this host by cloned genes may be used for modifications of the aglycone. The other interesting feature is that *S. galilaeus* produces a mixture of different glycosides, aclacinomycins (Acm), the main product of the wild type being Acm A, a trisaccharide. We have previously reported a series of Acm A nonproducing mutants which accumulate intermediates and shunt products of Acm biosynthesis including several strains producing differently glycosylated compounds.[2] The action of cloned genes modifying the aglycone in differently glycosylating mutant strains gives rise to a combinatorial method of producing hybrid molecules differing in structure.

Previously, we have reported the cloning of the rhodomycine (Rdm) biosynthetic gene cluster from *S. purpurascens*.[3] This gene cluster contains two activities modifying the aglycone moiety, the modification of position 10 and the 11-hydroxylating activity. In this study we report the structure of hybrid anthracyclines produced by *S. galilaeus* H038/pJN028. H038 is a mutant strain producing the aklavinone monosaccharide **1**, Acm T, as main product. pJN028 is a plasmid assembly containing Rdm genes responsible for the modification of position 10 of aklavinone. As expected, the strain H038/pJN028 accumulated the hybrid monosaccharide **2** with position 10 hydroxylated. Additionally, another product, **3**, was produced during fermentation.

Experimental

Strains H038 and H038/pJN028 were fermented for four to six days in 5,5 liters of production media with an aeration rate of 6 liters/min and stirring at 280 rpm. The medium was supplemented with thiostrepton for the hybrid strain. The mycelium was extracted with methanol and the methanol combined with the supernatant with methylene chloride. The extract was evaporated to give a gummy residue. The residue was dissolved in chloroform and the products were separated and purified using a two step MPLC procedure with gradients of chloroform:methanol:acetic acid and chloroform:methanolwater:acetic acid. The compounds in the fermentation broth and the purity of fractions from the chromatographic separation were characterised by TLC and HPLC. HPLC-analyses were conducted using a RP-18 column and a mobile phase of 0,05 M K_2HPO_4, pH 3,00 in acetonitrile (3:7). The NMR spectra of the compounds were recorded on a JEOL, JNM-6X 400 FT-NMR spectrometer in $CDCl_3$ and chemical shifts relative to TMS are reported in ppm (Table 1.).

Acknowledgements

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