Optimization of *Streptoalloteichus tenebrarius* for efficient and more sustainable tobramycin production

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Streptoalloteichus tenebrarius (ex *"Streptomyces tenebrarius*", Higgens and Kastner 1967 [1]) is an industrially important microorganism, producing an aminoglycoside antibiotic (AGA) complex also referred to as nebramycin factors I to XIII. The main compounds of the AGA complex are apramycin, carbamoyl tobramycin (direct precursor of tobramycin), and carbamoyl kanamycin B. Kanamycin and tobramycin are clinically used to cure many gram-negative bacterial infections. Tobramycin is especially effective against *Pseudomonas* spec. and therefore particularly valuable for treatment of chronic *P. aeruginosa* infections in patients with cystic fibrosis (CF). Furthermore, tobramycin is one of the few antibiotics, which have been approved for treatment of neonates. Despite the fact that tobramycin is used as antibiotic since decades, its biosynthesis and the interdependence between the AGA pathways in *S. tenebrarius* is not fully understood. In addition, the industrial production is often energy-, time- and cost-consuming due to the very laborious downstream processing, involving the separation and purification of tobramycin from the other aminoglycoside compounds produced by *S. tenebrarius*. Thus, efficient and bio-sustainable manufacturing of tobramycin relies on the provision of optimized producer strains.

In order to improve the production of carbamoyl tobramycin and eliminate or reduce the biosynthesis of aminoglycoside by-products, a heterologous expression of the tobramycin biosynthetic gene cluster (BGC) and a combined engineering approach including genetic modification, gene overexpression and media optimization were conducted.
Sequencing of the genome of *S. tenebrarius* and genome mining enabled the identification of the apramycin and tobramycin BGCs. A pESAC13 PAC library was constructed for a heterologous expression of the tobramycin BGC. The PAC containing the tobramycin BGC was transferred into *Streptomyces coelicolor* super hosts (M1145, M1152, and M1154). However, no tobramycin was detected in the heterologous hosts. Possibly, additional essential genes from other AGA gene cluster are missing on the PAC-DNA endorsing the crosstalk between the different AGA pathways. Therefore, the native producer strain *S. tenebrarius* was used for further engineering. The inactivation of a gene encoding a unique putative NDP-octodiose synthase (previously described as *aprK*[2]) resulted in a mutant that was abolished in the production of apramycin. Subsequent introduction of an additional copy of the tobramycin gene cluster and optimization of the fermentation medium improved the carbamoyl tobramycin production by 30%[3] compared to the production yields in the parental strain.
The phenotypic stability of the generated mutants was validated in a small scale production at industrial conditions which demonstartes the potential of the engineered *S. tenebrarius* derivatives for a more bio-sustainable manufacturing of tobramycin.

1. Tamura, T.; Ishida, Y.; Otoguro, M.; Hatano, K.; Suzuki, K. Classification of 'Streptomyces tenebrarius' Higgins and Kastner as Streptoalloteichus tenebrarius nom. rev., comb. nov., and emended description of the genus Streptoalloteichus. *Int J Syst Evol Microbiol* **2008**, *58*, 688-691.
2. Xiao, J.; Li, H.; Wen, S.; Hong, W. Concentrated biosynthesis of tobramycin by genetically engineered Streptomyces tenebrarius. *J Gen Appl Microbiol* **2014**, *60*, 256-261.
3. Musiol-Kroll, E.M.; Stiefel, A.; Wohlleben W. Optimization of *Streptoalloteichus tenebrarius* for more sustainable production of tobramycin (Manuscript in preparation).