KOLLOQUIUM
Institut für Molekulare Biowissenschaften
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GOETHE UNIVERSITÄT FRANKFURT AM MAIN

Science in progress

Tuesday, June 11th, 2024, 12:00, Biocentre, lecture hall B3

Ayesha Ahmed and Andreas Borst

Ayesha Ahmed

Functional redundancy and dual function of a hypothetical protein in the biosynthesis of eunicellane-type diterpenoids

Many complex terpenoids, predominantly isolated from plants and fungi, show drug-like physico-chemical properties. Recent advances in genome mining revealed actinobacteria as an almost untouched treasure trove of terpene biosynthetic gene clusters (BGCs). In this study, we characterized a terpene BGC with an unusual architecture. The selected BGC includes, amongst others, genes encoding a terpene cyclase fused to a truncated reductase domain and a cytochrome P450 monooxygenase (P450) that is split over three gene fragments. Functional characterization of the BGC in a heterologous host led to the identification of several new members of the *trans*-eunicellane family of diterpenoids, the euthailols that feature unique oxidation patterns. A combination of bioinformatic analyses, structural modeling studies, and heterologous expression revealed a dual function of the pathway-encoded hypothetical protein that acts as an isomerase and an oxygenase. Moreover, in the absence of other tailoring enzymes, a P450 hydroxylates the eunicellane scaffold, yielding a product with an unprecedented modification where it has not been observed before in eunillane derived diterpenoids Surprisingly, both hypothetical protein and P450 catalyzed modifications exhibit partial redundancy. Bioactivity assays revealed that some of the euthailols show growth inhibitory properties against Gram-negative pathogens. The characterization of the eunicellane BGC in this study provides unprecedented insights into the functional redundancy of tailoring enzymes in complex diterpenoid biosynthesis and highlights hypothetical proteins as an important and largely overlooked family of tailoring enzymes involved in the maturation of complex terpenoids.

More Information: https://tinygu.de/kolloqmbw

Andreas Borst

Analysis of the Low salt transcriptome in *Haloferax volcanii*

Archaea are well known for inhabiting areas of our planet that are usually hostile for any form of life, hence they are often termed as “extremophiles”. Although also found in common soil or the digestive tract of vertebrates, some species thrive on acidic or alkaline grounds, in submarine black smokers with temperatures surpassing 100°C or live in aquatic areas with a very high salinity.

Haloferax volcanii is a halophilic and mesophilic archaeon that was first discovered in the 1930s and isolated from the hypersaline environment of the Dead Sea. Its optimal growth conditions are at 42 °C in around 2 M NaCl and complex nutrient medium, however it can also grow in different synthetic media and at a wide range of temperatures and NaCl concentrations. While NaCl concentrations up to 0.7 M inhibit growth in synthetic glucose media, a concentration of 0.9 M is sufficient to sustain growth at a reduced rate.

Here we show for the first time a transcriptome-wide analysis of differential gene expression of cells grown under low salt condition. We cultivated cells at an optimal NaCl concentration of 2.1 M in glucose media and compared the expression profile of these to cells grown in 0.9 M NaCl after 26 and 68 h.

Using RNA-Seq, we identified a multitude of differentially regulated genes and gene-clusters that seem either to be of importance as a fast “stress-response” (after 26 h) to the change of ion concentrations in the environment or to enable continuous growth under these conditions (68 h). We generated in-frame deletion mutants of selected genes/gene clusters and investigated the impact of these deletions on growth under 0.9 M NaCl. To this end, four of twelve tested deletion mutants showed a growth defect under low salt while three of these having none or only a minor one under normal salt, indicating that these genes are involved in adaption to a low salt environment. Vector-based expression of these genes in the respective deletion mutant will be used for complementation assays and identification of potential interaction partners via Mass Spectrometry to further elucidate their function.

More Information: https://tinygu.de/kolloqmbw