**Use of Genome Scale Models to get New Insights into the Marine Actinomycete genus Salinispora: Metabolic Engineering and its Application in Secondary Metabolite Production**

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**Highlights**

* Bacteria within the genus *Salinispora* are a well-known source of natural products.
* *Salinispora tropica* is a marine actinomycete that produces diverse secondary metabolites
* The first manually curated genome-scale metabolic model for *Salinispora tropica* was constructed.
* GSMs were constructed for fully sequenced type strains of *S. arenicola*, and *S. pacifica.*

**1. Introduction**

Bacteria within the order Actinomycetales are a well-known source of natural products such as antibiotics and anticancer agents, and the genus *Salinispora* is no exception. *Salinispora tropica* is a marine actinomycete that produces diverse secondary metabolites, including many that possess pharmaceutical properties such as Salinosporamide A (NPI-0052), a potent anticancer agent, and sporolides, candidates for antiviral compounds. Few manually curated actinomycete reconstructions are currently available despite their important role in drug discovery. Recently, the first manually curated genome-scale metabolic model for *Salinispora tropica* strain CNB-440T was constructed [1]. This model provides a starting point to produce Genome Scale Models (GSMs) of closely related organisms such as other *Salinispora* species. This study is focused on new insights into the metabolism of the three-identified species using constraints-based modeling. GSMs were constructed for fully sequenced type strains of *S. arenicola*, and *S. pacifica*, strains CNH643T and CNR-114T, respectively. We also constructed a Salinispora core model that contains the genes shared by 93 sequenced strains. Functional differences between the developed metabolic networks were identified to have a glimpse into the unique metabolic differences attributable to each *Salinispora* species.

**2. Methodology**

To create metabolic reconstructions to represent each Salinispora species, the gene sequence from the metabolic model for *Salinispora tropica* CNB-440T was used to identify orthologs. We studied 89 genomes of *Salinispora* strains with high quality draft genomes together with the type strains. Phylogenetic estimation was carried out using the amino acid sequences of the proteins encoded by the core genes.

**3. Results and discussion**

The genome-scale metabolic model, *i*CC908 was used to study strain-specific capabilities in defined minimal media and to analize growth capabilities in 41 different minimal growth-supporting environments. These nutrient sources were evaluated experimentally to assess the acuracy of in-silico growth simulations. Here, we update, and expand the scope of the model of *Salinispora tropica* CNB-440T, and GSMs were constructed for two sequenced type strains covering the three-identified species. We also constructed a *Salinispora* core model that contains the genes shared by 93 sequenced strains and a few non-conserved genes associated with essential reactions. The models predicted no auxotrophies for essential amino acids, which was corroborated experimentally using a defined minimal medium (DMM). The Core metabolic content shows that the biosynthesis of specialised metabolites is the less conserved subsystem. Sets of reactions were analyzed to explore the differences between the reconstructions. Unique reactions associated to each GSM were mainly due to genome sequence data except for the ST-CNB440 reconstruction. In this case, additional reactions were added from experimental evidence. This reveals that by reaction content the ST-CNB440 model is different from the other species models. The differences identified in reaction content between models gave rise to different functional predictions of essential nutrient usage by each species in DMM. Furthermore, models were used to evaluate in silico single gene knockouts under DMM and complex medium. Cluster analysis of these results shows that ST-CNB440, and SP-CNR114 models are more similar when considering predicted essential genes.

Also, the GSM of *Salinispora tropica* has been used to define a production medium to improve Salinosporamide A production in a recombinant strain with increases compared to the wild type.

**4. Conclusions**

This study shows that strain-specific models of *Salinispora* can help to better understand the metabolism of *Salinispora* strains, and gain more knowledge about the physiology of the different species. *Salinispora* models would help researchers to establish links between genetic data and metabolic phenotypes. Additionally, the models developed can be used to systematically analyze the essential growth capabilities of Salinispora metabolism that delineate the adaptation process and enhance the production of specialised metabolites.

**References**

[1] Contador CA, Rodríguez V, Andrews BA, Asenjo JA. Genome-scale reconstruction of *Salinispora tropica* CNB-440 metabolism to study strain-specific adaptation. Antonie van Leeuwenhoek, Int. J. Gen. Mol. Microbiol. 2015;108:1075–90.

[2] Contador CA, Rodríguez V, Andrews BA, Asenjo JA. Use of Genome Scale Models to get New Insights into the Marine Actinomycete genus Salinispora. 2019; BMC systems Biology, in press.