GENOME-SCALE RECONSTRUCTION OF *SALINISPORA TROPICA* METABOLISM; MICROBIAL ENGINEERING AND ITS APPLICATIONS IN SECONDARY METABOLITE PRODUCTION

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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. S*alinispora 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. Here, we present the first manually curated genome-scale metabolic model (iCC908) for *Salinispora tropica* strain CNB-440. The reconstruction enables characterization of the metabolic capabilities for understanding and modeling the cellular physiology of this newly sequenced genome. The model was based on physiological and biochemical information of primary and secondary metabolism pathways. The reconstructed stoichiometric matrix consists of 1169 biochemical reactions, 204 transport reactions and 1317 metabolites. A total of 908 structural open reading frames (ORFs) were included in the reconstructed network. The number of gene functions included corresponds to 20% of all characterized ORFs in the *S. tropica* genome. The genome-scale metabolic model was used to study strain-specific capabilities in defined minimal media and to analyze growth capabilities in 41 different minimal growth-supporting environments. These nutrient sources were evaluated experimentally to assess the accuracy of in-silico growth simulations. The model predicted no auxotrophies for essential amino acids, which was corroborated experimentally. The strain is able to use 21 different carbon sources, 8 nitrogen sources and 4 sulfur sources from the nutrient sources tested. Cases where the model was incorrect provided opportunities to gain new insights into the physiology of this specie and generate hypotheses. The incorporation of modifications led to increased accuracy in predicting the outcome of growth/no growth experiments from 76 to 93%. New data, and modifications can be incorporated into the reconstruction to iteratively improve the reconstruction.

Since specialized pathways were included in the reconstruction, growth simulations and in silico gene deletions can be performed by using flux balance analysis (FBA) to dramatically increase secondary metabolites production and yield in *Salinispora* for possible “gene cluster identification” so specific pathways can be cloned in more efficient strains. For example, iCC908 has been used to define a production medium to improve Salinosporamide A production in a recombinant strain with increases over 20% compared to the wild type. This presentation will describe the main features of the metabolic flux analysis and microbial engineering methodology based on reconstruction of the whole metabolism and its applications in the optimization of secondary metabolite production.