Medium prediction of Actinobacteria from the sponge *Petrosia ficiformis*

2022-09-10

Shan Zhang

Actinobacteria are gram-positive bacteria. It was found in the microbiome of the sponge *Petrosia ficiformis*.

On the basis of a draft model of Actinobacteria (Actino\_5\_Burgsdorf\_2021-draft.RDS), the following substances were suggested to an *in silico* medium (Actino\_5\_Burgsdorf\_2021-medium.csv):

Table. The full list of predicted *in silico* medium for Actinobacteria predicted by using Gapseq.

|  |  |  |
| --- | --- | --- |
| compounds | name | maxFlux |
| cpd00001 | H2O | 100 |
| cpd00971 | Sodium | 10 |
| cpd00205 | K+ | 10 |
| cpd00099 | Cl- | 10 |
| cpd10515 | Fe2+ | 10 |
| cpd10516 | Fe3+ | 0.1 |
| cpd00009 | Phosphate | 10 |
| cpd00149 | Cobalt | 10 |
| cpd00254 | Mg | 10 |
| cpd00244 | Nickel | 1 |
| cpd00048 | Sulfate | 10 |
| cpd00239 | H2S | 1 |
| cpd00034 | Zn2+ | 10 |
| cpd00058 | Cu2+ | 10 |
| cpd00030 | Mn2+ | 10 |
| cpd00063 | Ca2+ | 10 |
| cpd00013 | Ammonium | 10 |
| cpd00007 | O2 | 12.5 |
| cpd00027 | D-Glucose | 5 |
| cpd00082 | D-Fructose | 5 |
| cpd00161 | L-Threonine | 0.1 |
| cpd00305 | Thiamin | 0.025 |
| cpd00159 | L-Lactate | 15 |
| cpd00029 | Acetate | 20 |
| cpd22614 | sulfoquinovose | 10 |
| cpd01020 | Gallate | 10 |
| cpd00067 | H+ | 54.975 |

According to the medium prediction rule (medium\_prediction\_rules.tsv from <https://github.com/jotech/gapseq/search?q=medium+prediction+rules>) used by the prediction function in gapseq (src/predict\_medium.R), D-glucose, L-fructose, L-lactate, acetate, sulfoquinovose and gallate were suggested in the predicted medium since Actinobacteria degrades these chemicals for its biomass accumulation (catabolism). While L-threonine and Thiamin, the essential compounds of microbial biomass, were suggested for the medium due to the absence of anabolism of these two chemicals in Actinobacteria.

D-glucose, L-fructose and L-lactate are found as common substrates in the cultivation medium, therefore, the above medium cannot be considered as a “minimum/selective medium” that can only enrich Actinobacteria from the sponge microbial community.

To generate a selective medium for Actinobacteria, we can train three gapseq models using three distinct media, and then extract the gapfilled reactions from each model. The comparison of these filled reactions could reveal how many of the compounds are necessary for the selective culture medium.

This is how the three models should be obtained::

1. Using ALL metabolites in above predicted medium and the Actino\_5\_Burgsdorf\_2021-draft.RDS to get a final model titled “Actino\_5\_Burgsdorf\_2021\_full.RDS” (This one was completed by Shan.)
2. Using the above medium but excluding D-glucose, L-fructose, L-threonine, Thiamin, L-lactate, acetate, sulfoquinovose and gallate (i.e. all the compounds with highlight) to get a final model titled “Actino\_5\_Burgsdorf\_2021\_excl.RDS”
3. Find a literature about culture medium for marine Actinobacteria, then modify it as your training diet (e.g. <https://reader.elsevier.com/reader/sd/pii/S0167701212000115?token=068258519F598C5DB4F3C492C999122BDAA64E6281D860EADDE0DBEE430B2BFEC6883B00B41759B836ABFE7A5D5BA2C7&originRegion=us-east-1&originCreation=20220910082658> ). Simply make a list before performing the “gapseq -fill” function. Rename the model “Actino\_5\_Burgsdorf\_2021\_liter.RDS”.

Then, simulate the single species culture for each model on BacArena.

All of the files and output are in the github repository:

Steps taken for each of the 3 models:

1. Gapseq was run again on the basis that Actinobacteria is a gram positive bacteria (there were a few other files and I didn’t want to risk choosing the wrong one)
   1. The .sh job “qsub\_GapSeq\_Petrosia\_pos\_mod\_1.sh” was conducted to get the final model titled “Actino\_5\_Burgsdorf\_2021\_full.RDS”
2. After obtaining the predicted medium and the Actino\_5\_Burgsdorf\_2021-draft.RDS from the previous job, D-glucose, L-fructose, L-threonine, Thiamin, L-lactate, acetate, sulfoquinovose and gallate were removed from the original predicted medium file
   1. This new medium file Actino\_5\_Burgsdorf\_2021-medium\_mod\_2.csv is created and gapfill of this file is conducted to get the final model titled “Actino\_5\_Burgsdorf\_2021\_excl.RDS”
3. From existing literature, compounds are added/removed according to the list below with their respective reason and relevant literature
   1. This new medium file Actino\_5\_Burgsdorf\_2021-medium\_mod\_3.csv is created and gapfill of this file is conducted to get the final model titled “Actino\_5\_Burgsdorf\_2021\_liter.RDS”

Based on a paper by [Mincer et. al. (2005)](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1287694/) and [Cheng et. al. (2015)](https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0138528#pone.0138528.ref031). They mentioned about medium M1 (1% starch, 0.4% yeast extract, 0.2% peptone, natural seawater, and 2% agar) however, since some of the mixtures, e.g., yeast extract, is not available in the modelseed database, its composition is inferred.

|  |  |  |  |
| --- | --- | --- | --- |
| compounds | name | maxFlux | Reason |
| cpd11657 | starch |  |  |
| cpd00065 | L-Tryptophan |  | Inferred from the mixture: [peptone](https://himedialabs.com/TD/M028.pdf) |
| cpd00971 | Na+ |  |
| cpd00099 | Cl- |  |
| cpd00027 | D-Glucose |  | Inferred from the mixture: [Yeast extract](https://www.neogen.com/globalassets/pim/assets/original/10007/ncm0218_ts_en-us.pdf) |
| cpd00423 | Vitamin B12r |  |
| cpd03612 | H2NR |  |
| cpd00001 | H2O |  |  |
|  |  |  |  |
|  |  |  |  |
|  |  |  |  |

Alternative medium, M2

|  |  |  |  |
| --- | --- | --- | --- |
| compounds | name | maxFlux | Reason |
| cpd00971 | Na+ |  | Inferred from M2 |
| cpd00099 | Cl- |  |
| cpd00100 | Glycerol |  |
| cpd00051 | L-Arginine |  |
| cpd00009 | Phosphate |  |
| cpd00205 | K+ |  |
| cpd00048 | Sulfate |  |
| cpd00254 | Mg |  |
| cpd00001 | H2O |  |
|  |  |  |  |
|  |  |  |  |