Imaging-guided extraction and separation of molecules produced by *S. coelicolor*

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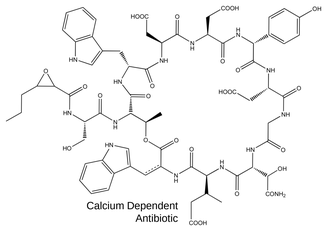
Overview

Streptomyces coelicolor produces many natural products which are useful in the making of today's antibiotics. 7ae, a compound synthesized by the Burkart Lab, was tested with *S. coelicolor*. MALDI images suggest that 7ae elicits the production of various known natural production in addition to unknown molecules in *S. coelicolor.* To identify the unknown molecules, an extraction was performed.

Introduction

S. coelicolor and its Natural Products

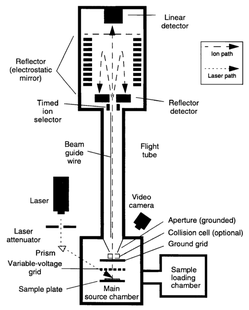


Though its structure is similar to that of a fungus, the *S. coelicolor* is a very helpful bacteria widely used today. It plays a key role in supplying over two-thirds of all natural antibiotics currently available. The *S. coelicolor* bacteria play key roles in producing antibiotics used in human and veterinary medicine and agriculture, as well as anti-parastitic agents, herbicides, and pharmaceutically active metabolites. Mostly found in soil, these bacteria produce antibodies to remove their competition for nutrients found in their habitat. By producing extracellular hydrolytic enzymes, the *S. coelicolor* metabolize many different compounds. These include sugars, alcohols, amino acids, and aromatic compounds. With mycelial growth and spore formation, the *S. coelicolor* have complex lifecycles. The Calcium Dependent Antibiotic (CDA) is an example of a natural product produced by *S. coelicolor*.

The Effects Of Using 7ae

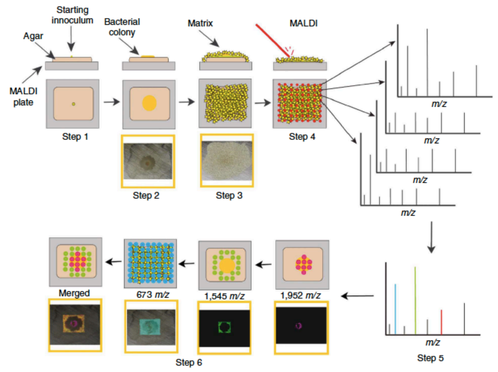
7ae is a compound synthesized by the Burkart Lab (UCSD) to inhibit PPTase activity. However, they observed an increase in actinorhodin production in *S. coelicolor.* 7ae may play an important role in eliciting secondary metabolite production in the *S. coelicolor.*

MALDI-TOFMS



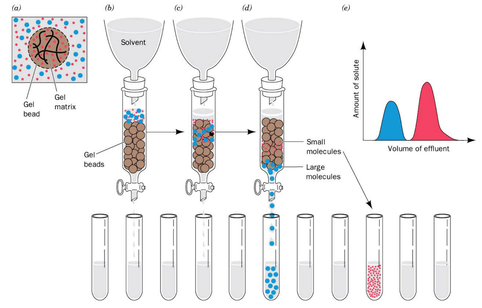
MALDI-TOF (Matrix-assisted laser desorption/ionization Time of Flight) is a mass spectrometer helpful in the analysis of biomolecules (biopolymers such as proteins, peptides, and sugars) and large organic molecules (polymers, dendrimers, and other macromolecules). As the MALDI laser shoots the sample, the matrix and laser ionize the sample, which desorbs into the time of flight tube. These ions hit the detector in order by mass according to their time of flight.

MALDI-Imaging



MALDI imaging is a technique used to observe the different compounds, biomarkers, metabolites, peptides, or proteins in a particular sample. One of the useful features of mass-spectrometric imaging is that it shows how proteins, peptides, and metabolites are distributed in animal and plant tissues, and also throughout bacterial colonies. MALDI spectra is recorded in x-y raster regions, generating a two-dimensional grid of spectra. Specifying a m/z results in a spatialand relatively qualitative image of where that ion exists. The mass spectrometer records the spatial distribution of the different molecules that appear in the sample.

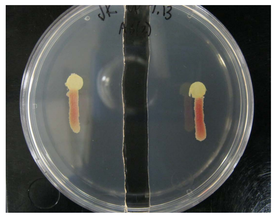
Size Exclusion Chromatography



Size Exclusion Chromatography (SEC) is a technique used to separate molecules in a certain sample based on size. Beads with pores inside a column allow large molecules to go through first with the smallest molecules following, as the smaller molecules get trapped inside the beads.

Methods

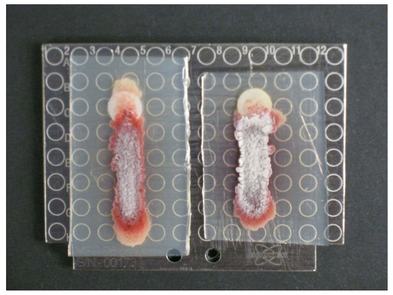
The Inoculation of Plates



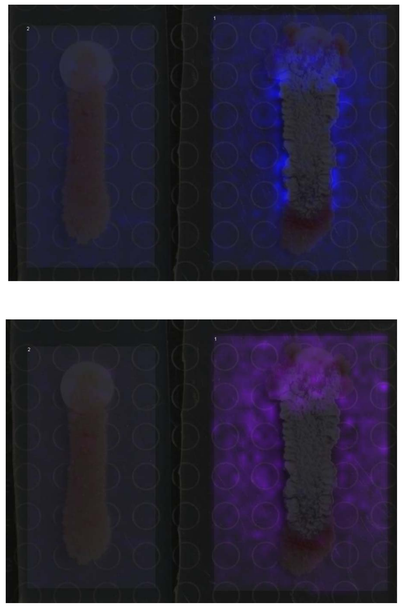
Before inoculating the bacteria, ISP2 plates were prepared. ISP2 is used for bacteria growth, and its ingredients include yeast extract, malt extract, water, dextrose, and agar. ISP2 media were developed specifically to enhance the growth of the *S.* species. The yeast and malt extracts provide nitrogen, amino acids, and vitamins. Dextrose is the carbon source while agar is the solidifying agent. When inoculating Petri dishes with the bacteria, it is important to keep the plate as sterile as possible. Each plate contains two separate colonies of *S. coelicolor:* one with 7ae and one without (control). An agar piece is cut through the middle of the plate so that the two bacteria on the plate do not have the chance to communicate with one another (quorum sensing).

Preparing the MALDI Plate For Imaging

The first step in the preparation of the MALDI plate is to cut out rectangles around the specific bacteria. Next is to place the agar piece on the MALDI plate carefully. After transferring the samples to the plate, the matrix is applied using a sieve. Finally, the plate is placed in the incubator.



MALDI-Imaging Of S. coelicolor With 7ae



These are MALDI images of *S. coelicolor* with a control filter disc (left) and with 7ae (right) at 867 m/z and 1518 m/z. 867 m/z is an unknown ion and 1518 m/z is CDA. Other unknown m/z were observed at a higher concentration in the *S. coelicolor* with 7ae than in the control. What those molecules are and how 7ae elicits them is currently unknown.

To identify these molecules, they need to be extracted from the *S. coelicolor* and isolated.

Extraction of S. coelicolor Molecules

Seven days after the inoculation of the *S. coelicolor*, an n-butanol extraction must be performed. The bacteria along with the surrounding agar was cut out from the plates and transferred in a tube filled with n-butanol. Using a sharp edge, the agar and bacteria were into minuscule pieces. The pink solution (mixture of n-butanol and extracted molecules produced by the bacteria) was subjected to rotary evaporation to evaporate the solvent.

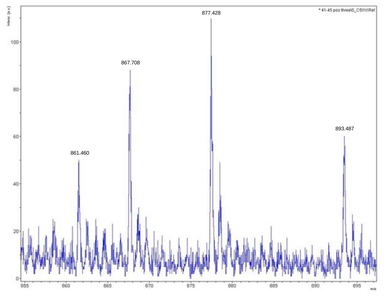
Size Exclusion Chromatography

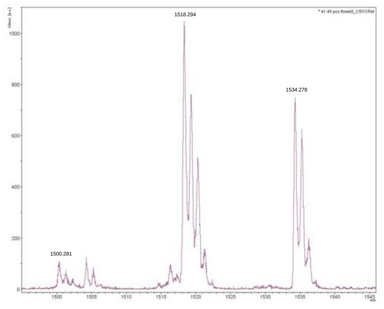
The crude extract was subjected to SEC with Sephadex beads, which is designed for separation of natural products. 60 fractions were collected, and the MALDI was used to check these fractions.

Results

MALDI Of the SEC Fractions

MALDI was used to evaluate the content of each SEC fraction, and indicated which fractions contain the target molecules. These spectra display the peaks from the ions identified in the previous MALDI images, 867 m/z and CDA.





Conclusions

7ae elicits the production of many molecules in *S. coelicolor,* and some of these molecules can be extracted and isolated.

Acknowledgments

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