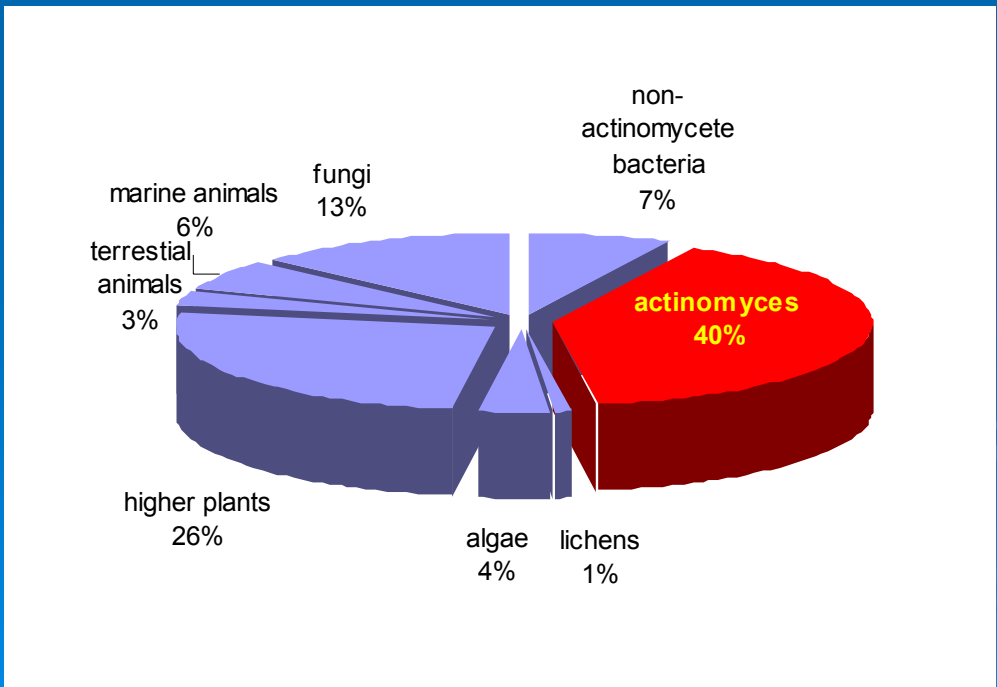
Microgravity effect on the growth, actinorhodin production and gene expression levels of the *Streptomyces coelicolor*

By
Sawat Tantiphanwadi
National Science and Technology Development Agency (NSTDA)
Thailand

APRSAF-12, ISS Working Group
11-13 October 2005, Kitakyushu, Fukuoka, Japan

Background

- Bacteria produce almost half (47%) of all antibiotics.
- Actinomycete bacteria produce a large majority (85%) of these antibiotics

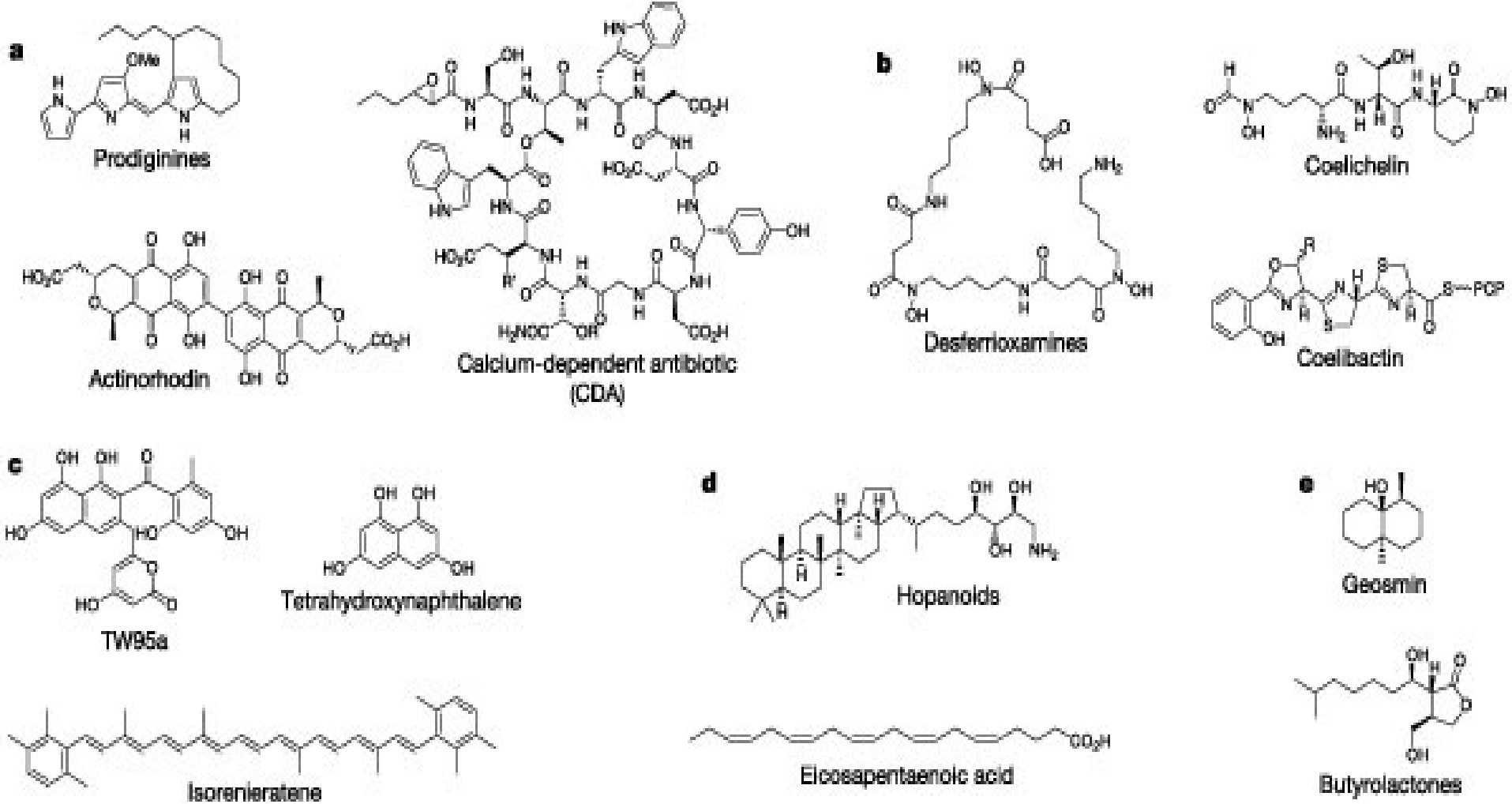


Background

- The genus of *Streptomyces spp.* produces over 80% of actinomycete-derived antibiotics,
- *S. venezuelae* produces chloramphenicol,
- *S. griseus* produces streptomycin,
- *S. plicatus* produces actinomycin D

Background

- *Streptomyce coelicolor,* produces four antibiotics
  - prodiginines,
  - actinorhodin,
  - methylenomycin, and
  - calcium-dependent antibiotic



Examples of secondary metabolites produced by *Streptomyces*. (**a**) antibiotics, (**b**) siderophores, (**c**) pigments, (**d**) lipids, (**e**) and other molecules (Bentley, Chater et al. 2002).

- In addition to antibiotics, *Streptomyces spp.* synthesize many biologically important molecules such as antitumor agents, fungicides, antiparasitics, herbicides, and insecticides (Hodgson 2000).

- Due to their complicated structures, most of these molecules cannot be synthesized cost-effectively using traditional organic chemistry techniques.

- Thence, pharmaceutical companies have been investing continuously to find new strategies to increase production of the antibiotics.

This project aims to utilize the knowledge obtained from the altered antibiotic production in microgravity to improve the production on earth; thereby, lowering cost of antibiotics for mankind.

- It has been previously reported that microgravity had positively affected on the microbial behaviors such as increased growth rate, shortened lag phase duration, extended exponential phase, and enhanced antibiotic production (Benoit, Li et al. 2005).

- Lam (2002) has also confirmed that in microgravity, the intertwine of growth rate and antibiotic production in *Streptomyces plicatus* was observed. They found that *S. plicatus* reduced the colony-forming unit and increased the specific productivity of actinomycin D.

- To better understand molecular biology of organisms in space, gene expression study is a great tool for this purpose.

- DNA Microarrays can facilitate study of gene expression profiles in an organism.

  It can look at all the genes in the genome simultaneously with a resolution of a single gene.

- Due to the lack of genome sequence of *S.plicatus*, it is cumbersome to employ DNA Microarrays in this study.

- Therefore, *Streptomyces coelicolor*, whose genome was completely sequenced in 2002 (Bentley, Chater et al. 2002), can serve as a better model to study gene expression response in microgravity environment.

- The DNA Microarrays for the *S. coelicolor* are readily available through a collaboration with Dr. Camilla Kao, Chemical Engineering Department, Stanford University, Stanford, USA.

Specific Objectives

- To study growth rate and antibiotic production of *S. coelicolor* in the ISS JAXA module.
- To study gene expression profiles corresponding to the changes in growth rate and antibiotic production using DNA Microarrays
- To characterize gene functions responsible for the changes
- To employ the genetic-level knowledge from microgravity to improve the production of antibiotic on earth

3 Phases of the Project

- *<u>Phase I.</u> Comparison of growth rates and antibiotic production between under microgravity and on earth ground.*

  Two sets of identical experiments, in space and on earth

<u>Expected output: PAPER # 1</u>

Microgravity effect on growth and actinorhodin production in *Streptomyces coelicolor*

- *Phase II. Gene expression profile and gene characterization study*

  RNA extraction and DNA Microarrays study will be performed on ground using molecular biology techniques.

Expected output: PAPER # 2

Microgravity effect on gene expression profiles in *Streptomyces coelicolor*

- *Phase III. Genetic engineering a better antibiotic producer*

  Characterization of gene functions will allow better understanding of how they are involved in antibiotic production.

Expected output: PAPER # 3

Design a better actinorhodin producer to serve as a workhorse for other antibiotic production.

Experiment

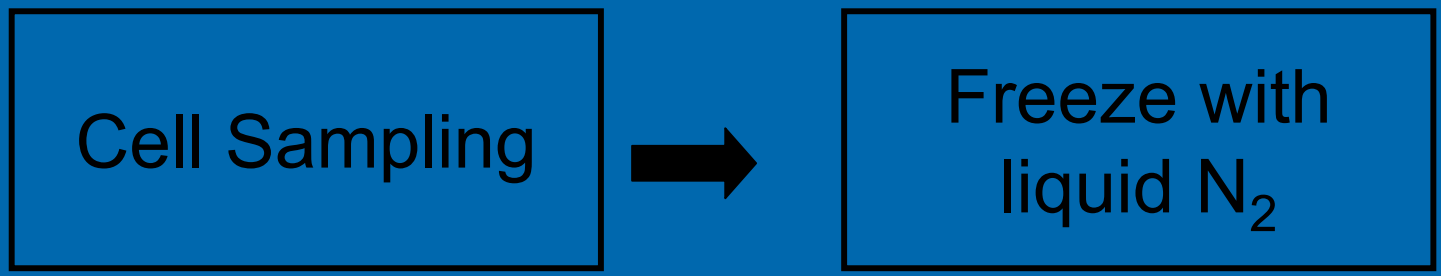
- *Microorganism*

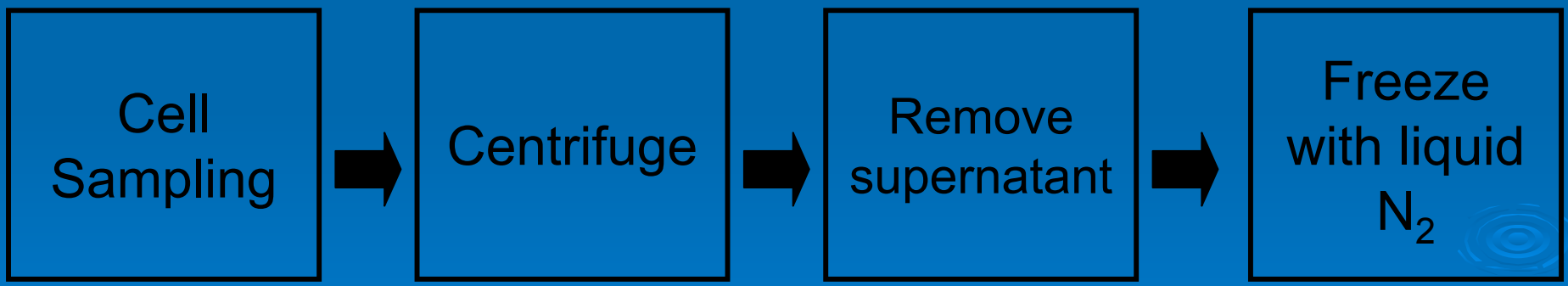
  The wide-type *Streptomyces coelicolor* strain M600 will be used

- *Culture condition*

  Cultures grown at 30°C in rich medium (R5)

Cell collection for the gene expression study

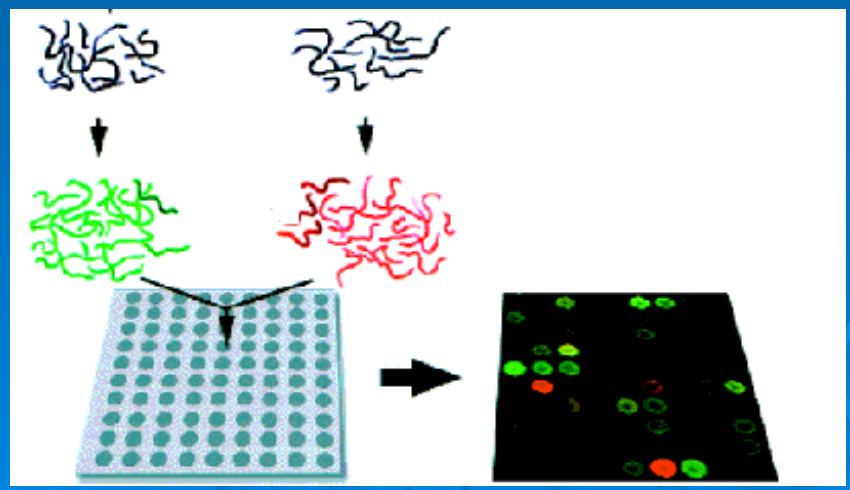




Post-flight process

- *Actinorhodin production measurement*
- *RNA Extraction*
- *DNA Microarrays*

Reference Sample (12hr) **Test Sample (24hr)**



Thank you.