

This piece was received as an unsolicited contribution to *XX* at ASM from a group of high school students in Dubai UAE. The form is that of a generally written research proposal. We opted to take it and publish it as a Letter to the Editor here, in order to serve as an example of the type of research-based assignments in STEM curricula internationally. Please direct all queries or questions to Ms. Sarah Groves, teacher using email provided.

LETTER TO THE EDITOR:

GENETIC EDITING OF SECRETORY
PATHWAY OF *PENICILLIUM CHRYSOGENUM*
AFTER OBSERVATION OF INCREASED
SECRETORY RATES IN AN INCREASED
STRESS ENVIRONMENT (MICROGRAVITY),
A RESEARCH PROPOSAL BY HIGH SCHOOL
STUDENTS IN DUBAI

AKIO SHIRALI ^{1,*}, WILSON MINTER HUIJSMANS ^{2,*}
AND MATTEO SOTTOCORNOLA ^{3,*}

¹THE CRANLEIGH SCHOOL, ABU DHABI, UNITED
ARAB EMIRATES

* AUTHORS HAVE CONTRIBUTED EQUALLY TO THIS WORK

MANUSCRIPT RECEIVED 12 DECEMBER 2017

CORRESPONDING AUTHORS

Akio Shirali
Student, Cranleigh AD, Abu Dhabi, UAE
Email: shiraliakio@gmail.com
Ms. Sarah Groves
Science Teacher, the Cranleigh AD, PO BOX
126888 Abu Dhabi, UAE
Email: sgroves@cranleigh.ae
Wilson Minter Huijsmans
Student, Cranleigh AD, Abu Dhabi, UAE
Email: wilbuoy@gmail.com
Matteo Sottocornola
Student, Cranleigh AD, Abu Dhabi, UAE
Email: m.sottocornola@icloud.com

KEYWORDS

- *Penicillium Chrysogenum*
- CRISPR cas-9
- miniPCR
- RTQ-PCR
- microgravity
- beta – lactam antibiotics
- pcbAB
- pcbC
- penDE

ABSTRACT

In this study, we aim to amplify the secretory pathway of *Penicillium Chrysogenum* within the ISS or similar simulated microgravity using the miniPCR and/or RTQ-PCR and then optimizing *Penicillium Chrysogenum* function using CRISPR cas-9 (Clustered Regularly Interspaced Short Palindromic Repeats), a new technology in the genetics which can help in gene alteration for better drug production. The secretory pathway of *Penicillium Chrysogenum* is controlled by genes pcbAB , pcbC and penDE

INTRODUCTION

The secretory pathway of microorganisms have shown promising results in regard to increased secretory rates when simulated by microgravity. Therefore, in microgravity, this microorganisms can be used to obtain higher amount of microorganisms' secondary metabolites. Beta lactam bacteria are of great biotechnological interest due to their ability to secrete bioactive antibiotica as secondary metabolites. Secondary metabolites are organic compounds produced through the modification of primary metabolite synthesis. Secondary metabolites do not

play a role in growth, development, and reproduction like primary metabolites do, because they are typically formed during the end of log phase or near the stationary phase of growth. The secretory pathway of *Penicillium Chrysogenum* is controlled by genes pcbAB, pcbC and penDE. These genes control the production of penem, a type of beta lactam antibiotics. Space flight experiments have been suggested to affect cellular processes in microorganisms. For instance, preliminary reports on the effects of spaceflight on secondary

metabolism. In our study, we plan to fill the gap between same quality higher yields versus same quality lower yields of secondary metabolites during space flights. Furthermore, *Penicillium Griseofulvum* is found to have a penicillin gene cluster similar to that of *Penicillium Chrysogenum*. No other species among the studied fungi were found to produce penicillin or to possess the penicillin biosynthetic genes, except *P. verrucosum*, which contains the *pcbAB* gene but lacks *pcbC* and *penDE*. Hence *Penicillium Chrysogenum* is our fungi

of choice. The process of regulation of penicillin biosynthesis has been studied for many years. Specifically, the improvement of *P. chrysogenum* strains to obtain higher penicillin yields is a main intense objective in industrial research. To simulate the microgravity environment on earth, several models have been developed and applied to examine the effect of microgravity on secondary metabolism. The purpose of our study is to use *Penicillium Chrysogenum* for production of higher penicillin yields in microgravity environment.

METHODS

We divided the method of the experiments into two parts:

1- Freeze and fly: A simple experiment where the *Penicillium Chrysogenum* is monitored in a low gravity environment on earth. This acts as a proof of concept experiment.

2- Further plan: Use of data obtained by the freeze and fly experiment to edit the microbe gene structure using CRISPR technology in order to produce a permanent artificially induced stress condition to increase penicillin production rate by *Penicillium Chrysogenum*.

1- FREEZE AND FLY EXPERIMENT:

The “Freeze and fly experiment” will be a proof of concept and a chance to gather information on how the expression rate of genes is different to the ones on plain gravity. Genes for the biosynthesis of secondary metabolites are arranged in clusters together with genes for resistance to the toxic action of secondary metabolites on the producer organisms. Likewise, mRNA templates of the Penicillin biosynthesis cluster of *P. Chrysogenum* – *pcbAB*, *pcbC*, and *penDE* will be used on the miniPCR. These templates will be prepared and purified on earth.

The frozen mRNA templates will be sent on the ISS to run the miniPCR. The freeze and fly will be used with the three genes (*pcbAB*, *pcbC* and *penDE*) and their primers (from Ref 15):

pcbAB:

FWD: GAA GAC GTC ATA CTT ATT CTC TG
REV: CGG CAT CGG ATA AAG AGA TCT GG

pcbC:

FWD: GAT TGG CGC TCC TCG TTC
ACC REV: CCA TTA TTT TTC TAG TCG
ACA TGG CAT CGA TTC CCA AGG CCA
ATG TCC CC

penDE:

FWD: CCC GCA GCA CAT ATG CTT CAC
ATC CTC TGT CAA GGC

REV: ATG ACA AAC ATC TCA TCA GGG

How can this help the world? Our Idea is there to fill any of two voids:

1. The idea can be used to increase the production of stock which then can help

decrease the problem of a low inventory. This will make sure that in a financial sense, the constant fear of not being able to accommodate the high demand with a low supply can be cancelled or at the least reduced.

2. The increase in the stock of medicine can be help full as any charity buys the medicines at either a lower cost or in a greater quantity at the same cost which will help the underprivileged to help procure a medicinal cure to ailments that they may suffer from.

We hope that our work can have a massive impact on the lives of others, as the blessing and happiness of a person is far greater than

any sort monetary gain that anyone or we could gain.

Considering the method that we have tried to introduce here we believe that this will have many implications on the pharmaceutical business as there are many types of medicines procurable from bacterial secondary metabolites. Here are some examples:

1. Cancers- the chemotherapy drugs come from secondary metabolites of plant.
2. Diabetes Type 1 – Humulin, the most successful medicine to treat type 1 diabetes is the recombinant insulin produced in bacteria Escherichia coli and yeast.

The experiment will be done in a miniPCR in duplicate, with 8 sample reactions as following:

Sample 1 – Control: no polymerase	Sample 5 – Primers pair: pcbC
Sample 2 – Control: no template	Sample 6 – Primers pair: pcbC
Sample 3 – Primers pair: pcbAB	Sample 7 – Primers pair: penDE

Table 1: PCR experiment will be run on the genes pcbAB, pcbC and penDE from *Penicillium Chrysogenum*

While this is being done in microgravity there would be another batch from the same strain of *Penicillium chrysogenum* would be monitored on normal gravitational conditions.

2- FURTHER PLAN EXPERIMENT:

The expression rates of the three genes on earth would be compared to their expression rates in microgravity. This would be done using a reverse transcription method form their RNA to create a cDNA, a technique known as RT PCR. When this is done, we plan to create a draft plasmid containing an edited version of the DNA to account for the changes that have been made in the expression rates in microgravity. This

plasmid would then be implanted into a cell which can express the plasmid; like F+ cell. Once F+ cell is placed with a group of F- cells, the DNA as a plasmid can move from the F+ cell to the F- cell via the help of the protein relaxosome and relaxase. This is the process of conjugation. Once complete the F+ cell could be placed in a colony with massive amounts of F- cells. This would then allow the F- cells to procure the same traits of the F+ cell, which is an increased secretory rate.

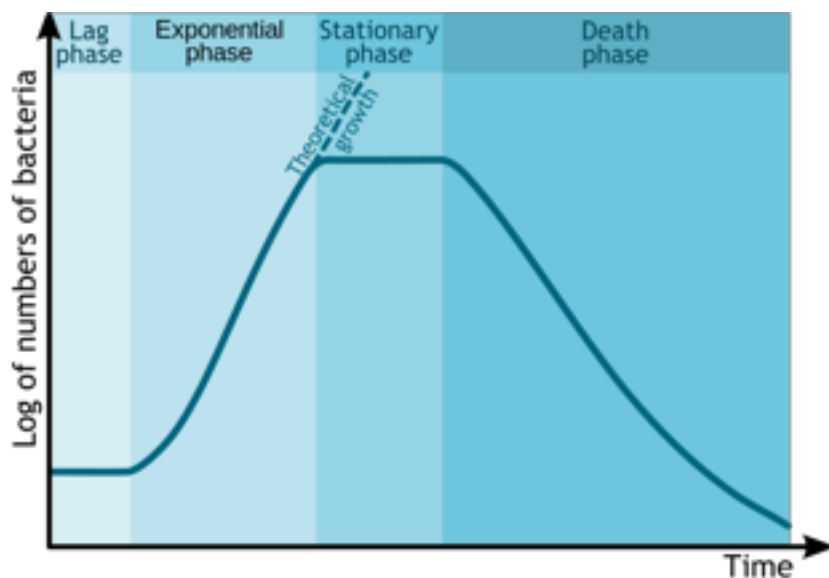
Why the stationary phase is important for secondary metabolites production?

First, we would like to reemphasize the objective of the experiment “Genetic editing of secretory pathway of *Penicillium chrysogenum*, after observation of secretory rates in increased stress environment”. This idea is based on multiple facts:

1. Stress that is induced on micro bacteria increases the rate of secondary metabolite production¹.
2. Genetic editing is now quite widely used.
3. Other studies done on bacterium with similar genomic structures have shown

promising results^{2,4}.

To understand the process of secondary metabolite production, it is important to take into account that secondary metabolite production is only temporary as a results of adaptation to stress by bacteria and fungi. This best to compare to puts of Charles Darwin, one of history’s best biologist, “it is not the strongest that survives, but the species that survives is the one that is able to adapt to and to adjust best to the changing environment in which it finds itself”. To ensure that the fungi cannot adapt to the stress we propose that the edited stress is added to the micro bacteria in their stationary phase (see graph 1).



Graph 1: The Bacterial growth curve^{1,4} This is to ensure that the bacterium does not create offspring that are resistant to the stress because they die at the end of the graph due to lack of nutrients and food.

DISCUSSION

How can this help the world?? Our Idea is there to fill any of two voids:

1- The idea can be used to increase the production of stock which then can help decrease the problem of a low invento-

ry. This will make sure that in a financial sense, the constant fear of not being able to accommodate the high demand with a low supply can be cancelled or at the least reduced.

2- The increase in the stock of medicine can be help full as any charity buys the medicines at either a lower cost or in a greater quantity at the same cost which will help the underprivileged to help procure a medicinal cure to ailments that they may suffer from .

We hope that our work can have a massive impact on the lives of others, as the blessing and happiness of a person is far greater than any sort monetary gain that anyone or we could gain.

Considering the method that we have

tried to introduce here we believe that this will have many implications on the pharmaceutical business as there are many types of medicines procurable from bacterial secondary metabolites. Here are some examples:

1- Cancers- the chemotherapy drugs come from secondary metabolites of plant. 2- Diabetes Type 1 - Humulin, the most successful medicine to treat type 1 diabetes is the recombinant insulin produced in bacteria Escherichia coli and yeast.

ACKNOWLEDGEMENTS

- Dr. Wael Mohammed Osman (Khalifa University, Abu Dhabi, UAE)
- Dr. Claudia Schafer (MIT, Boston, USA)
- Mr. Matthew MacNaught (The Cranleigh School, Abu Dhabi, UAE)
- Ms. Sarah Groves (The Cranleigh School, Abu Dhabi, UAE)
- Mr. Damien Ward (The Cranleigh School, Abu Dhabi, UAE)
- miniPCR (Amplifyus 1770 Massachusetts Avenue Cambridge MA 02140 USA)

ABOUT THE AUTHORS

Akio Shirali

Hello, my name is Akio Shirali, I am a 13 year old student at Cranleigh Abu Dhabi. I have always been interested in the sciences and have always excelled in mathematics. One of my main motivations is my Grandmother who was a microbiologist. My area of specialty is biology.

Wilson Minter Huijsmans

My name is Wilson Minter Huijsmans and I am 15 years old. I have always excelled at Mathematics and the Sciences, specifically Physics. I have won/achieved multiple things in all these areas, with the most recent being a finalist prize in the Genes in Space UAE competition, the motivation for this paper, alongside my science obsessed teammates, the co-authors of this paper.

Matteo Sottocornola

Hello, my name is Matteo Sottocornola. I'm Italian and French and speak fluently three languages. I'm currently studying for my A levels as I am a 16 years old. My main interests lie in the fields of Physics and Mathematics although I also take interest in subjects such as biology and chemistry.

.....

REFERENCES

1. Ana M. Calvo, Richard A. Wilson, Jin Woo Bok, and Nancy P. Keller. 2002. Relationship between Secondary Metabolism and Fungal Development. *Microbiol Mol Biol Rev*; 66(3): 447–459.
2. Hong Gao, Zhiheng Liu, Lixin Zhang. Secondary metabolism in simulated microgravity and spaceflight. *Protein Cell* 2011. 2(11): 858–861 DOI 10.1007/s13238-011-1125-z
3. Microbiology Book Series number 2: Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology (Volume 1), Publisher: FORMATEX RESEARCH CENTER, Badajoz, Editors: A. Méndez-Villas, pp.577–588.
4. Nitin Kumar Singh, Adriana Blachowicz, Aleksandra Chęcinska, Clay Wang, Kasthuri Venkateswaran. Draft Genome Sequences of Two *Aspergillus fumigatus* Strains, Isolated from the International Space Station. *Genome Announc* . 2016 Jul–Aug; 4(4): e00553–16. Published online 2016 Jul 14. doi: 10.1128/genomeA.00553–16
5. Efimov VP, Morris NR. The Lis1-Related Nudf Protein of *Aspergillus nidulans* Interacts with the Coiled-Coil Domain of the Nude/Rol1 Protein. *J Cell Biol* . 2000 Aug 7; 150(3): 681–688.
6. Marco A van den Berg, Richard Albang, Kaj Albermann, Jonathan H Badger *et al*. 2008. Genome sequencing and analysis of the filamentous fungus *Penicillium chrysogenum*. *Nature Biotechnology* 26:1161–1168.
7. Eric Fourest, Catherine Canal and Jean-Claude Roux. 1994. Improvement of heavy metal biosorption by dead biomasses and *Penicillium* pH control and mycelial (*Rhizopus arrhizus*, *Mucor miehei chrysogenum*). *FEMS Microbiol. Rev.* 14(4):325–332.
8. David J. Smith, Martin K. R. Burnham, John H. Bull *et al*. B-Lactam antibiotic biosynthetic genes have been conserved in clusters in prokaryotes and eukaryotes.
9. Henry F. Chambers, Delphine Moreau, David Yajko *et al*. 1990. Can Penicillins and Other β -Lactam Antibiotics Be Used To Treat Tuberculosis? *EMBO J.* 9(3):741–747.