Science and Technology Group Annual Report FY2019

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1. Introduction

Molecular biology is widely used to create synthetic microorganisms designed to produce high value biochemicals such as polypeptides (insulin precursors, growth hormones, antimicrobial peptides, antibodies), small bioactive molecules (antibiotics, food additives, antimalarials), lipids and other specialty chemicals. With plummeting costs of DNA synthesis and novel forms of genes editing the aspirations of metabolic engineers in companies and academic labs to design, test, and build biological systems are being unleashed.

By entrapping engineered microorganisms in a specialized matrix, we are able to halt growth, arrest the cell cycle and thus stabilize the genomes and extend the life cycle of recombinant microorganisms. This increases production rates and yields and, over a longer period, may decrease overall production cost. This technique can be applied to a variety of microbiology platforms, such as bacterial coliforms, budding and related yeast, molds and even metazoan cells.

2. Activities and Findings

As a proof of concept, we turned to production of astaxanthin, a potent UV protector and a dye used in salmonid farming. While currently astaxanthin is obtained via synthesis from petroleum sources, bio-based sources do exist, such as the yeast Pfaffia rhodozyma (Xanthophyllomyces dendrorhous Golubev) (Fig. 1). However, the cost of production is too high for bio-astaxanthin to enter the market.

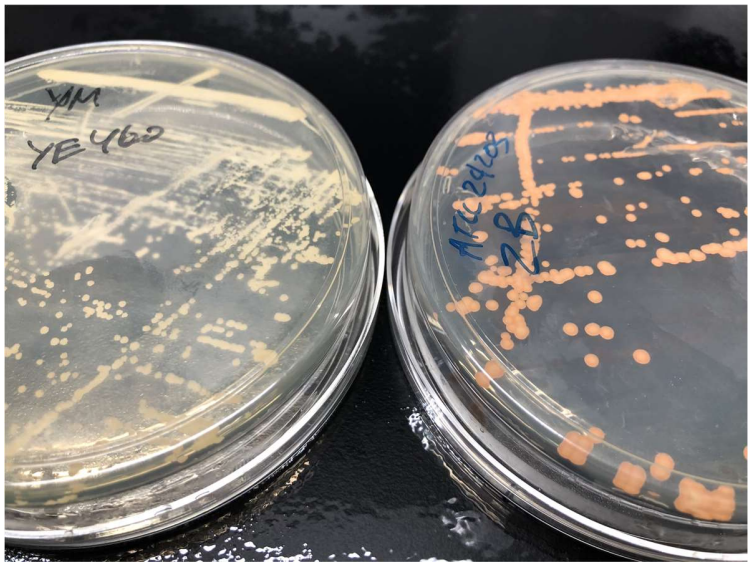

Fig. 1. On the left a typical *Saccharomyces cerevisiae* strain YE460. On the right is the astaxanthin–producing species *Pfaffia rhodozyma.* Note the orange color brought about by the dye. Besides the obvious color difference, the species a very different in their carbohydrate tolerance, growth rate and optimal cultivating temperature.

We aim to decrease the cost of producing astaxanthin in yeast as a natural product using our platform of entrapped cells that increase the yield and/or production rate of a product of interest. To this end we obtained several strains of Pfaffia rhodozyma that are characterized with high production yield of astaxanthin and hope that cell entrapment will increase production yield and rate, analogous to our previous experiments[1]. The major step is to extract the dye from cell membranes without physical cell disruption, we will use a combination of natural oils and non-ionic and ionic detergents. Once we optimize the non-disruptive yield and production rate in free cell cultures, we will entrap cells in alginate, a matrix that is transparent for small molecules but immobilizes cells inside and establish whether astaxanthin is produces at higher rate as in the free cells in the same conditions.

3. Collaborations

Prof. Frank Rosenzweig, Georgia Institute of Technology, Atlanta GA USA
Dr. Pamela Broussard, University of Montana, Missoula MT USA
Prof. Leonid Kalachev, University of Montana, Missoula MT USA
Dr. Lesley Ellies, UC San Diego, San Diego, CA USA
Dr. Zach Bell, Shoreditch, Onna-son, Kunigami-gun, Okinawa, Japan

4. Publications and other output

Encapsulation enhances protoplast fusant stability
J Gulli, E Kroll, F Rosenzweig
Biotechnology and Bioengineering

Diverse conditions support near-zero growth in yeast: Implications for the study of cell lifespan
J Gulli, E Cook, E Kroll, A Rosebrock, A Caudy, F Rosenzweig
Microbial Cell 6 (9), 397

5. External Funding
A combination therapy for interventional treatment of triple-negative breast cancer
National Cancer Institute R15CA242394 (PI) 2019-2020

Applied for: *Yeast as a model for Chromosome Catastrophe in cancer*

All life forms routinely encounter severe environmental conditions. To adapt to these stressful conditions, evolution has favored genetic mechanisms that increase population variation via point mutations and recombination. It is well-known that that genetic mutations and recombination happens more frequently in some cells than in others. In turn, unequal recombination that leads to genome restructuring and loss of heterozygosity, is also a hallmark of cancer. Initially, loss of heterozygosity in cancer was presumed to be slow and progressive; however, it has been shown that aggressive cancers often undergo chromosome catastrophe, where multiple chromosome rearrangements and chromosome loss occur within a few cell generations. While double-strand breaks (DSBs) have been proposed as the precursors, the pertinent question still remains: what causes multiple DSBs in the first place?

My group developed a simple to use but effective model of stress-associated population evolution3. In our model, cell populations starved for one to three months developed a wide range of chromosomal rearrangements and aneuploidies (stress-associated genomic restructuring, or SAGR) that can be detected by pulsed-field gel electrophoresis and sequencing. We discovered that some of these rearrangements led to adaptive phenotypes and some led to reproductive isolation. Together, these may lead to rapid speciation. We also discovered that the development of SAGR is nonlinear, i.e. there is an "avalanche" of SAGR events later in the starvation process. This yeast process is caused by unrepaired double strand breaks and is reminiscent of a characteristic cancer genome restructuring. Can the genes that control SAGR in yeast be potential drug targets in human cancers?

The objective of this study is to identify the molecular mechanisms of stress-associated genome restructuring that are common between yeast and man. We will employ a simple to use but effective method we developed to induce chromosomal rearrangements and aneuploidy in yeast and study the genes involved in genome restructuring. Specifically, we will (1) detect multiple stress-associated genome restructuring events in single cells; (2) discover mutants that prevent or lower the incidence of genomic restructuring; and (3) study a model of metazoan somatic cells to link molecular mechanisms of genome restructuring in yeast to higher eukaryotes. By identifying the genes involved in genome restructuring in yeast in response to stressful environments, we will move closer to understanding the role that large-scale genome restructuring plays in the emergence of antibiotic resistance and the progression of cancer.