VITAYEAST

The Transformation of *S. cervisiae* for the Production of Vitamin D3
OVERVIEW OF PRESENTATION

• What is Vitamin D and why is it a problem.
• Our approach to solving the problem of vitamin D deficiency
• Method we used to solve this problem
• Ongoing work
WHAT DOES VITAMIN D3 DO?

• Used in for a wide variety of functions in the human body
  • Assists in absorption of calcium
  • Helps prevent diabetes
  • May reduce hypertension
  • Linked to cancer prevention
  • Decreases the negative effects of alcohol
ABSORPTION OF CALCIUM

- Essential for both phosphorus and calcium metabolization.
- Absorbed with the aid of bile after bonding to calcium.
- Regulates the cellular absorption of calcium in bone and skeletal muscle tissue.
- Stimulates reabsorption of phosphate in the nephrons.
WHY IS VITAMIN D DEFICIENCY A PROBLEM

• Most people do not get enough sun.
• Supplements
WHAT CAUSES VITAMIN D DEFICIENCY

“Anything that diminishes the transmission of solar UVB radiation to the earth’s surface or anything that interferes with the penetration of UVB radiation into the skin will affect the cutaneous synthesis of vitamin D3”
LESS KNOWN CAUSES OF UVB DEFLECTION

• Sunscreen with an spf of 15 is enough to block 99% of vitamin D3 production.

Image courtesy of the Skin Cancer Foundation
LESS KNOWN CAUSES OF UVB DEFLECTION

- Geography

**Fig. 4.** Annual change in broadband UVB radiation measured in Boston using polysulfone film badges for periods of 1 h (■—■) and 3 h (●—●) from 1130 h EST. Each point is the mean of two or three badges. - - - , The level of broadband UV radiation below which no conversion of 7-DHC occurred.
OUR SOLUTION

• Modify the vitamin D2 path in *Saccharomyces cerevisiae* to produce Vitamin D3
  • *S. cerevisiae* already has most of the genes necessary to make D3
  • Multiple applications for distributing the D3 after production
    • *Bread and other leavened products*
    • *Supplements*
    • *Fermented liquids*
GENES FOR PRE-VITAMIN D3 SYNTHESIS

- 7-Dehydrocholesterol is the precursor to D3
- Only three Genes are needed to alter the D2 pathway to produce 7-Dehydrocholesterol. DHCR24, EBP, and SC5DL
- It turns out that SC5DL is already present in *S. cerevisiae* under the name ERG3 in the D2 pathway.
METHODS

• We used Gateway® Recombination Cloning Technology from Invitrogen to insert the necessary genes into the *S. cerevisiae* cells.
  • pENTR221 entry vector from Genecopia
  • transformed into One Shot® OmniMAX™ 2TI® E. Coli competent cells
METHODS CONT.

• LR Clonease™ II Enzyme Mix To transfer The genes into the Gateway® vector, pYES2-DEST52
ONGOING WORK

• Transform the yeast
• Confirm expression of genes and protein production
• Confirm synthesis of 7-Dehydrocholesterol
QUESTIONS FOR THE FUTURE

• Will the yeast uptake the genes and will the genes be expressed?
• Will the yeast survive if some of the resources for D2 production are aliquot to D3 production?
• Will the final product be stable?
• Will it be able to be added into a food product?
PROBLEMS ENCOUNTERED

- Deciding on an appropriate project
- Funding
- Establishing the club
- Organizing the club
- Funding
- Finding a good meeting time
- Filling officer positions
- And Funding yet again
- Finding time to work on the project
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THANK YOU

Questions?