VitaYeast - Transformation of S. cerevisiae for the production of Vitamin D3

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Abstract

Vitamin D3 deficiency is a prevalent problem in the world today. We sought to create a yeast strain that was capable of creating vitamin D3. We chose yeast for the facts that it already contains much of the pathway to making vitamin D3 and because of the easy distribution possibilities of yeast as it is used in several food production methods. We identified the genes necessary for vitamin D3 synthesis in yeast and are in the process of transforming them into the yeast.

Introduction

Vitamin D: A Serious Problem

Vitamin D is an important nutrient in everyday bodily functions. It is essential for calcium absorption, in bone growth and remodeling, protects against osteoporosis, and helps maintain homeostasis in the body. Without enough vitamin D, a person is can develop a skeletal muscle defect, such as osteoporosis, muscle weakness, and can have an increased risk of falling. Vitamin D also has a possible links to increase in cancer risk, diabetes, and hypertension (1).

The problem that we face today is that many people do not get enough exposure to the sun to result in sufficient vitamin D synthesis. The National Institutes of Health recommend 5-30 minutes of sun exposure between 10AM and 3PM on the face, arms, legs, and back at least twice a week to produce enough vitamin D for everyday functions. The problem is that most people do not get this much exposure or there are factors preventing their exposure.

What Prevents Vitamin D Synthesis in Human Skin

"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" (1)

One of the things that limit UVB exposure is sunscreen. An SPF of 15 will block 99% of UVB light from the skin and not allow vitamin D production. (1)

Another factor that will limit vitamin D synthesis is geographical latitude. As you can see below in Figure 1, in people living in Boston are only able to utilize sunlight to synthesize vitamin D during the months of March through October. During the winter months the intensity of UVB is not strong enough for sufficient vitamin D synthesis.

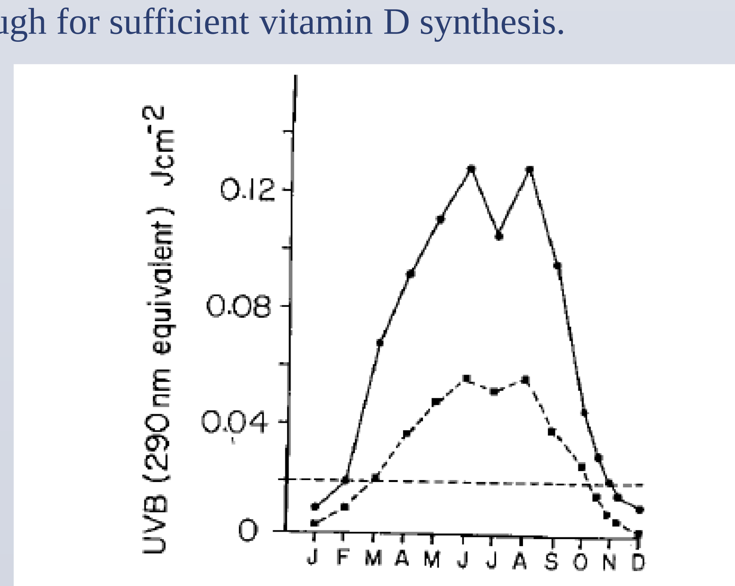

FIG. 4. Annual change in broadband UVB radiation measured in Boston using polysulfone film badges for periods of 1 h (■- -■) and 3 h (●—●) from 1130 h EST. Each *point* is the mean of two or three badges. – – –, The level of broadband UV radiation below which no conversion of 7-DHC occurred.

Figure 1 – UVB exposure by Month (3)

Also a factor that will affect your ability to synthesize vitamin D is skin tone. People with darker skin will block UVB light more effectively then people with lighter skin. (1)

Why Vitamin D3?

There are two types of vitamin D that exist and are used by humans: vitamin D3 and D2. Humans synthesize vitamin D3 in the skin. Therefore, humans can use vitamin D3 more effectively. Figure 2 shows us that after 30 days of treatment with D3 or D2, that the human body retained vitamin D3 much more effectively then vitamin D2.

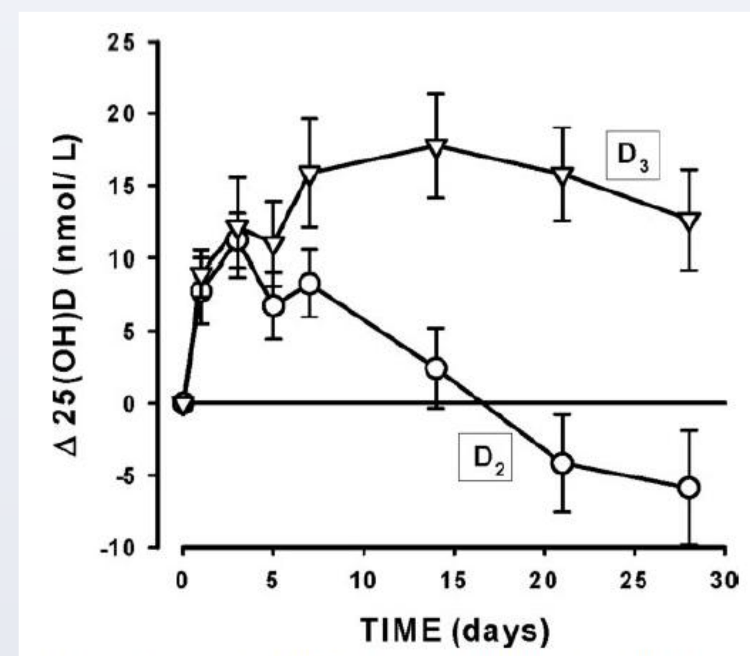

FIG. 2. Time course of the rise in serum 25OHD after a single oral dose of 50,000 IU of either cholecalciferol (vitamin D_3) or ergocalciferol (vitamin D_2) to two groups of 10 normal men each. Error bars are 1 SEM. The zero-change line incorporates a correction for the seasonal rise in 25OHD occurring at the time this study was performed. (To convert from nmol/liter to ng/ml, multiply by 0.4.) [Copyright Robert P. Heaney, 2004. Used with permission.]

Figure 2 (4)

Why Yeast?

Yeast was chosen as a suitable organism for this project as it already synthesizes the genes necessary for vitamin D2 and therefore has some of the enzymes for vitamin D3 synthesis. Also it makes for an ideal candidate organism for distribution of the vitamin D as it is already used in several food products on the market today.

Genes Necessary for Pre-Vitamin D3 Synthesis

Unfortunately it is not possible to directly synthesize vitamin D3 without the aid of the body. But it is possible to synthesize the precursor to vitamin D3: 7-Dehydrocholesterol. This is then converted in the body to vitamin D3 inside the body. As shown in Figure 3, in order to create 7-Dehydrocholesterol, 3 genes are required: DHCR24, EBP, and SC5DL.

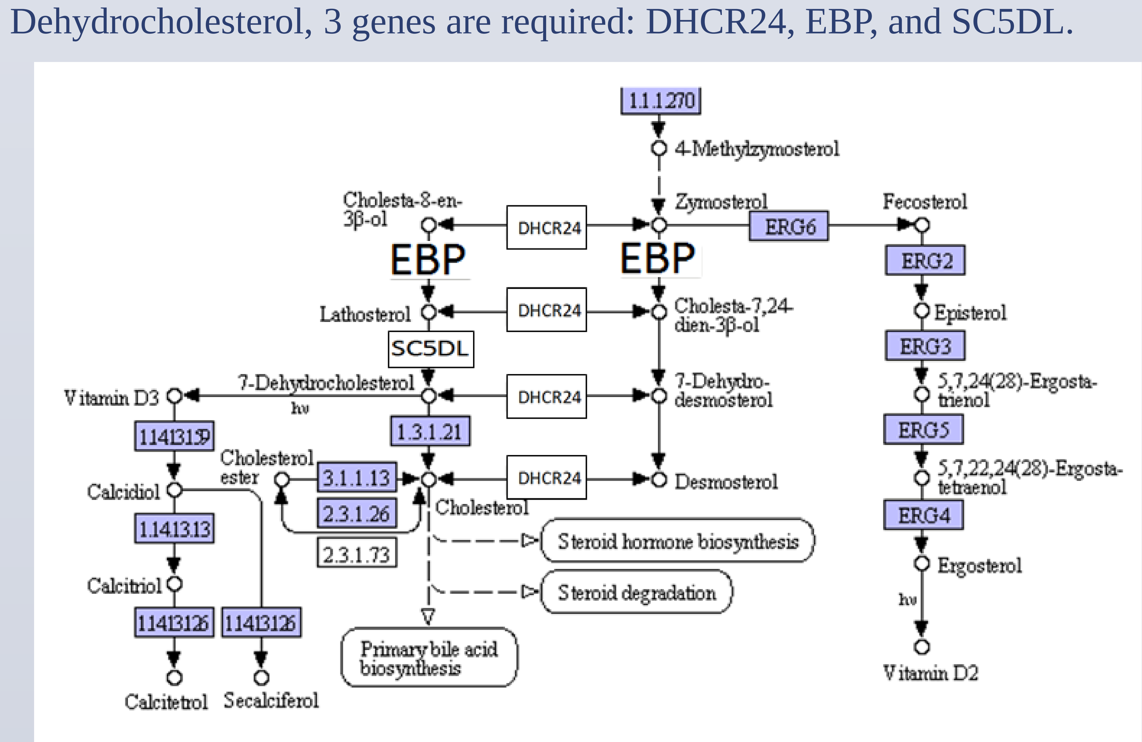

Figure 3 (5)

Yeast is already capable of synthesizing vitamin D2. This means that it is capable of using all the genes in the vitamin D2 Pathway. ERG3 is the same gene as SC5DL. So to synthesize vitamin D3 we only need to give yeast the genes for DHCR24 and EBP.

Methods

We are using Gateway® Recombination Cloning Technology by Invitrogen. The Gateway® Technology is a very versatile method that is able to easily able to clone the genes into yeast cells.

Entry Vector with Genes:

The entry vector used was pENTR221 ordered from Genecopia.

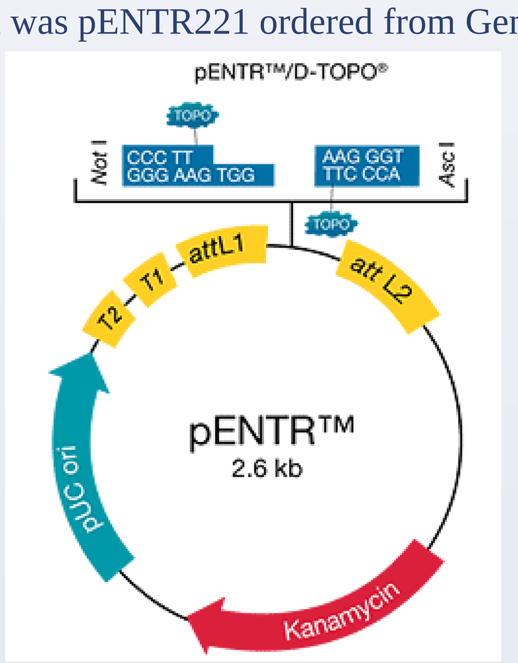

Figure 4 – pENTR vector for cloning genes into E. Coli (6)

These vectors were transformed into One Shot® OmniMAX™ 2 T1[R] Cells and grown overnight.

LR Clonase™ II Enzyme Mix was used to transfer the gene.

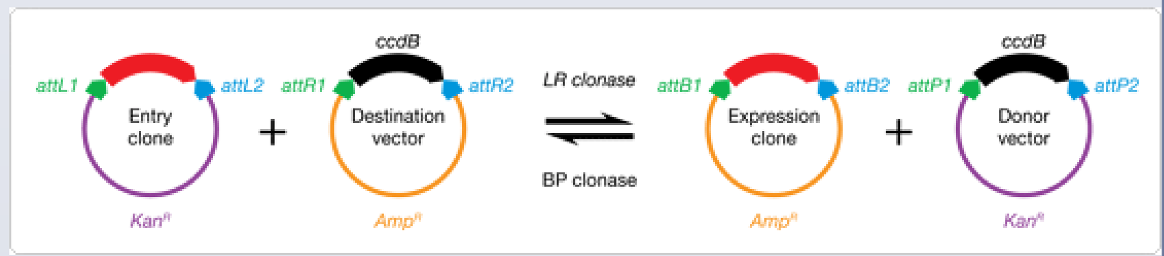

Figure 5 – Method by which LR Clonase™ II Enzyme Mix works (6)

The Genes were transferred into the Gateway® destination vector pYES2-DEST52. This vector is a reliable shuttle vector between e. coli and yeast.

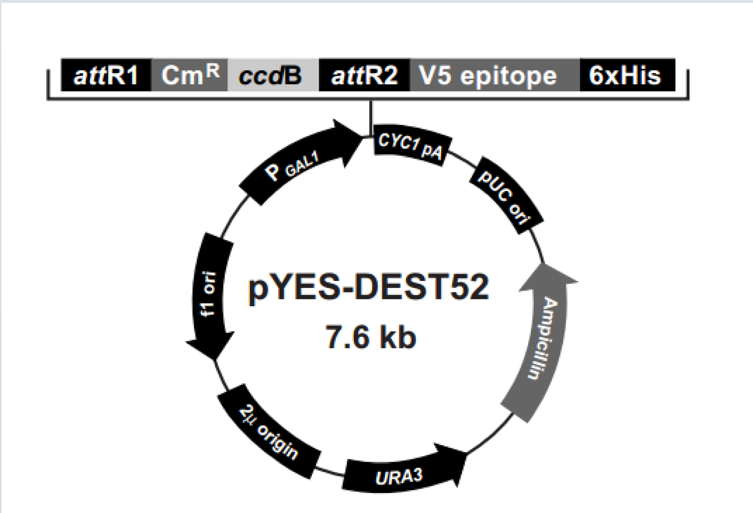

Figure 6 (6)

Ongoing Work

We are currently attempting to transform the yeast with our newly made vectors. Each gene has been cloned into the same version of the Gateway® Destination vector. This presents a problem in that the vectors have identical selectable marker. Once the vectors have been transformed into yeast, we will be met with the challenge of confirming that both genes have been transformed into the same yeast cell and not just one of the genes. Then we will need to confirm expression of the genes in yeast and confirm if the yeast is creating vitamin D3.

Alternate Approach

An Alternate approach that might be explored is PCR the genes into the vectors with two separate markers. These vectors have markers for Leucine and Histamine. When these vectors are transformed into the yeast we will be able to tell right away if both genes have been transformed into the yeast.

Results (How we plan to obtain them)

We will either DNA sequence the yeast to determine if the vectors are incorporated into the yeast or preform a western blot test for the proteins that the vectors are expressing. After confirming presence or expression of the genes, we will use mass spectrometry to determine if the yeast is synthesizing 7-Dehydrocholesterol, the precursor to vitamin D3.

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