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, bone growth and remodeling, protects against osteoporosis, and helps maintain homeostasis in the body. Without enough vitamin D, a person 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.

The problem that we face today is that many people do not get enough exposure to the sun to result in sufficient vitamin D3 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 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 UV exposure is sunscreen. An SPF of 15 will block 99% of UVB light from the skin and not allow vitamin D synthesis. The other factors that limit vitamin D synthesis in human skin are the amount of time exposed and the intensity of the sun. People with lighter skin will be able to synthesize vitamin D more effectively than people with darker skin.

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.

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 2

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, EPB and SC5DL.

Growth in Presence and Absence of sunlight

Figure 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.

Figure 4

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.

Ongoing Work

Figure 6

Alternates 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.

Figure 5

References

6. Invitrogen.com

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