Increasing Vitamin D2 with Ergosterol for Calcium Absorption in Sugarcane

Ivy Nguyen
Cluster 1: Biotechnology
COSMOS Final Project
July 2009

Terms to Know

- Genetic engineering – uses recombinant DNA technology to transform the genome of an organism by piecing together genes from different sources
- Transgenic – having gene(s) transferred from another species [ex: Bt corn – bacterial gene in corn to kill pests]
- Cations – positively charged elements, more protons than electrons; elements toward the left side of periodic table
- GOI – gene of interest

Calcium (Ca^{2+}) and Plants

- Calcium along with potassium and nitrogen are necessary nutrients for a plant to grow
- Component in cell walls and cell membranes, helps maintain structure
- Plants obtain calcium from the environment, most particularly from minerals in the soil
  - Maintains the chemical balance with other elements, reduces salt content of soil, and enhances water transportation to plant

Calcium Deficiency

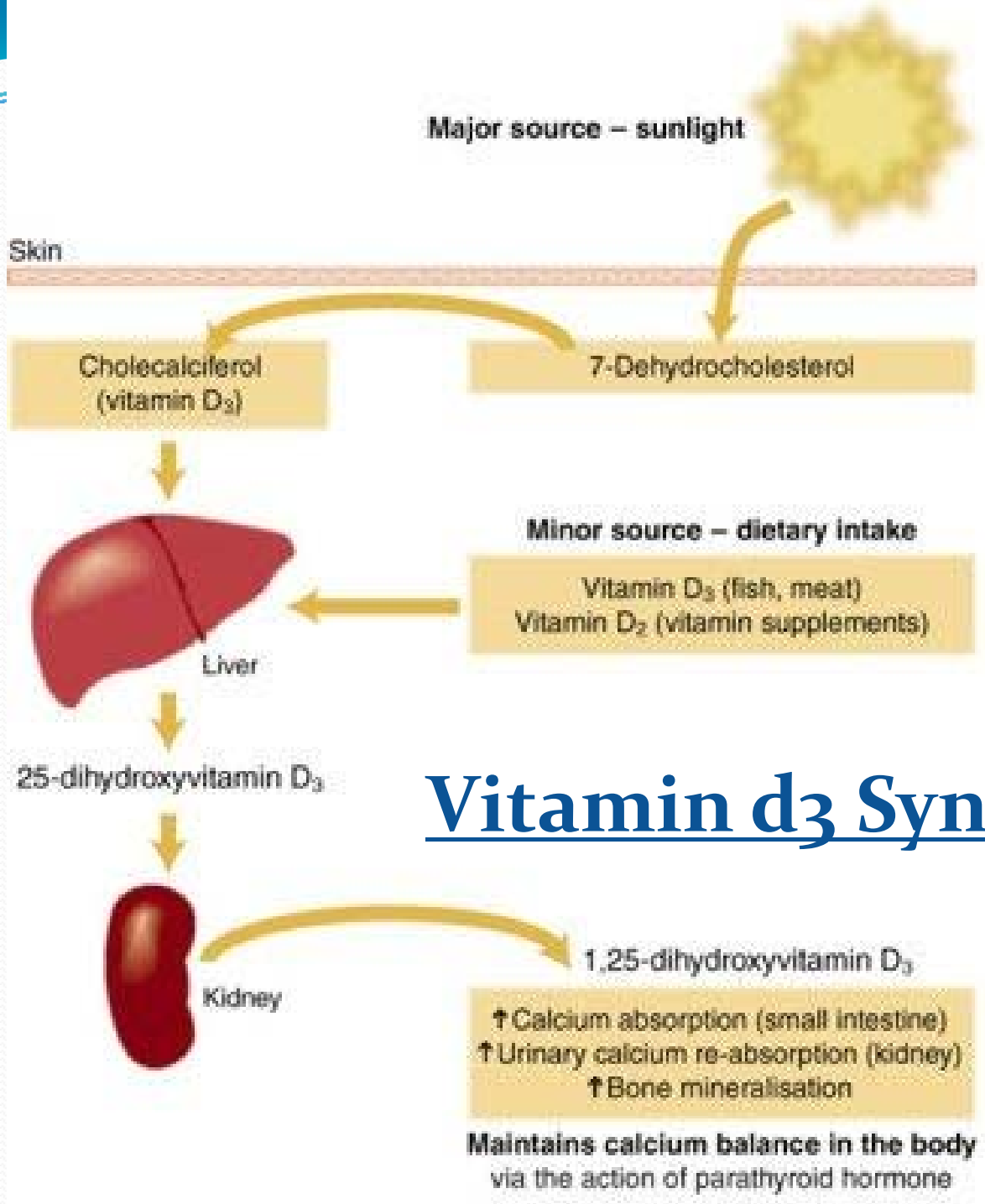
Causes:

- Some soils may contain only small amounts of calcium especially those that are sandy, course, acidic
- Soil can be full of calcium, but calcium uptake could still be hindered → calcium deficit in plant tissues
- Some soils contain many minerals with calcium or calcium carbonate, but are often insoluble
- Overuse of nitrogen and potassium fertilizers reduces calcium absorption
  - High levels of other cations: magnesium (Mg), iron (Fe), aluminum (Al)

Vitamin D

- General purpose in experiment – to absorb calcium
- 2 major forms –
  - Vitamin D2 – ergocalciferol
    - Produced in invertebrates, fungus, and plants
    - Produced when ultraviolet radiation is applied to ergosterol
  - Vitamin D3 – cholecalciferol
    - Produced in skin, formed when ultraviolet light (sunlight has enough wavelenghts) reacts with the vitamin d3 precursor, 7-dehydrocholesterol (derived from cholesterol)
    - In humans – longer process of synthesis, must also be hydroxylated in the liver and kidneys by enzymes

Vitamin d3 Synthesis

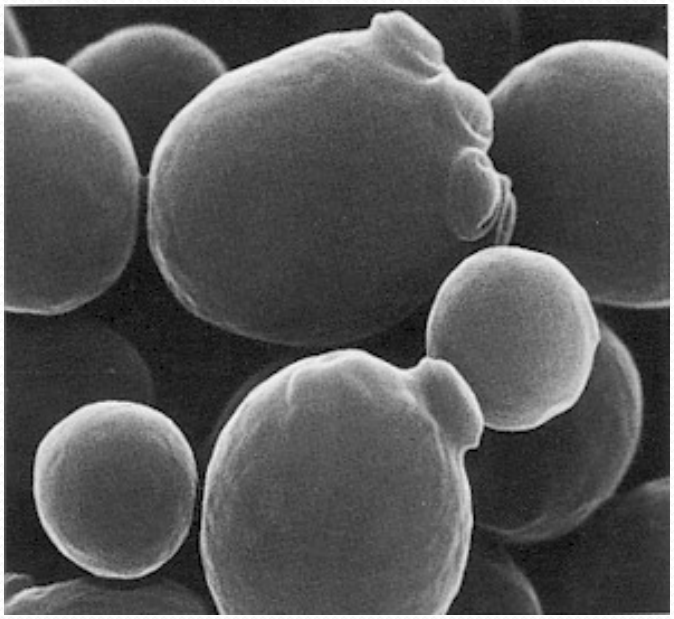


Ergosterol

- A precursor/provitamin to vitamin d2
- When irradiated, converts into viosterol and then ergocalciferol = vitamin d2
- Found in cell membranes of fungus
- Function is analogous to cholesterol in animal cells – building/maintaining membrane
- In this experiment, will isolate two genes from yeast.

Saccharomyces cerevisiae

- Species of budding yeast
- Commonly known as Baker's/Brewer's yeast
- Model eukaryote to study [E.coli – prokaryote]
- Will isolate two genes from the yeast that each code for a different enzyme in ergosterol synthesis
  - Gene ERG24 – C-14 sterol reductase
  - Gene ERG 4 – C-24(28) sterol reductase
    - Catalyzes the final step of ergosterol biosynthesis



Sugarcane

- *Saccharum officinarum*
- Tropical grass species from Graminaceous plant family
- Sugarcane is not a fungi but should have ergosterol based on previous research on other Graminaceous plants (ryegrass, oatgrass, orchard grass)

Purpose

- To genetically engineer a plant to have increased amounts of the vitamin d2 precursor, ergosterol.
- Increased levels of ergosterol → increased vitamin d2 → increased calcium absorption
- Plants would then be able to absorb the necessary calcium from any type of environment/soil quality.
- In this experiment, I will use sugarcane plants, a grass species with ergosterol

Researchable Question

Can a sugar cane plant be genetically engineered to produce increased quantities of ergosterol, a provitamin to vitamin d2 which will then help with the absorption of calcium?

Materials

- *Agrobacterium tumefaciens* (soil-borne bacteria) with pTi plasmid
- Genes of interest - Gene ERG24 and Gene ERG 4 obtained from budding yeast (*Saccharomyces cerevisiae)*
- 35S Promoter – from CaMV [cauliflower mosaic virus]
- Antibiotic resistance gene to prevent non-transformed tissue from growing
- Sugarcane plants
- Soil with different concentrations of calcium to grow the transgenic plants
- Atomic absorption spectroscope – detects presence and concentration of metals in sample

Methods

Four group of sugar cane plants:

- Non-transgenic sugar cane plants grown in calcium sufficient soil
- Non-transgenic sugar cane plants grown in calcium deficient soil
- Transgenic sugarcane plants grown in calcium sufficient soil
- Transgenic sugarcane plants grown in calcium deficient soil

Procedure

1. Identify and isolate genes of interest from *Saccharomyces cerevisiae,* ERG24 and ERG 4
2. Cut out GOI and insert into Ti plasmid in the *A. tumefaciens*
3. Bacteria with plasmid and GOI is then mixed with plant cells
   - Plasmid will move into plant cell and insert its DNA into plant chromosome
4. Newly transformed cells will first be grown in culture medium and then grown in soils of different calcium levels → observe the general health of plants
5. Use an atomic absorption spectroscope to specifically measure the concentration of calcium in the plants

Predicted Results

- If the transformation is successful then there would be an overall increase in calcium levels in the plants.
- The transgenic plants should have a higher production of ergosterol → more vitamin d2 production → better absorption of calcium
- Overall, the success of producing this transgenic plant would allow for plants to obtain the needed amount of calcium from any type of environment.

Potential Problems

- Ergosterol gene fails to insert into the Ti plasmid
  - Can remedy problem by inserting into several plasmids
- Soil may contain other minerals that could affect overall uptake of calcium by the plants
  - Add more calcium to soil, to hopefully block out uptake of the other minerals

Bibliography

- Alcazar-Fuoli, Laura, Emilia Mellado, Guillermo Garcia-Effron, Jordi F. Lopez, Joan O. Grimalt, J. Manuel Cuenca-Estrella, & Juan L. Rodriguez-Tudela. "Ergosterol biosynthesis pathway in *Aspergillus fumigatus*." Steroids 73 (2008): 339-347.
- Bowen, R. "Vitamin D (Calcitriol)." (2007). 28 July 2009. <http://www.vivo.colostate.edu/hbooks/pathphys/endocrine/otherendo/vitamind.html>.
- "Calcium Deficiency." International Rice Research Institute (2003). 28 July 2009. <http://www.knowledgebank.irri.org/RiceDoctor/Fact_Sheets/DeficienciesToxicities/Calcium_Deficiency.htm>.
- "Dietary Supplement Fact Sheet: Vitamin D." Office of Dietary Supplements (2008). 28 July 2009. <http://ods.od.nih.gov/factsheets/vitamind.asp>.
- "ERG24/YNL280C Summary." SGD (2000). 28 July 2009. <http://www.yeastgenome.org/cgi-bin/locus.fpl?locus=erg24>.
- He, Xiuping, Borun Zhang, & Huarong Tan. "Overexpression of a sterol C-24(28) reductase increases ergosterol production in *Saccharomyces cerevisiae*." Biotechnology Letters 25 (2003): 773-778.
- Lai, Margaret H, Martin Bard, Charles A. Pierson, John F. Alexander, Mark Goebl, Guy T. Carter, and Donald R. Kirsch. "The identification of a gene family in the *Saccharomyces cerevisiae* ergosterol biosynthesis pathway." Elsevier B.V. 140.1 (1994): 41-49.

Bibliography cont.

- See A. C. Barnes, *The Sugar Cane* (2d ed. 1973); B. Albert and A. Graves, ed., *World Sugar Economy in War* (1988).
- Skladanka, Jiri, Vlastimil Dohnal, Petr Dolezal, Alena Jezkova, & Ladislav Zeman. "Factors Affecting the Content of Ergosterol and Zearalenone in Selected Grass Species at the End of the Growing Season." Acta Veterinaria Brno 78 (2009): 353-360.
- "The Importance of Calcium." TETRA Technologies, Inc. (2005). 28 July 2009. <http://www.tetrachemicals.com/Products/Agriculture/The_Importance_of_Calcium.aqf>
- Tissue, Brian M. "Atomic-Absorption Spectroscopy (AA)." The Chemistry Hypermedia Project (2000). 28 July 2009. <http://www.files.chem.vt.edu/chem-ed/spec/atomic/aa.html>.
- Veen, Markus, Ulf Stahl, and Christine Lang. “Combined overexpression of genes of the ergosterol biosynthetic pathway leads to accumulation of sterols in *Saccharomyces cerevisiae*.” Federation of Microbiological Societies (2003). 28 July 2009. <www.fems-microbiology.org>.

Picture Bibliography

- Sugarcane [Slide 1] http://blogs.princeton.edu/chm333/f2006/biomass/ethanol%20SUGAR%20CANE2.jpg
- Vitamin d3 Synthesis [Slide 6] http://www.megasun.co.nz/images.php?oid=703
- *Saccharomyces cerevisiae* [Slide 8] http://www.chateauneuf.dk/artikler/vini15.jpg
- Sugarcane [Slide 9] http://upload.wikimedia.org/wikipedia/commons/4/4a/Saccharum_officinarum_Blanco1.18.png