FRESHELLENT YEAST

Valencia-CIPF 2013 iGEM team

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OBJETIVES

The aim is to create a biological platform within *S.cerevisiae* ERG20 K197G to develop an alternative method for the production of several aromatic monoterpenoids.

The yeast will be able to produce two different aromas (linalool and geraniol) and a repellent (1,8-cineole), each one controlled by a promoter: the expression of S-linalool syntase will be induced by Cup1 promoter and the geraniol syntase by a H_2O_2 promoter. The 1,8-cineole syntase will be expressed in a constitutive way using a ADH promoter.

Moreover, genomic-scale metabolic models will be used to estimate the behavior of the organism. Our team will do a modeling based on the model iFF708. This has been validated experimentally by other researchers.

MATERIAL AND METHODS

Linalool synthase gene (LIS) was isolated using specific primers from the ER85 plasmid. Once it was amplified, the TOPO® vector strategy was used in order to increase the amplicon's concentration. Then, LIS gene was isolated digesting with the restriction enzimes BamHI and SalI, and it was ligated with the pYEX-4T plasmid. Finally, it was transformed in the *S.cerevisae* ERG20 K197G yeast.

Regarding the modeling, the metabolic network and the Flux Balance Analysis have been used for assessing the productive capacity of the yeast in the production of 1,8-cineole y S-linalool.

RESULTS

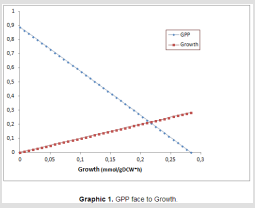
WET LAB

LIS was cloned into pYEX-4T which contained Cup1 promoter. The construction was transformed afterwards into *S. cerevisiae* ERG20 K197G yeast.

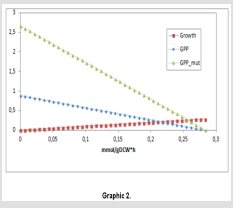
Starting from the initial objectives, good results were not achieved in geraniol synthase and 1,8-cineole synthase. They could not be cloned into their respective plasmids neither expressed in yeast. In the first case, the cDNA could not be obtained; in the second one, the gene could not be amplified from the *A. thaliana* cDNA.

DRY LAB

In one of the simulations GPP was excreted to the medium. As this simulation was in steady-state conditions, the accumulation of this product was perceived. If there is a decrease in the yeast growth, there will be an excess of GPP. At the point of optimal growth, production value of GPP is zero, since all the GPP produced is used for growth. However, it should be highlighted that the points near to the optimum have a higher accuracy, since they are closer to the point validated experimentally (Figure 1).



Graphic 1. GPP face to Growth



Graphic 2.

In another simulation, the ERG20_2 mutant enhances the Geranyl diphosphate (GPP) production and decreases its transformation to Farnesyl diphosphate (FPP).

The production of GPP in the mutant has been maximized, as it is clearly seen that the production is always better than in the wild type, which means that the production of interesting products (1,8-cineole and S-linalool) has increased significantly.

CONCLUSIONS

The ability of the yeast as a biologic device to harvest foreign genes capable to synthesize monoterpenoids was proved.

In the study, the level of expression of the gene could be measured, varying the concentration of copper and measure the time the gene takes to express, in an optimum copper concentration.

On the other side, yeast expressing genes which are capable of inducing the synthesis of other monoterpenic compounds could have been obtained, both in a constitutive way or induced by other substances.

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