CRISPR YEAST:

HOW CAS9 IS REPLACING THE NEED FOR PLANT DERIVED METABOLITES THROUGH YEAST ENGINEERING.

OVERVIEW

A vast amount of important molecules of interest originate from plant specialized metabolites, most of which are synthesized via complex biosynthetic pathways. These compounds play an essential role in plant defence mechanisms and have uses across pharmaceutical, cosmetics, food, fashion and agricultural industries. Despite the vast chemical diversity of natural compounds, their content in plants is often very low, and, as a consequence, this lowers the possibility of high-yielding production of these secondary metabolites from plants. Therefore, microorganisms are widely used as cell factories by industrial biotechnology in the production of different non-native compounds. Among microorganisms commonly used in such applications, yeast is a prominent host for the diverse secondary metabolite biosynthetic pathways.

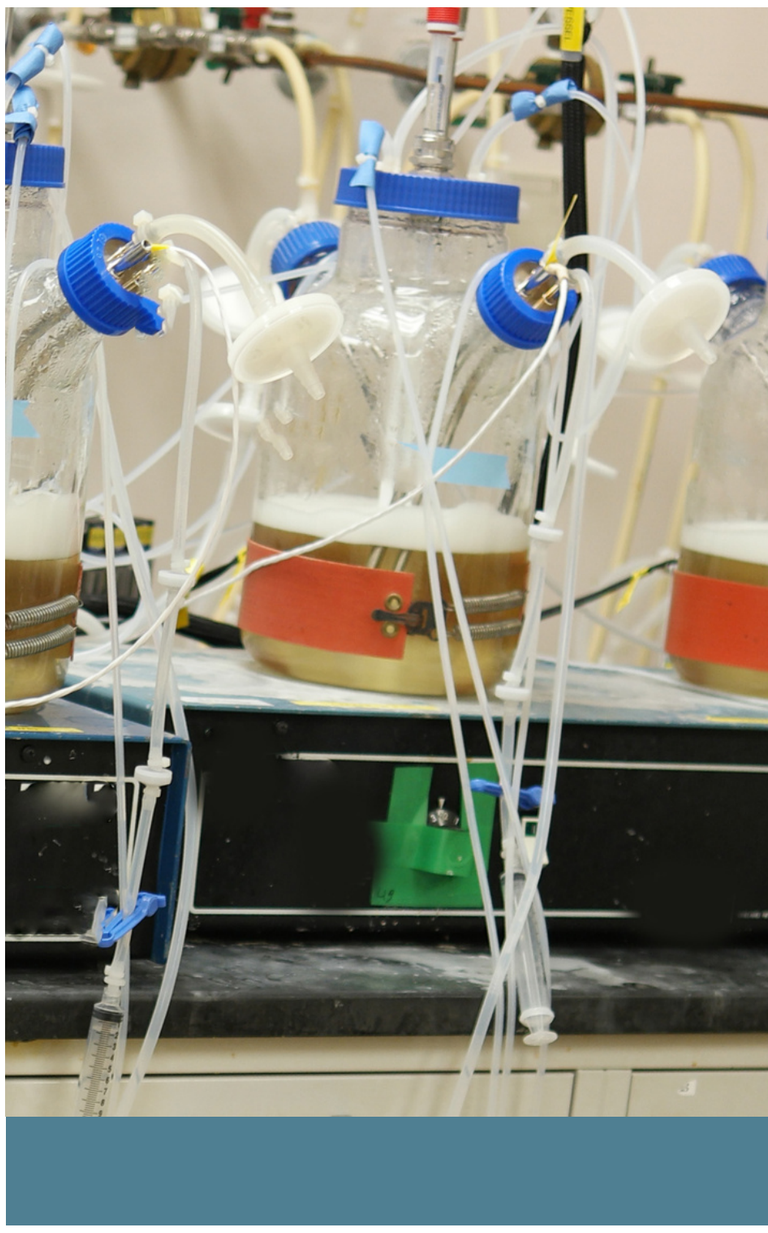
Why employ yeast as a biosynthesis system?

- Efficient yeast transformation techniques simplify the day-to-day use of yeast.
- Yeast has efficient homologous recombination machinery allowing targeted stable incorporation of DNA into its chromosomes.
- Yeast has limited endogenous specialized metabolism pathways, which minimizes competition with introduced pathways. (Pyne et al., 2019).
- Yeast partially shares primary metabolism pathways with plants, which means plant-specialized metabolism pathways can be easily plugged into the existing pool of yeast primary metabolite precursors, albeit some flux enhancement may be required.
- It is relatively safe to work with non-pathogenic yeast, recognized as one of the generally safe microbes.
- Finally, yeast can carry out certain post-translational modifications, something generally lacking in most bacterial strains. (Siddiqui et al., 2012)

Why CRISPR/Cas9?

Despite all the advantages of yeast biosynthesis, reconstructing complex pathways in yeast can still be challenging using traditional methods. Use of plasmids is plagued by plasmid instability and imbalance. It is often difficult to achieve consistent levels of recombinant expression in individual cells, which can lead to different degrees of toxicity and metabolic burden in each yeast cell (Da Silva and Srikrishnan, 2012). Thus, the integration of heterologous genes into the yeast genome is preferred for stable expression. But using homologous recombination, scientists are constrained by a limited number of selection markers for stable transformation.

Gene integration techniques in yeast, such as in vivo homologous recombination and pre-CRISPR nuclease-based systems (e.g., I-SceI, HO endonuclease, ZFNs, and TALENs), have been extensively developed. (David and Siewers, 2015). However, these integration techniques are laborious and are limited by the availability of efficient integration sites, the need for selection markers, or the complexity of design and delivery to target multiple sites.



CRISPR/Cas9 is an elegant and simple technique that has been demonstrated to be effective in a variety organisms. The simplicity, efficiency, and flexibility of CRISPR/Cas9 has greatly stimulated metabolic engineering in yeast. A practical use of CRISPR/Cas9 in yeast is multiplex genome editing aimed at reconstructing complex metabolic pathways (Mali et al., 2013). This system has the capability of efficiently integrating multiple genes of interest in a single transformation without the express need for selection markers, simplifying the reconstruction of complex pathways. As plant specialized metabolites usually have complex multigene biosynthetic pathways, the multiplex CRISPR/Cas9 system in yeast is suited well for functional genomics research in plant specialized metabolism.

USES:

Pharmaceuticals:

Flavonoids play an important role in healthcare with their antioxidant, antibacterial, and anti-inflammatory activities (Adamczak et al, 2020). However, yeast does not naturally produce phenylpropanoid phenolics, where its metabolism provides the necessary aromatic amino acids precursors for the further phenolic biosynthesis pathway. Coumaric acid is a phenolic acid of the hydroxycinnamic acid family, and it has many biological functions such as anti-oxidant, anti-inflammatory, antidiabetic, anti-ulcer, anti-platelet, anti-cancer activities. (Ilavenil et al, 2016)

Koopman et al reported that S. cerevisiae is capable of de novo production of naringenin by co-expressing the naringenin production genes from A. thaliana and optimization of the flux towards the naringenin pathway. In doing so, they demonstrated that engineered yeast naringenin production host provides a metabolic chassis for production of a wide range of pharmaceutically important flavonoids. (Koopman et al., 2012).

Dyes: Cosmetics, Fashion, Food

Anthocyanins are one of the most important plant pigments, and they are responsible for most of the red, blue, and purple colours of leaves, fruits, and flowers with applications in the cosmetic, food and fashion industries.

Anthocyanins are considered as flavonoids due to their C6-C3-C6 chemical structure, although they have a positive charge at the oxygen atom of the C-ring of the basic flavonoid structure. (Kong et al,. 2003).

Anthocyanins are produced in a specific branch of the flavonoid pathway. From naringenin, they are biosynthesized by flavanone 3-hydroxylase, dihydroflavonol 4-reductase, and anthocyanidin synthase. This requires the insertion of a minimum of 3 three genes.

Eichenberger et al. successfully reconstituted the full pathway of the biosynthesis of pelargonidin-3-O-glucoside (P3G), cyanidin-3-O-glucoside, and delphinidin-3-O-glucoside within S. cerevisiae using flanked 60 base pair homologous recombination. (Eichenberger et al,. 2018)

Brewing:

In brewing, additions of Humulus lupulus plant (Hops) are made as a source of bitterness, aroma, and flavour in beer. Hops contain volatile thiols that impart a variety of desirable flavours and aromas in beer. Two volatile thiols of particular interest are 3-mercaptohexan-1-ol and its acetate ester, 3-mercaptohexyl acetate, which impart guava and passionfruit flavours, respectively. Yeasts also produce a variety of volatile organic compounds during their primary and secondary metabolism which also contribute to the flavour and aroma profile of the final product like beer, wine and spirits. These metabolic routes allow for the insertion of new genes responsible for flavour compounds that can then be synthesised by the yeast. (Molitor et al., 2022). Molitor et al. achieved the integration of a gene encoding a highly active cysteine S-conjugate beta-lyase enzyme that converts thiol precursors into the volatile thiol, 3MH. The newly engineered yeast strain produced beer that had up to 73-fold higher 3MH. The beers were described as “intensely tropical and fruity”, achieved without Hops but instead with genetically engineered yeast. (Molitor et al., 2022)

Other Uses:

Other than gene integration, multiplex CRISPR/Cas9 can also be used to optimize yeast strains via metabolic engineering strategies, such as gene disruption for eliminating competing pathways, gene downregulation for diminishing competing but important pathways, and gene upregulation for boosting endogenous yeast pathways (Sander and Joung, 2014; Pyne et al., 2019).

CONCLUDING REMARKS

CRISPR/Cas9 allows for simplicity in multiplex genome editing in yeast with the aim of reconstructing complex metabolic pathways quickly and efficiently. This system has the capability of integrating multiple genes of interest in a single transformation, simplifying the reconstruction of these complex pathways. As plant specialized metabolites usually have complex multigene biosynthetic pathways, the multiplex CRISPR/Cas9 system in yeast is excellent in functional genomics plant specialized metabolism for molecules of interest to the pharmaceutical, cosmetic, fashion, brewing and food industries.

REFERENCES:

Molitor, R.W. et al. (2022) “The sensorial and chemical changes in beer brewed with yeast genetically modified to release polyfunctional thiols from Malt and hops,” Fermentation, 8(8), p. 370. Available at: https://doi.org/10.3390/fermentation8080370.

Ilavenil, S. et al. (2016) “Potential application of P-coumaric acid on differentiation of c2c12 skeletal muscle and 3T3-L1 preadipocytes—an in vitro and in silico approach,” Molecules, 21(8), p. 997. Available at: https://doi.org/10.3390/molecules21080997.

Koopman, F. et al. (2012) “De novo production of the flavonoid naringenin in engineered saccharomyces cerevisiae,” Microbial Cell Factories, 11(1). Available at: https://doi.org/10.1186/1475-2859-11-155.

Siddiqui, M. S., Thodey, K., Trenchard, I., and Smolke, C. D. (2012). Advancing secondary metabolite biosynthesis in yeast with synthetic biology tools. FEMS Yeast Res. 12, 144–170. doi: 10.1111/j.1567-1364.2011.00774.x

Pyne, M. E., Narcross, L., and Martin, V. J. J. (2019). Engineering plant secondary metabolism in microbial systems. Plant Physiol. 179, 844–861. doi: 10.1104/pp.18.01291

Da Silva, N. A., and Srikrishnan, S. (2012). Introduction and expression of genes for metabolic engineering applications in Saccharomyces cerevisiae. FEMS Yeast Res. 12, 197–214. doi: 10.1111/j.1567-1364.2011.00769.x

David, F., and Siewers, V. (2015). Advances in yeast genome engineering. FEMS Yeast Res. 15, 1–14. doi: 10.1111/1567-1364.12200

Yang, Z., and Blenner, M. (2020). Genome editing systems across yeast species. Curr. Opin. Biotechnol. 66, 255–266. doi: 10.1016/j.copbio.2020.08.011

Doudna, J. A., and Charpentier, E. (2014). The new frontier of genome engineering with CRISPR-Cas9. Science 346:1258096. doi: 10.1126/science.1258096

Mali, P., Yang, L., Esvelt, K. M., Aach, J., Guell, M., DiCarlo, J. E., et al. (2013). RNA-guided human genome engineering via Cas9. Science 339, 823–826. doi: 10.1126/science.1232033

Adamczak A., Ożarowski M., Karpiński T.M. Antibacterial Activity of Some Flavonoids and Organic Acids Widely Distributed in Plants. J. Clin. Med. 2020;9:109. doi: 10.3390/jcm9010109. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

Marienhagen J., Bott M. Metabolic engineering of microorganisms for the synthesis of plant natural products. J. Biotechnol. 2013;163:166–178. doi: 10.1016/j.jbiotec.2012.06.001.

Kong J.-M., Chia L.-S., Goh N.-K., Chia T.-F., Brouillard R. Analysis and biological activities of anthocyanins. Phytochemistry. 2003;64:923–933. doi: 10.1016/S0031-9422(03)00438-2.

Eichenberger M., Hansson A., Fischer D., Dürr L., Naesby M. De novo biosynthesis of anthocyanins in Saccharomyces cerevisiae. FEMS Yeast Res. 2018;18 doi: 10.1093/femsyr/foy046.

Sander, J. D., and Joung, J. K. (2014). CRISPR-Cas systems for editing, regulating and targeting genomes. Nat. Biotechnol. 32, 347–355. doi: 10.1038/nbt.2842

Pyne, M. E., Narcross, L., and Martin, V. J. J. (2019). Engineering plant secondary metabolism in microbial systems. Plant Physiol. 179, 844–861. doi: 10.1104/pp.18.01291