Dow College of Biotechnology

GREEN YOUTH MOVEMENT CLUB

DOW UNIVERSITY OF HEALTH SCIENCES

PROPOSAL

THE CLEAN AND GREEN KARACHI

DEPARTMENT: DOW COLLEGE OF BIOTECHNOLOGY

5th SEMESTER

FRAGRANCE FROM MICROBES
PRODUCTION OF 2-PHENYLETHANOL FROM SACCHAROMYCES CEREVISIAE

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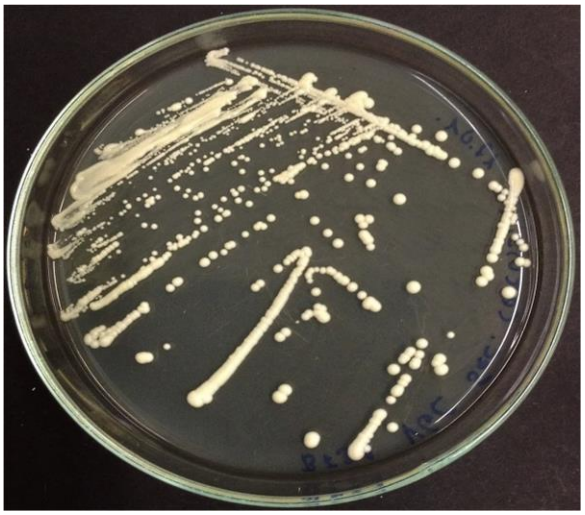


Introduction

2-Phenylethanol is an organic compound known for its mild, warm, and rose-honey-like aroma. It consists of a phenethyl group attached to a hydroxide. It has been used as a fragrance ingredient in many products from cosmetic to cleaning, perfumery, and the food industry. It is naturally found in essential oils and plant from where it can be extracted. This mode of extraction and purification of the compound can have detrimental effects to the flora, as tons of plants are processed for the production of a minimal amount of pure 2-Phenylethanol.

Conventionally, it has been produced using chemical methods which are harsh and often cause harm to the environment. Recent advancements have been made by biotechnologists to produce 2-Phenylethanol by the microbial fermentation process. The technique involves the conversion of L-phenylalanine into 2-Phenylethanol by a biotransformation process called the Ehrlich pathway. Saccharomyces cerevisiae will be used as a starter culture because it is nonpathogenic, inexpensive and simple to handle the whole-cell system.

One of the drawbacks of the microbial production and extraction of 2-Phenylethanol through Ehrlich pathway method is that product inhibition limits the final product concentration and space-time yields because 2-Phenyethanol is toxic in larger concentrations to the yeast cells, which stops its production if the levels reach a certain threshold. This is why it is important to add a reliable in-situ product removal method that would ensure the concentration of 2-Phenylethanol remains under a certain threshold in the culture, and the produced 2-Phenylethanol is extracted rapidly to ensure high product concentration. We propose to add polypropylene glycol as an in-situ product extractant, employing liquid-liquid extraction method. Through the use of polypropylene glycol, the limitation caused by feedback inhibition can be reduced significantly and will result in increased yield of the desired compound.

Problem to be addressed

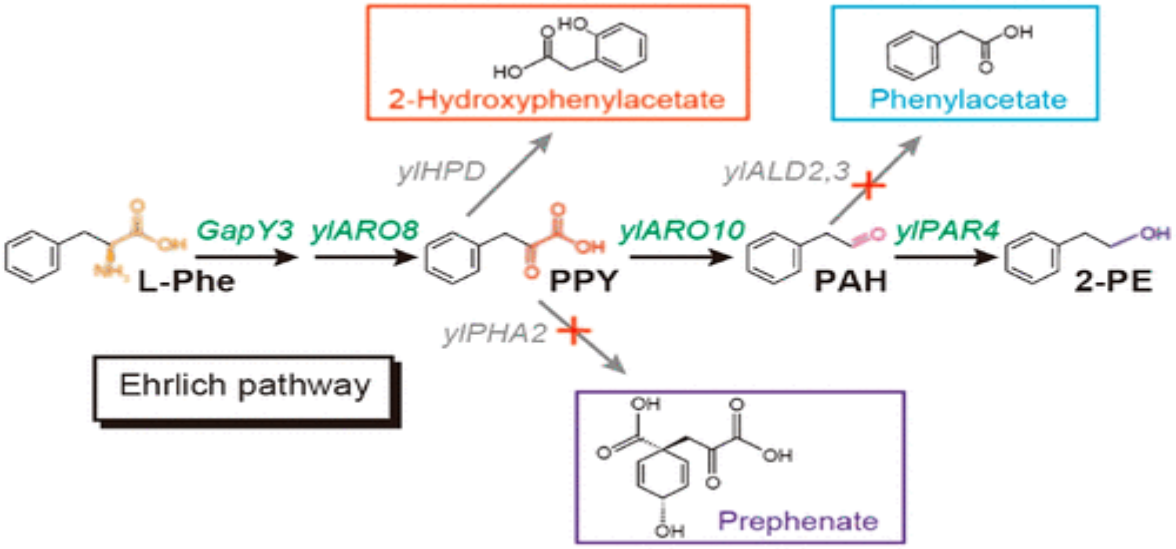
The stoichiometric method of producing 2-PE from rose petals on an industrial level utilizes petrochemicals, certain catalysts and high-temperature conditions. The limitations of this chemical synthesis include undesirable odors, such as chloride-derived species or other unwanted byproducts, into the final product, resulting in a distinct smell.

However, the increasing demand for natural flavors has led to a growing interest in producing 2-PE by biotechnology, the products of which are identified as 'natural'. According to a research Institute based in Faisalabad, 5000kg of roses give nearly 1kg of rose oil. This method causes dismantling of the Flora of Pakistan. Due to the limited availability of naturally occurring 2-PE in flowers, meeting the substantial market demand becomes challenging, resulting in high prices. Therefore, there is a requirement to develop a more efficient, cost-effective, and environmentally sustainable biotechnological approach as an alternative to traditional industrial methods. Extraction of natural 2-PE from the essential oil of some flowers is much more expensive compared to the chemically synthesized 2-PE with an estimated price of about 1000 US$/kg (285,000 Pakistani Rupee) while the estimated cost of bioproduction of 2-PE via a microbial route would actually cost around 220 US$/kg (62700 Pakistani Rupee).

Furthermore, the purification process of 2-PE is complex and intricate due to the presence of unwanted byproducts. Purification techniques involve adsorption, extraction with supercritical CO2, liquid–liquid extraction and membrane processes using oleic acid, heptane, ethyl acetate, toluene and other organic compounds as extractants. Limitations using these extractants are selectivity issues and concerns regarding toxicity, environmental impact, and cost. Careful consideration of alternative extraction methods and optimization of extraction conditions can help address these limitations.

Methodology

We will be using the baker's yeast as a source of *Saccharomyces cerevisiae.* A fed-batch process will be used for the bioconversion of L-phenylalanine to 2-phenylethanol by Ehrlich Pathway and Polypropylene glycol 1200 will be used as an in-situ extractant as product inhibition limits the final product concentrations.



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- Isolating *Saccharomyces cerevisiae* from baker's yeast

Baker's yeast will be inoculated in a sterile seed culture medium containing glucose (20g/L), peptone (5g/L), NaCl (5g/L), and YPD (5 g/L). The yeast will be grown at 30 degrees Celsius in a shaking incubator (180 rpm) until it reaches the mid-logarithmic stage. The culture will be centrifuged for several minutes at a low speed till the yeast cells pellet down. After removing the supernatant, the pellets will be redissolved in buffer or DI water. The yeast cells will be taken out of the remaining medium after a second centrifugation process and transferred to biotransformation media.

- Biotransformation system

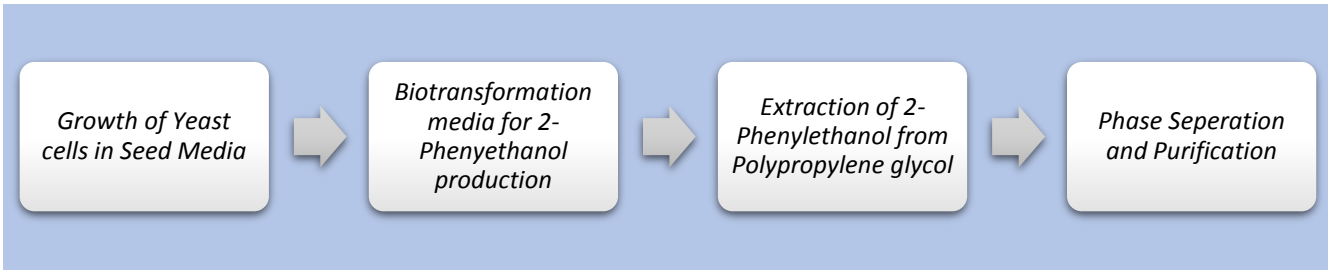
6 % v/v preculture will be introduced in the biotransformation system. This system is composed of molasses (50 g/L), L-phenylalanine (7 g/L), K2HPO4 (0.75 g/L), KH2PO4 (0.5 g/L), for optimal biotransformation conditions and product extraction. Polypropylene glycol 1200 will be added in a 1:1 ratio (aqueous: organic) and it will form an emulsion upon stirring. Glucose, phosphate, and L-phenylalanine will be kept at optimum concentration throughout the fed-batch process to avoid exhaustion of nutrients. The biotransformation will be conducted at 180 rpm in a shaking incubator at 35 degrees Celsius and pH 5.

- Ehrlich pathway

L-Phenylalanine is the sole nitrogen source and is a precursor for the Ehrlich pathway. L-Phenylalanine converts into phenylpyruvate through transamination, phenylpyruvate converts into phenylacetaldehyde through decarboxylation, and in the final step, phenylacetaldehyde converts into 2-Phenylethanol through reduction.

- Phase separation

The liquid-liquid extraction method is employed to separate the phases. The sample will be centrifuged for 2 min at 16,000×g. The aqueous phase will be analyzed directly, while the organic phase will be diluted at least fivefold with methanol prior to HPLC analysis. Further vacuum distillation will be employed for more purification of the final product.



Budget:

PRODUCT	PRODUCT NUMBER ON SIGMA ALDRICH	QUANTITY	PRICING
Yeast from Saccharomyces cerevisiae	YSC2	1KG	43511.26 PKR
L-Phenylalanine, reagent grade	P2126	1KG	283285.29 PKR
Polypropylene Glycol	81380	1L	36483.71 PKR
D-(+)-Glucose	G7021	5KG	64996.19 PKR
Peptone from Glycine max	P0521	500G	72354.25 PKR

YPD Agar	Y1500	250G	35257.37 PKR
Deionized Water	38796	1L	5395.91 PKR
Sucrose (Molasses)	S9378	500G	25354.65 PKR
Potassium Phosphate dibasic	60353	250G	35257.37 PKR
Potassium Phosphate monobasic	P5655	100G	12570.02 PKR
TOTAL:			614466.02 PKR

Executive summary

Through this project, we will explore a novel method of the creation of compound 2-Phenylethanol using yeast, Saccharomyces cerevisiae. 2-Phenylethanol is an organic compound used widely in a variety of products and procedures, ranging from flavors, perfumery to additives and in preservation. It also has microbial properties, making it a useful compound. Over the past few years, a high elevation in its demand has been seen all around the globe and in Pakistan. It had an annual demand of 1,000 tons in 2011, equal to a market value of $700 million. The demand needs to be met in an environmentally friendly way.

The extraction of 2-Phenylethanol is usually done by utilizing plant matter that produce it naturally, this disrupts our ecosystem and diminishes our flora, as tons of plant matter is used to extract the compound. The chemical methods for extraction are just as harmful to the environment.

Our project proposes an alternative to these two current processes by developing a microbial mode of production of 2-Phenylethanol that will be safe and non-hazardous. Through this procedure, we may produce more products in less time and less toxicity. It will bring about and expand the use of microbes and biotechnology. The success of our project could open more gates into producing other commonly used compounds through the use of microbial biotechnology, which is a much rapid, and environmentally sustainable approach to production. The applications

of such technology would be vast and helpful. By eliminating the only drawback of the Ehrlich pathway, the feedback product inhibition of 2-Phenylethanol by using Polypropylene glycol as an in-situ product extraction material, this process of 2-Phenylethanol production through Saccharomyces cerevisiae will be advanced and enhanced.

References

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