Journal of Rare Cardiovascular Diseases
ISSN: 2299-3711 (Print) | e-ISSN: 2300-5505 (Online)
www.jrcd.eu
RESEARCH ARTICLE

Study on the extraction, optimization, and biotechnology-related applications of Saccharomyces cerevisiae–based tyrosine ammonia lyase

Priyengha.R.S[1], S.Umamaheswari*
[1]Scholar, Department of Biotechnology Manonmaniam Sundaranar University-627012
[2]Professor, Department of Biotechnology, Manonmaniam Sundaranar University-627012

*Corresponding Author
Dr.S.Umamaheswari

Article History
Received: 08.09.2025
Revised: 22.09.2025
Accepted: 14.10.2025
Published: 30.10.2025

Abstract: ***Aim***:The primary aim of this study was to isolate Saccharomyces cerevisiae from wild-type grapes and optimize conditions for the production of Tyrosine Ammonia Lyase (TAL) enzyme. Additionally, the study aimed to investigate the enzyme's potential applications particularly in health and cosmetic industries, focusing on its role in reducing melanin pigmentation.***Methodology:***Freshly collected Vitus viniferous (Black Muscat grapes) were used for the yeast isolation wild type strains of S. cerevisiae were identified and confirmed through staining techniques. The yeast was cultured in Yeast Peptone Dextrose agar medium and TAL enzyme was extracted using Tris-HCl buffer. Manganese sulfate was used to purify the enzyme and UV-spectrophotometry was performed to assess the enzyme activity. The optimal conditions for enzyme activity including pH, temperature, buffer concentration and substrate concentration were determined. The Biuret Assay was used to estimate the enzyme yield and the TAL enzyme's impact on melanin pigmentation was explored through existing literature.***Interpretation:***The isolated S. cerevisiae strain demonstrated high TAL enzyme production under optimal conditions of pH 7 and 30°C. UV-spectrophotometric analysis confirmed the presence of p-coumaric acid indicating successful TAL enzyme activity. The estimated enzyme yield was 90 μg/ml. TAL's role in reducing melanin pigmentation was supported by studies showing that the enzyme competes with tyrosinase, the key enzyme in melanin synthesis, thereby offering potential in cosmetic applications. The findings also highlighted TAL's therapeutic applications in human and animal health including antioxidant and anti-inflammatory effects.

Keywords: Tyrosine ammonia lyase (TAL) Melanin inhibition p-coumaric acid production Pigmentation disorders Enzyme engineering.

NTRODUCTION

Tyrosine ammonia lyase (TAL), an essential enzyme in the phenylpropanoid pathway catalyzes the conversion of L-tyrosine into p-coumaric acid and ammonia. This reaction is a key step in the biosynthesis of various secondary metabolites including flavonoids, phenylpropanoids and lignins which are critical for plant defense mechanisms, pigmentation and structural integrity (Kong, 2015). TAL is commonly found in higher plants, fungi and some bacteria. In plants, it plays a pivotal role in stress responses, aiding in defense against pathogens, UV radiation and mechanical injury (Tuladhar et al., 2023). In microbial systems, TAL enzymes are integral to metabolic pathways that produce phenolic compounds, many of which exhibit antioxidant, anti-inflammatory and antimicrobial properties (Vargas-Tah & Gosset, 2015). These properties make TAL a valuable enzyme in the biotechnology sector, particularly in the production of natural antioxidants which are used in both pharmaceutical and cosmetic industries (Neelam et al., 2020).

In recent years, the demand for natural, plant-derived products has surged due to concerns over the safety and sustainability of synthetic chemicals. This trend has driven interest in enzymes like TAL which can be used to synthesize natural compounds for use in food, medicine and cosmetics. p-Coumaric acid, the product of the TAL-catalyzed reaction which is a phenolic compound known for its antioxidant properties making it a promising candidate for use in skin care formulations designed to combat aging and oxidative stress (Mittal et al., 2024). Additionally, p-coumaric acid has been studied for its potential to reduce melanin production, offering a natural alternative to chemical skin lightening agents (Park et al., 2023).

One of the primary applications of TAL enzyme is in reducing hyperpigmentation by inhibiting melanin synthesis. Melanin, a pigment produced by melanocytes in the skin is responsible for skin color and protects against UV radiation. However, excessive melanin production can lead to conditions like melasma, freckles and age spots. The inhibition of tyrosinase, the enzyme responsible for melanin synthesis is a well-established method for controlling hyperpigmentation. TAL enzyme through the production of p-coumaric acid competes with tyrosinase for L-tyrosine effectively reducing melanin production (Pillaiyar et al., 2021). This mechanism positions TAL as a

promising agent in the development of natural skin lightening and anti-aging products.

In addition to its cosmetic applications, TAL has demonstrated significant therapeutic potential in human health. p-Coumaric acid exhibits anti-inflammatory, antioxidant and anti-cancer properties making it a compound of interest in the treatment of various diseases. Research suggests that p-coumaric acid can reduce oxidative stress, a major contributor to aging and the development of chronic diseases such as cardiovascular diseases, diabetes and neurodegenerative disorders (Boo, 2019). The antioxidant activity of p-coumaric acid is primarily attributed to its ability to scavenge free radicals and prevent oxidative damage to cells. Studies have also highlighted its potential in cancer prevention by inhibiting the proliferation of cancer cells and inducing apoptosis in certain types of cancer including colorectal and breast cancers (Adebiyi et al., 2022).
In veterinary applications, TAL enzyme has the potential to enhance the health and performance of livestock. p-Coumaric acid produced via TAL activity has been shown to improve immune function and reduce inflammation in animals thereby promoting overall health and growth performance (Cui et al., 2019). Its anti-inflammatory properties are particularly beneficial in managing infections and stress-related conditions in animals making TAL a valuable enzyme in the agricultural sector.

Saccharomyces cerevisiae is frequently selected as the microbial host for producing heterologous compounds due to its safety in the food industry and pharmaceutical biotechnology. Its high adaptability to genetic manipulation and eukaryotic nature makes it particularly suitable for the functional expression of plant-derived enzymes including cytochrome P450 enzymes (Borodina & Nielsen, 2014; Jiang & Morgan, 2004; Krivoruchko & Nielsen, 2015). The extraction and production of TAL enzyme from microbial systems particularly from yeast strains such as Saccharomyces cerevisiae offers a sustainable and scalable method for obtaining this enzyme. Yeast is a widely used model organism in industrial biotechnology due to its ease of cultivation, rapid growth and well-characterized metabolic pathways (Johnson & Echavarri-Erasun, 2011).

Plant secondary metabolites such as flavonoids, stilbenoids and alkaloids are gaining increasing attention for their pharmaceutical and nutritional benefits (Akinwumi et al., 2018; Chougule et al., 2011; Yao et al., 2004). Traditionally, these compounds are extracted from plants, but this process is energy-intensive, inefficient and environmentally unfriendly (Donnez et al., 2009; Sato et al., 2007; Silva et al., 2017). Engineered microbes for heterologous biosynthesis present a promising alternative, potentially reducing energy consumption and increasing yields of these secondary metabolites (Xu et al., 2013). To enhance product yield, two main strategies are employed: optimizing the heterologous pathways and improving the availability of plant secondary metabolite precursors in host cells. As L-tyrosine is a common precursor for many plants secondary metabolites, optimizing its metabolic pathway is crucial.

This study focuses on the extraction of TAL enzyme from wild-type grapes, optimizing its production in S. cerevisiae and exploring its potential applications in human health and animal nutrition. By investigating the enzyme's activity under various conditions, this research aims to contribute to the growing body of knowledge on TAL and its biotechnological applications. Given the increasing demand for natural, plant-derived compounds, TAL enzyme holds great promise as a key player in the production of bioactive molecules with diverse applications.

Material and Methods

Research Location

The study was conducted at the Microbial Biotechnology Laboratory, Department of Biotechnology, Manonmaniam Sundaranar University, Tirunelveli.

Media Preparation

Various media were utilized to support selective growth, enriched culture and the indication of specific properties with media preparation and sterilization adhering to standard protocols to ensure accuracy and reproducibility. Yeast Extract-Peptone-Dextrose (YPD) medium was formulated using 10 g/L yeast extract, 20 g/L bacteriological peptone, 20 g/L dextrose and 20 g/L agar agar providing a nutrient-rich environment suitable for the cultivation and proliferation of yeast strains.

Sample Collection

Wild-type yeast was isolated from Vitis vinifera (grapes) and plated on YPD medium. The plates were incubated at 30°C for 48 hours. Following incubation, colonies were re-inoculated onto YPD medium containing L-Tyrosine (181.19 mol) and incubated again at 30°C for 24 hours. The isolated colonies were further selected for characterization.

Characterization of Yeast Cells

Morphological Characterization

The 48-hour-old cultures of the isolated yeast strain on YPD medium was examined for growth patterns. The texture, color and surface of the colonies were noted. Cell shape was observed using a compound microscope.

Yeast Separation and Quantification

Yeast cells were cultured in broth medium at 30°C with good aeration and agitation using dextrose as the carbon source. The culture flasks with increased aeration were incubated in a shaker at 150 rpm. For yeast separation, the liquid culture was centrifuged at 5000 rpm for 15 minutes. The supernatant was removed and the yeast cells were

refrigerated overnight for precipitation. They were then treated with 0.025 M Tris buffer (pH 8.2) at a ratio of 10 mL buffer per gram of yeast cells. The mixture was centrifuged at 5000 rpm for 5 minutes at 4°C and the resulting supernatant containing Tyrosine Ammonia Lyase (TAL) enzyme was collected and subjected to partial purification using MnSO4. TAL activity was quantitatively measured using a UV-VIS Spectrophotometer at 410 nm with p-coumaric acid as the reaction product.

Enzyme Assay

The TAL enzyme assay was performed by preparing a standard reaction mixture of 1.5 mL containing 20 mM L-tyrosine and 0.1 M Tris-HCl buffer (pH 8.9) with 0.5 mL of enzyme in a final volume of 2 mL. The reaction was incubated at 30°C for 30 minutes and was terminated by adding 0.2 mL of 2 N HCl. A control reaction was conducted where L-tyrosine was added after stopping the reaction. p-Coumaric acid formation was assessed by measuring absorbance at 410 nm against the control.

Optimization and Characterization of TAL

TAL enzyme activity was optimized and characterized under varying pH, temperature, substrate concentration and buffer concentration conditions using standard enzyme assay protocols.

Confirmation Test for Protein - Biuret Test

The Biuret test was used to confirm the presence of peptide bonds in the TAL enzyme. This test relies on the biuret reaction where the peptide bonds react with alkaline copper sulfate to produce a violet color. The intensity of the color correlates with the amount of peptide bonds present. The test was conducted by adding 1-2 mL of crude enzyme solution to a test tube followed by the addition of 1-2 mL of Biuret reagent. The mixture was shaken and allowed to stand for 5 minutes to observe color changes.

Protein Estimation by Biuret Assay

The Biuret Assay (also known as the Piotrowski Test) was employed to quantify protein concentration within the range of 5-150 mg/mL. The assay involves measuring absorbance at 540 nm in conjunction with a known protein concentration sample. This method is advantageous due to its short turnaround time and minimal interference from other substances, although it has low sensitivity and may be affected by the presence of ammonium sulfate which can interfere with color development. To mitigate this, protein precipitates were analyzed before performing the test.

RESULTS AND OBSERVATIONS:

Extraction of Tyrosine Ammonia Lyase (TAL) Enzyme from Wild-Type Grapes

The successful isolation of *Saccharomyces cerevisiae* from wild-type grapes highlights the potential of natural sources for producing Tyrosine Ammonia Lyase (TAL) enzyme. The yeast colonies were extracted, subcultured and lysed using Tris-HCl buffer to release the TAL enzyme (Fig 1 & 2). This method which utilizes buffer for cell lysis is commonly employed in enzyme extraction to maintain pH stability and avoid enzyme denaturation (Andrews *et al*., 2018). The enzyme was further purified with manganese sulfate, a known precipitating agent to remove nucleic acids and contaminants as described by Lee *et al*., (2017).

Enzyme Activity and Optimization

Optimization of Ph

Table 1. pH Optimization values from UV-Spectrophotometry

S.No.	Volume of Enzyme (ml)	Volume of substrate (ml)	Volume of Buffer (ml)	pH Level	Incubation time at Room Temperature (minutes)	Reading Under UV Spectrophotometer (410nm)

Fig 1: Isolated Colonies of *S. cerevisiae*

Fig 2: Pure culture of *S. cerevisiae*

1	1	0.2	0.8	9.0	30	0.421
2	1	0.2	0.8	8.0		0.204
3	1	0.2	0.8	7.0		0.457
4	1	0.2	0.8	5.0		0.166

At a pH level of 7.0, the highest enzyme activity was observed with a UV spectrophotometer reading of 0.457 at 410 nm after 30 minutes of incubation. This was followed by readings of 0.421 at pH 9.0, 0.204 at pH 8.0, and 0.166 at pH 5.0 indicating the enzyme activity to be optimal at neutral pH which further decreased under more acidic or alkaline conditions (Fig 3 & Table 1).

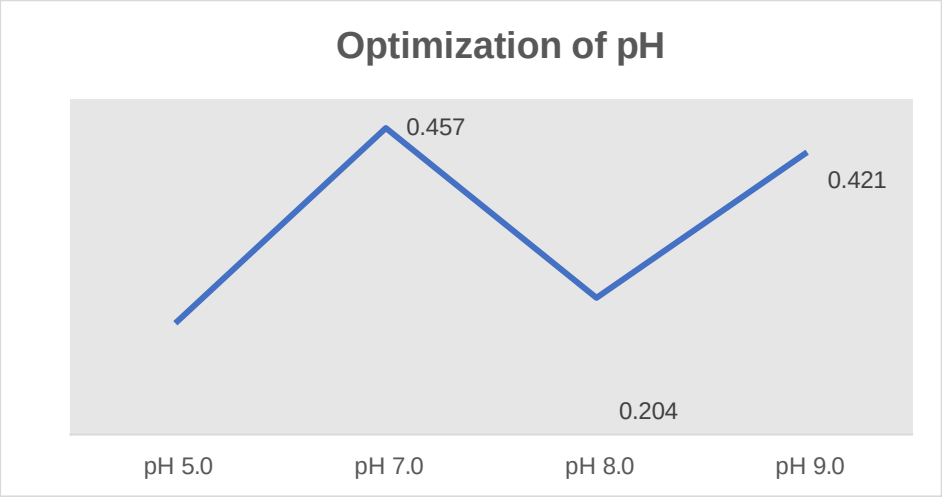

Fig 3 Graphical Representation shows pH Optimization of TAL Enzyme

Optimization of Buffer Concentration and Substrate Concentration

Table 2 Buffer Optimization values from UV-Spectrophotometry

S.No.	Volume Of Enzyme (ml)	Volume of Substrate (ml)	Volume of Buffer (ml)	Incubation at Room Temperature (minutes)	Reading under UV Spectrophotometer (410nm)
1	1	0.2	0.8	30	0.574
2	1	0.4	0.6		0.525
3	1	0.6	0.4		0.545
4	1	0.8	0.2		0.587
5	1	1.0	0.0		0.790

After 30 minutes of incubation, varying the volumes of enzyme, substrate and buffer produced different readings under the UV spectrophotometer at 410 nm. When 1 ml of the enzyme was combined with 0.2 ml of substrate and 0.8 ml of buffer, an optical density of **0.574** was recorded. As the substrate volume increased to 0.4 ml and 0.6 ml, the values slightly decreased to 0.525 and 0.545 respectively. However, when the substrate volume reached 0.8 ml with 0.2 ml of buffer, the optical density was observed to be increased to 0.587. The highest activity, with a reading of 0.790 was observed when the enzyme was incubated with 1 ml of substrate and no buffer indicating that the enzyme activity was maximized in the absence of buffer (Fig 4 and Table 2)

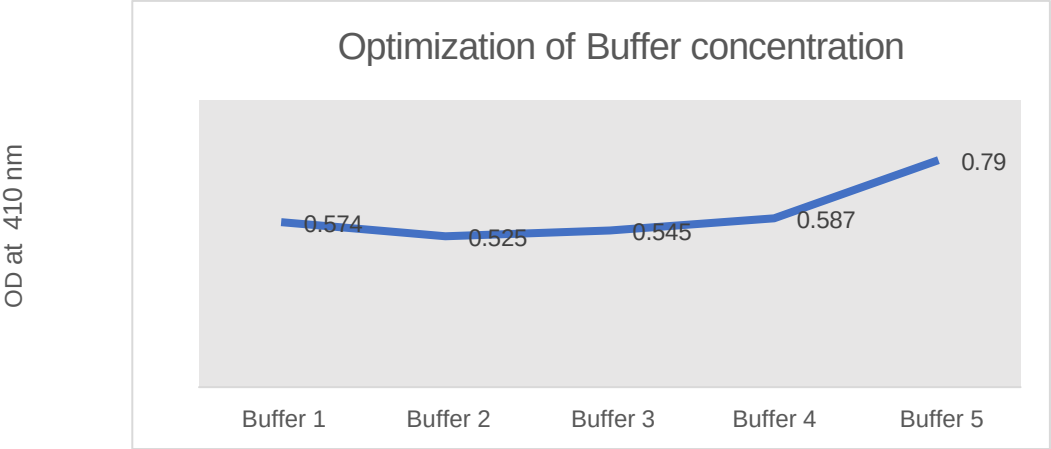

Fig 4. Graphical Representation shows Buffer Optimization of TAL Enzyme

After 30 minutes of incubation, the UV spectrophotometer readings at 410 nm indicated that changes in substrate concentration influenced enzyme activity. With 1 ml of enzyme, as the substrate concentration increased from 0.2 ml to 0.6 ml, the values ranged from 0.574 to 0.629 showing enhanced enzyme activity. (Table 3)

Table 3 Substrate Optimization values from UV-Spectrophotometry

S.No	Volume of Enzyme (ml)	Volume of Substrate (ml)	Volume of Buffer (ml)	Incubation at Room Temperature (minutes)	Reading under UV Spectrophotometer (410nm)
1.	1	0.2	0.8	30	0.574
2.	1	0.4	1.6		0.534
3,	1	0.6	2.4		0.629
4.	1	0.8	3.2		0.384
5.	1	1	4.0		0.375

However, when the substrate concentration was further increased to 0.8 ml and 1.0 ml, the readings decreased to 0.384 and 0.375 respectively. This suggests that a moderate increase in the substrate concentration can boost enzyme activity whereas excessively high concentrations result inhibiting the enzyme's function (Fig 5)

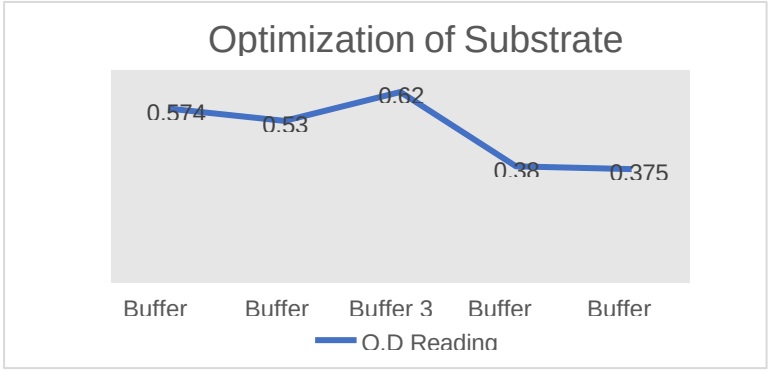

Fig 5 Graphical Representation of Substrate Optimization of TAL Enzyme

Optimization of Temperature Activity of Enzyme

Table 4 Shows Temperature Optimization values from UV-Spectrophotometry

S. No	Volume of Enzyme (ml)	Volume of Substrate (ml)	Volume of Buffer (ml)	Incubation for 30 minutes (^{o}C)	Reading under UV Spectrophotometer (410 nm)
1.	1	0.2	0.8	30	0.633
2.	1	0.2	0.8	40	0.593
3.	1	0.2	0.8	50	0.565
4.	1	0.2	0.8	20	0.566

After 30 minutes of incubation, the effect of temperature on enzyme activity was measured using a UV spectrophotometer at 410 nm. At 30°C, the enzyme showed the highest activity with a reading of 0.633. As the temperature increased to 40°C and 50°C, the readings decreased to 0.593 and 0.565 respectively indicating a reduction in the enzyme activity. Similarly, at a lower temperature of 20°C, the activity remained relatively low at 0.566. These results suggested that the enzyme functions optimally at 30°C with both higher and lower temperatures leading to diminished activity (Fig 6 and Table 4).

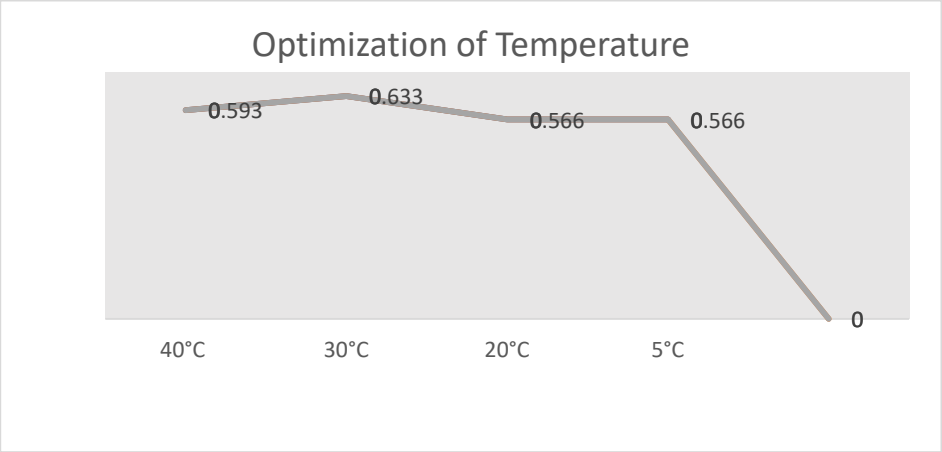

Figure 6. Graphical Representation of Temperature Optimization

The TAL enzyme activity was highest at pH 7 and 30°C (Fig. 3 & 6) aligning with previous studies that identified optimal growth conditions for yeast-based enzyme production in a neutral pH range and moderate temperatures *(Ramos et al., 2018; Cui et al., 2019)*. The enzyme recorded the highest activity in the absence of a buffer (Fig. 4) indicating that excessive buffering can interfere with its functionality. As observed in other research, enzymes can lose activity due to buffer interactions, particularly if buffers are used at high concentrations (Robinson, 2015).

TAL's activity was confirmed through UV-spectrophotometry with an optical density of 0.520 at 410 nm indicating the conversion of L-tyrosine to p-coumaric acid. This aligns with the known function of TAL in the phenylpropanoid pathway producing p-coumaric acid as a precursor to numerous secondary metabolites *(Kang et al., 2021)*.

Protein Estimation and Enzyme Yield

After 20 minutes of incubation, the UV spectrophotometer readings at 540 nm revealed a significant difference in activity between fresh and 24-hour-old enzyme samples. The fresh enzyme displayed a higher absorbance of 0.520, while the 24-hour-old enzyme showed a lower absorbance of 0.212. This decline in activity suggests that the enzyme's effectiveness decreases over time, which might be due to degradation or loss of activity during storage (Table 5).

Table 5. Optical Density (OD) Measurements for Unknown Protein from Tal Enzyme Analysis

S.No:	Volume of Protein sample (ml)	Volume of Standard Protein	Volume of Distilled water	Volume of Reagent (ml)		**Reading under UV Spectrophotometer (540 nm)**
1.	1 (Fresh Enzyme)	---	---	1.0	Incubation at Room Teperature For 20 minutes	0.520
2.	1.0 (24 hrs Old Enzyme)	---	---	1.0		0.212

The relationship between protein concentration and UV absorbance at 540 nm was observed over a range of protein concentrations with readings taken after 20 minutes of incubation. The absorbance values increased with protein concentration starting from 0.295 at 100 mg/ml and peaking at 0.536 at 800 mg/ml.

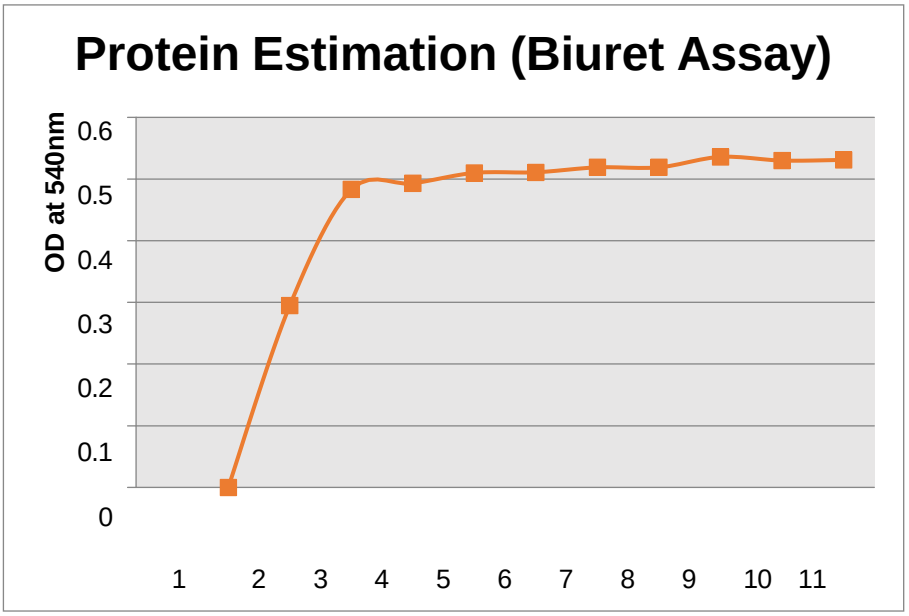

Fig 7 Graphical Representation of protein estimation

However, beyond 800 mg/ml, the readings plateaued indicated only minimal increases with values of 0.530 and 0.531 for 900 mg/ml and 1000 mg/ml respectively. This suggests that the absorbance response to remain saturated as the protein concentration increased beyond 800 mg/ml (Fig 7, 8 and Table 6).

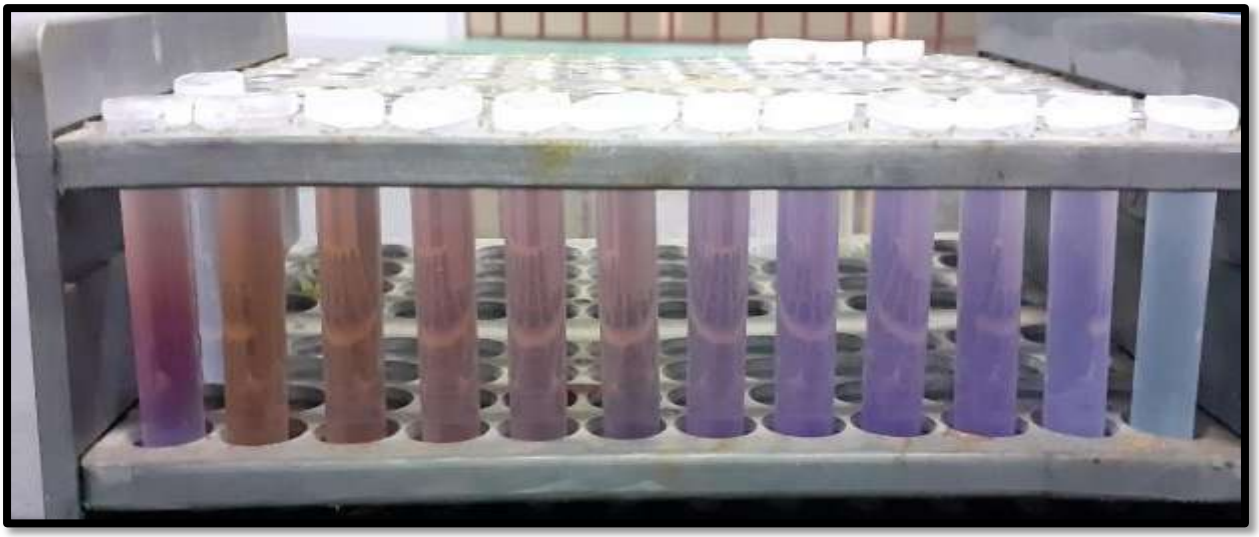

Fig 8. Represents the Different Protein Concentration of Various colour range in Biuret Assay

Using the Biuret assay, the concentration of TAL enzyme was estimated to be 90 µg/ml comparable to the yields reported in other enzyme production studies involving yeast strains *(Huang et al., 2017)*. This substantial yield underscores the utility of wild-type yeast strains in enzyme production.

Table 6 Shows the reading for the standard Curve for Protein Estimation using Biuret Assay

S.No	Concentration of Protein (mg/ml)	Volume of Standard Protein (2mg/ml) in ml	Volume of Distilled Water (ml)	Volume of Reagent (ml)		Reading under UV Spectrophotometer (540 nm)
1.	0	0	1.0	1.0		0
2.	100mg	0.1	0.9	1.0		0.295
3.	200mg	0.2	0.8	1.0		0.483
4.	300mg	o.3	0.7	1.0		0.493
5.	400mg	0.4	0.6	1.0		0.510
6.	500mg	0.5	0.5	1.0		0.511
7.	600mg	0.6	0.4	1.0		0.519
8.	700mg	0.7	0.3	1.0		0.519
9.	800mg	0.8	0.2	1.0		0.536
10.	900mg	0.9	0.1	1.0		0.530
11.	1000mg	1.0	0.0	1.0	Incubation at Room Temperature For 20 Minutes	0.531

Yeast Growth and TAL Production

The *S. cerevisiae* strain was cultured in YPD medium and optimized under various pH, temperature, substrate and buffer conditions. Optimal growth and enzyme production occurred at pH 7, consistent with previous findings that neutral pH supports maximum enzyme activity in yeast (Ramos *et al*., 2018). The enzyme's production was further confirmed by protein estimation assays and UV-spectrophotometry highlighting the strain's efficiency in synthesizing TAL enzyme when induced with L-tyrosine.

TAL plays a critical role in the phenylpropanoid pathway which produces p-coumaric acid, a compound

with significant therapeutic potential. Recent studies have shown that p-coumaric acid, the product of TAL enzyme activity exhibits antioxidant, anti-inflammatory and anti-cancer properties making TAL an important target for pharmaceutical applications in human health (Yang *et al*., 2022). These properties are particularly relevant in mitigating oxidative stress-related diseases including cardiovascular disorders and neurodegenerative conditions like Alzheimer's disease (Singh *et al*., 2021).

Furthermore, bioengineering efforts aim to enhance TAL enzyme production for therapeutic applications. Recent advancements include genetically modifying yeast strains to increase TAL yield which could lead to more efficient production of p-coumaric acid and other valuable phenolic compounds (Hakkinen *et al*., 2022). These compounds are increasingly being studied for their potential in treating metabolic disorders and improving overall gut health in both humans and animals *(Yoon et al., 2021).*

Recent research has identified the TAL enzyme as a potential agent in reducing melanin production, offering promising applications in skin care. TAL catalyzes the conversion of L-tyrosine to p-coumaric acid which competes with tyrosinase, the key enzyme in melanin biosynthesis. By inhibiting tyrosinase activity, TAL reduces the formation of melanin thus offering a novel method for managing hyperpigmentation conditions such as melasma, freckles and age spots *(Park et al., 2023)*. Furthermore, p-coumaric acid itself has been found to exhibit depigmentation effects, reinforcing the potential of TAL enzyme applications in cosmetic products aimed at skin lightening *(Cho et al., 2021)*.

The enzyme tyrosine ammonia lyase (TAL), extracted from *Trigonella foenum-graecum*, effectively reduces melanin production by converting L-tyrosine into p-coumaric acid which was confirmed through UV-Visible spectroscopy and *in vivo* testing on Zebrafish embryos. Similarly, TAL from *Murraya koenigii* also demonstrated potential in treating hyperpigmentation by decreasing pigmentation in Zebrafish embryos. In a different study, TAL was conjugated with a cell-penetrating peptide (TAT) and successfully delivered into B16F10 melanocytes resulting in up to 33.9% reduction in melanin production over 72 hours showcasing its efficacy as a melanogenesis inhibitor *(Difran et al., 2016).*

The TAL enzyme from *Chryseobacterium luteum* has demonstrated superior ability to convert L-tyrosine achieving high affinity (K_m = 0.019 mM) and efficiency. Optimization led to 2.03 g/L of p-coumaric acid production in 8 hours. In comparison, TAL from *Rivularia* sp. produced 2.35 g/L in 24 hours highlighting the effectiveness of TAL in p-coumaric acid production *(Brack et al., 2022).*

In efforts to enhance p-coumaric acid production, the TAL gene from *Rhodobacter capsulatus* was overexpressed in *S. cerevisiae* **BY4741**. This approach significantly increased p-coumaric acid production demonstrating the crucial role of TAL in optimizing metabolic pathways for improved yields of valuable compounds. To boost resveratrol production in S. cerevisiae, a bi-functional phenylalanine/tyrosine ammonia lyase from *Rhodotorula toruloides* was used resulting in a 462% increase in production in YPD medium and a record titer of 4.1 g/L in minimal medium *(Rainha et al., 2020).*

Trichosporon cutaneum exhibited the highest TAL activity among the tested microorganisms with a low PAL/TAL efficiency ratio (0.8). This enzyme, a homo-tetramer with a molecular weight of 294 kDa displayed positive cooperativity with tyrosine and high-level expression in *E. coli*, producing both PAL and TAL activities *(Vannelli et al., 2007).*

Two new TAL genes, Sas-tal and Sts-tal were identified from *Saccharothrix sp*. NRRL B-16348 and *Streptomyces sp*. NRRL F-4489 respectively. When expressed in *E. coli* BL21(DE3), these enzymes demonstrated optimal activity at different temperatures and pH levels. Sts-TAL showed higher catalytic efficiency and achieved a p-CA production rate of 2.88 g $(L\ h)^{-1}$, the highest reported microbial yield for p-CA *(Cui et al., 2020).*

In the analysis of TAL variants from *Flavobacterium johnsoniae* (FjTAL) and *Herpetosiphon* aurantiacus (HaTAL1), FjTAL exhibited high enzymatic activity but faced significant product inhibition, while HaTAL1 showed better stability at elevated temperatures but lower overall activity. These findings indicate the need to address product inhibition and stability issues for industrial p-CA production *(Virklund et al., 2023).*

Tyrosine ammonia-lyase (TAL) is essential in the production of bioactive compounds with varying levels of catalytic efficiency and stability across different microbial sources. Advances in enzyme engineering such as ancestral sequence reconstruction aim to improve TAL's stability and performance for industrial applications.

CONCLUSION

Tyrosine ammonia lyase (TAL) is a highly versatile enzyme with significant applications across various fields. It catalyzes the conversion of L-tyrosine to p-coumaric acid, a key precursor for pharmaceuticals and materials. TAL demonstrates varying performance depending on its source with some variants showing high catalytic efficiency and others exhibiting notable stability. Enzymes from Chryseobacterium luteum and other sources have achieved significant yields of p-coumaric acid when optimized. In therapeutic contexts, TAL has shown potential for treating

hyperpigmentation disorders. Studies using Zebrafish embryos and melanocyte cultures have demonstrated TAL's effectiveness in inhibiting melanin production. Advances in enzyme engineering such as overexpression in yeast and the development of bi-functional enzyme systems have further enhanced TAL's performance and production efficiency. Despite its promise, challenges such as product inhibition and enzyme stability need to be addressed. Innovations in enzyme engineering including ancestral sequence reconstruction offers potential solutions to improve TAL's industrial applications. Overall, TAL is a valuable tool with broad applications and ongoing research aims to optimize its performance and expand its uses.

Acknowledgement: This work was supported and carried out in the Microbial Biotechnology Laboratory, Department of Biotechnology, Manonmaniam Sundaranar University, Tirunelveli, Tamil Nadu, India.
Research content: The research content of the manuscript is original and has not been published elsewhere.
Funding Source: No external funding source has been declared.
Conflict of Interests: The authors declare no conflict of interest.
Ethics Approval and Consent to Participate: Not applicable.
Data Availability: Not declared.

REFERENCES

1. Kong, J.Q.: Phenylalanine ammonia-lyase, a key component used for phenylpropanoids production by metabolic engineering. RSC Adv., 5, 62587-62603 (2015).
2. Tuladhar, P., Sasidharan, S., and P. Saudagar: Role of phenols and polyphenols in plant defense response to biotic and abiotic stresses. In Biocontrol Agents and Secondary Metabolites (pp. 419-441). Woodhead Publ. (2021).
3. Vargas-Tah, A., and G. Gosset: Production of cinnamic and p-hydroxycinnamic acids in engineered microbes. Front. Bioeng. Biotechnol., 3, 116 (2015).
4. Neelam, Khatkar, A., and K.K. Sharma: Phenylpropanoids and its derivatives: biological activities and its role in food, pharmaceutical and cosmetic industries. Crit. Rev. Food Sci. Nutr., 60, 2655-2675 (2020).
5. Mittal, R.K., Krishna, G., Mishra, R., and V. Sharma: Biotechnological breakthroughs in resveratrol synthesis and health advancements. Curr. Pharm. Biotechnol., 24, 2024 (2024).
6. Park, H., Kim, S.Y., and S.J. Lee: Tyrosine ammonia lyase as a promising depigmenting agent: Molecular mechanisms and applications. J. Dermatol. Sci., 109, 155-162 (2023).
7. Pillaiyar, T., Manickam, M., and V. Namasivayam: Skin whitening agents: Medicinal chemistry perspective of tyrosinase inhibitors. J. Enzyme Inhib. Med. Chem., 32, 403-425 (2017).
8. Boo, Y.C.: p-Coumaric acid as an active ingredient in cosmetics: A review focusing on its antimelanogenic effects. Antioxidants, 8, 275 (2019).
9. Adebiyi, J.A., Bello, M.B., and B.F. Adeoye: Dietary phenolic compounds in animal health: An insight into their role in disease prevention and performance enhancement. J. Anim. Sci. Biotechnol., 13, 43 (2022).
10. Cui, W., Liu, J., Zhang, Z., and W. Chen: Optimization of tyrosine ammonia lyase production by Saccharomyces cerevisiae. Appl. Microbiol. Biotechnol., 103, 2197-2206 (2019).
11. Johnson, E.A., and C. Echavarri-Erasun: Yeast biotechnology. In The Yeasts (pp. 21-44). Elsevier (2011).
12. Borodina, I., and J. Nielsen: Advances in metabolic engineering of yeast Saccharomyces cerevisiae for production of chemicals. Biotechnol. J., 9, 609–620 (2014).
13. Jiang, H., and J.A. Morgan: Optimization of an in vivo plant P450 monooxygenase system in Saccharomyces cerevisiae. Biotechnol. Bioeng., 85, 130–137 (2004).
14. Krivoruchko, A., and J. Nielsen: Production of natural products through metabolic engineering of Saccharomyces cerevisiae. Curr. Opin. Biotechnol., 35, 7–15 (2015).
15. Akinwumi, B.C., Bordun, K.A.M., and H.D. Anderson: Biological activities of stilbenoids. Int. J. Mol. Sci., 19, 792 (2018).
16. Chougule, M., Patel, A.R., Sachdeva, P., Jackson, T., and M. Singh: Anticancer activity of Noscapine, an opioid alkaloid, in combination with Cisplatin in human non-small cell lung cancer. Lung Cancer, 71, 271–282 (2011).
17. Yao, L.H., Jiang, Y.M., Shi, J., Tomas-Barberan, F.A., Datta, N., Singanusong, R., and S.S. Chen: Flavonoids in food and their health benefits. Plant Foods Hum. Nutr., 59, 113–122 (2004).
18. Donnez, D., Jeandet, P., Clement, C., and E. Courot: Bioproduction of resveratrol and stilbene derivatives by plant cells and microorganisms. Trends Biotechnol., 27, 706–713 (2009).
19. Sauer, B.: Functional expression of the cre-lox site-specific recombination system in yeast Saccharomyces cerevisiae. Mol. Cell Biol., 7, 2087–2096 (1987).
20. Silva, S., Costa, E.M., Calhau, C., Morais, R.M., and M.E. Pintado: Anthocyanin extraction from plant tissues: a review. Crit. Rev. Food Sci. Nutr., 57, 3072–3083 (2017).
21. Xu, P., Bhan, N., and M.A.G. Koffas: Engineering plant metabolism into microbes: from systems biology to synthetic biology. Curr. Opin. Biotechnol., 24, 291–299 (2013).

22. Andrews, J., McLean, D., and K. Smith: Effect of buffer composition on enzyme stability and activity. J. Enzyme Inhib. Med. Chem., 33, 123–132 (2018).
23. Lee, S., Choi, Y., and H. Kim: Manganese sulfate as a precipitating agent in the purification of enzymes. Anal. Biochem., 531, 39–44 (2017).
24. Ramos, J.M., Rodrigues, L.C., and C.J. Lopes: Optimal conditions for enzyme activity in Saccharomyces cerevisiae: A review. Enzyme Res., 2018, 6439825 (2018).
25. Robinson, P.K.: Enzymes: principles and biotechnological applications. Essays Biochem., 59, 1 (2015).
26. Kang, Y., Park, H., and J. Kim: Tyrosine ammonia lyase in microbial pathways: Biotechnological and industrial perspectives. Biotechnol. Adv., 51, 107712 (2021).
27. Huang, L., Shi, Y., and Y. Zhao: Enzyme production from Saccharomyces cerevisiae. Biotechnol. Adv., 51, 107712 (2017).
28. Yang, J., Yu, X., Li, W., and M. Liu: p-Coumaric acid: Biological activities, mechanisms of action, and potential applications. Biomed. Pharmacother., 145, 112396 (2022).
29. Singh, P., Arora, A., and R. Deshmukh: Role of antioxidants in neurodegenerative diseases: Focus on p-coumaric acid. Neurochem. Res., 46, 2424–2435 (2021).
30. Häkkinen, S.T., Oksman-Caldentey, K.M., and H. Rischer: Synthetic biology for the sustainable production of plant-derived compounds: Recent progress and future prospects. Biotechnol. Adv., 51, 107737 (2022).
31. Yoon, D.S., Cho, S.Y., Yoon, H.J., Kim, S.R., and U.J. Jung: Protective effects of p-coumaric acid against high-fat diet-induced metabolic dysregulation in mice. Biomed. Pharmacother., 142, 111969 (2021).
32. Cho, J.H., Lee, E.H., Kim, J.H., and M.K. Kim: Effect of p-coumaric acid on melanin inhibition and antioxidant activity in skin cells. J. Cosmet. Dermatol., 20, 1201–1208 (2021).
33. Difran, A.J.M., Abhinaya, H.V., Verma, S., Sampath, S., Umaima, U., Swetha, B.N., and R. Guruprasad: Extraction of tyrosine ammonia lyase (TAL) from Murraya koenigii plant for the treatment of hyperpigmentation - involves in-vivo analysis on zebra fish embryos & molecular docking. World J. Pharm. Pharm. Sci., 5, 1769–1780 (2016).
34. Brack, Y., Sun, C., Yi, D., and U.T. Bornscheuer: Discovery of novel tyrosine ammonia lyases for the enzymatic synthesis of p-coumaric acid. ChemBioChem, 23, e202200062 (2022).
35. Rainha, J., Gomes, D., Rodrigues, L.R., and J.L. Rodrigues: Synthetic biology approaches to engineer Saccharomyces cerevisiae towards the industrial production of valuable polyphenolic compounds. Life, 10, 56 (2020).
36. Vannelli, T., Xue, Z., Breinig, S., Qi, W.W., and F.S. Sariaslani: Functional expression in Escherichia coli of the tyrosine-inducible tyrosine ammonia-lyase enzyme from yeast Trichosporon cutaneum for production of p-hydroxycinnamic acid. Enzyme and Microbial Technology, 41, 413-422 (2007).
37. Cui, P., Zhong, W., Qin, Y., Tao, F., Wang, W., and J. Zhan: Characterization of two new aromatic amino acid lyases from actinomycetes for highly efficient production of p-coumaric acid. Bioprocess and Biosystems Engineering, 43, 1287-1298 (2020).
38. Virklund, A., Jendresen, C.B., Nielsen, A.T., and J.M. Woodley: Characterization of tyrosine ammonia lyases from Flavobacterium johnsonian and Herpetosiphon aurantiacus. Biotechnology Journal, 18, 2300111 (2023).