ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25jul226

# Extraction Partial Purification and Characterization of Tyrosinase Enzyme from Isolated Microorganisms

Sheetal Patil[1], Neelakanth M Jeedi[2*,] Savita S Desai[3]

[1,2,3]Department of pharmacology, KLE College of Pharmacy, Hubballi - 580031 (A Constituent Unit of KLE Academy of Higher Education and Research, Belagavi, Karnataka), India

Corresponding Author: Dr. Neelakanth.M. Jeedi[*]

Publication Date: 2025/07/09

**Abstract: Tyrosinase (EC 1.14.18.1), a copper-containing bifunctional enzyme, catalyzes the hydroxylation of monophenols to o-diphenols and their oxi- dation to o-quinones, playing key roles in melanin synthesis, L-DOPA pro- duction, and bioremediation of phenolic compounds. This study aimed to isolate, purify, and characterize tyrosinase-producing microorganisms from freshwater soil and water samples in Ahmednagar, India. Samples were enriched on tyrosine-containing media, and black-brown pigment for- mation was used to screen for tyrosinase activity. Among the isolates, the most promising was identified as *Brevundimonas diminuta* by 16S rRNA gene sequencing. Tyrosinase was partially purified via ammonium sulfate precipitation and dialysis, and characterized for pH, temperature, metal ion effects, and substrate concentration. The enzyme showed optimal ac- tivity at pH 7.0 and 37°C, with increased activity in the presence of $Cu^{2+}$. Kinetic analysis revealed Michaelis–Menten constants in line with other bacterial tyrosinases. This work highlights the biotechnological potential of *B. diminuta* tyrosinase in environmental and pharmaceutical applica- tions.**

***Keywords:*** *Tyrosinase, Brevundimonas Diminuta, L-DOPA, Bioremedia- Tion, Enzyme Characterization.*

**How to Cite:** Sheetal Patil; Neelakanth M Jeedi; Savita S Desai (2025) Extraction Partial Purification and Characterization of Tyrosinase Enzyme from Isolated Microorganisms. *International Journal of Innovative Science and Research Technology*, 10(7), 145-149, https://doi.org/10.38124/ijisrt/25jul226

## I. INTRODUCTION

Tyrosinase (EC 1.14.18.1) is a copper-metalloenzyme exhibiting monophenolase and diphenolase activities central to melanin biosynthesis and the browning of plant-derived materials. Its applications span pharmaceuticals, where it assists L-DOPA synthesis for Parkinson's treatment, to environmental bioremediation of phenolic pollutants, and in food processing and cosmetics. However, sourcing tyrosinase from traditional plant or animal origins poses challenges including low yields, instability, and high purification costs. Microbial tyrosinases are of particular interest, offering scalable, eco-friendly, and genetically adaptable sources. Among microbial sources, bacteria have been underexplored compared to fungi, but recent studies have highlighted their simpler purification profiles and superior stability. The present work focuses on the isolation of tyrosinase- producing bacteria from freshwater lakes of Ahmednagar, India, aiming to iden- tify robust producers suitable for downstream industrial processes. Particular attention was given to optimizing production parameters and partially purifying the enzyme to evaluate its operational stability and kinetic characteristics.

## II. MATERIALS AND METHODS

- *Sample Collection and Isolation:*

  Samples were collected aseptically from soil, mud, and water at Kapurwadi, Dongargan, and Pimpalgaon lakes, transported at 4°C, and processed within 24 hours. Serial dilutions were plated on nutrient agar (bacteria) and potato dex- trose agar (fungi) supplemented with 0.5 g/L L-tyrosine and 0.004 g/L $CuSO_4$. Incubation was at 28–30°C for 3–7 days. Pigmented colonies were purified by streaking.

- *Screening and Identification:*

  The black-brown pigmented colonies were stored as slants and glycerol stocks. Biochemical tests (Gram stain, catalase, oxidase) and morphological features were recorded. 16S rRNA sequencing was performed with universal primers, and phylogenetic trees constructed using neighbor-joining methods.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25jul226

➢ *Production of Tyrosinase:*

Isolates were grown in a production medium containing starch (25 g/L), glucose (10 g/L), yeast extract (2 g/L), $CaCO_3$ (3 g/L), L-tyrosine (1 g/L) and a trace metal mix. Cultivation was performed at 28°C, 150 rpm for 7 days, then the culture supernatant was harvested.

➢ *Enzyme Assay:*

Tyrosinase activity was assayed by dopachrome formation with L-DOPA as substrate, measuring absorbance at 475 nm. Activity units were calculated using $\varepsilon = 3600\ M^{-1}cm^{-1}$.

➢ *Partial Purification:*

The crude enzyme was precipitated with 40% ammonium sulfate overnight at 4°C, centrifuged, resuspended in phosphate buffer pH 7, and dialyzed.

➢ *Characterization:*

Enzyme activity was tested over pH 5–9, temperatures 30–70°C, and in pres- ence of metal ions ($Hg^{2+}$, $Ca^{2+}$, $Mn^{2+}$, $Mg^{2+}$, $Cu^{2+}$). Kinetic constants were determined by Michaelis–Menten and Lineweaver–Burk methods.

## III. RESULTS AND DISCUSSION

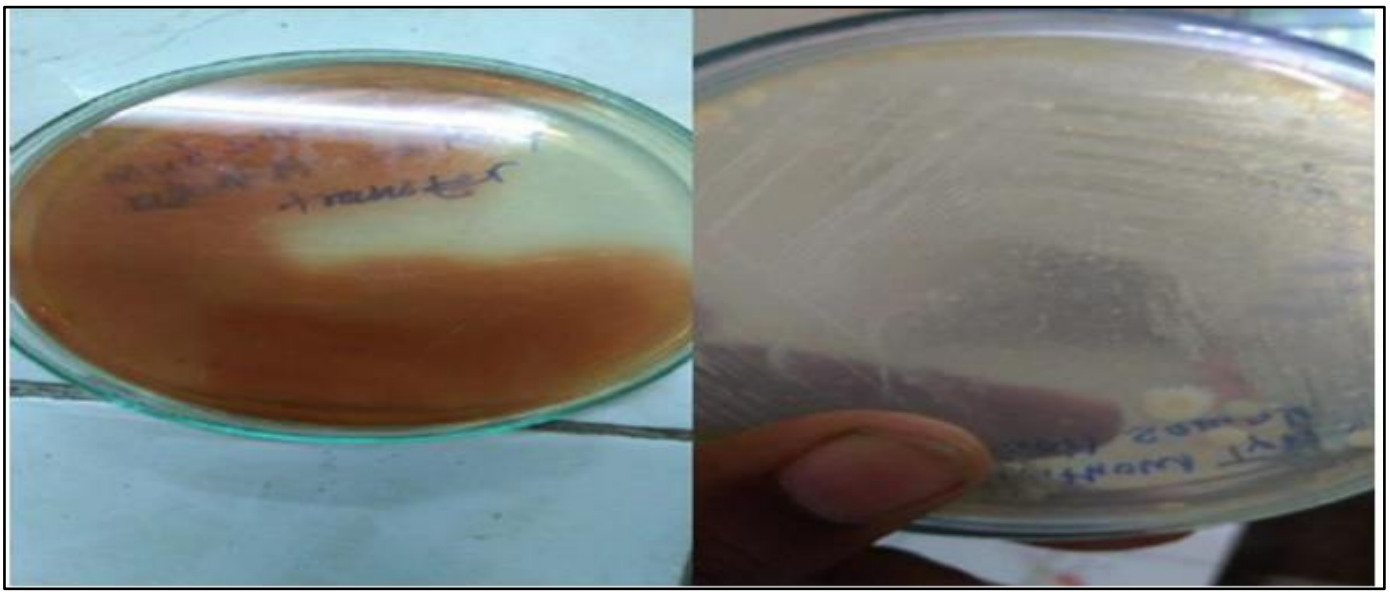


Fig 1 Growth of Tyrosinase-producing Organism on Agar Medium with and without L-Tyrosine.

➢ *Isolation and Screening:*

Out of nine environmental samples, three showed dark-brown pigmentation on tyrosine media, indicating tyrosinase activity. The Kapurwadi mud isolate (K.M.) was the most potent.

➢ *Enzyme Activity:*

K.M. showed 43 U/mL/min tyrosinase activity, the highest among the isolates.

➢ *Optimization of Production:*

Activity was highest at pH 7 and 37°C, declining sharply outside these con- ditions. Enzyme activity rose with L-tyrosine up to 20 mM, then plateaued.

➢ *Partial Purification and Characterization:*

Ammonium sulfate precipitation retained most activity. Enzyme was copper- dependent, inhibited by mercury ions, stable at pH 7 and moderate temperature.

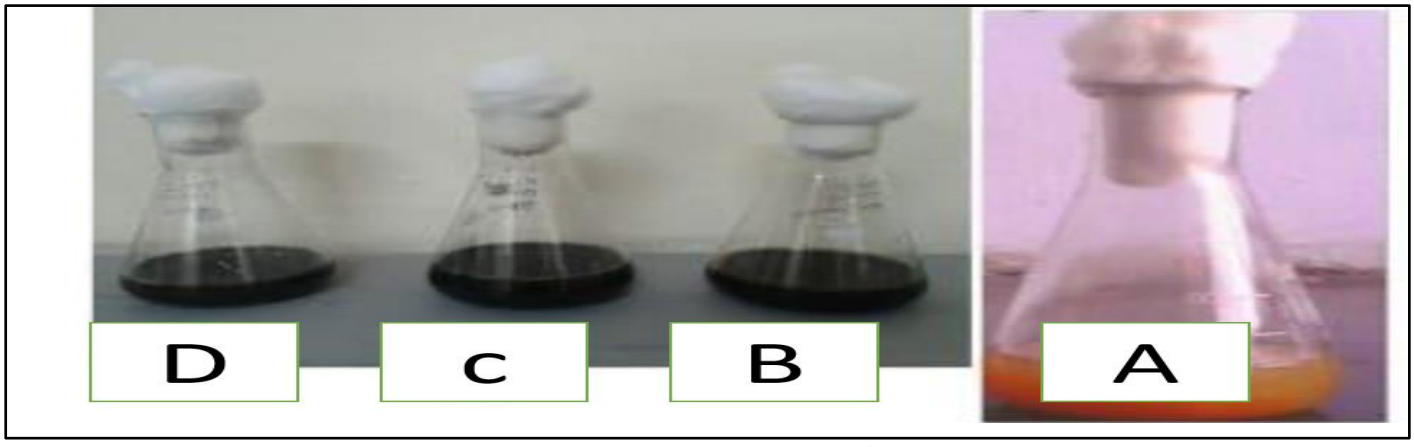



Fig 2 Screening Images of Isolates

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25jul226

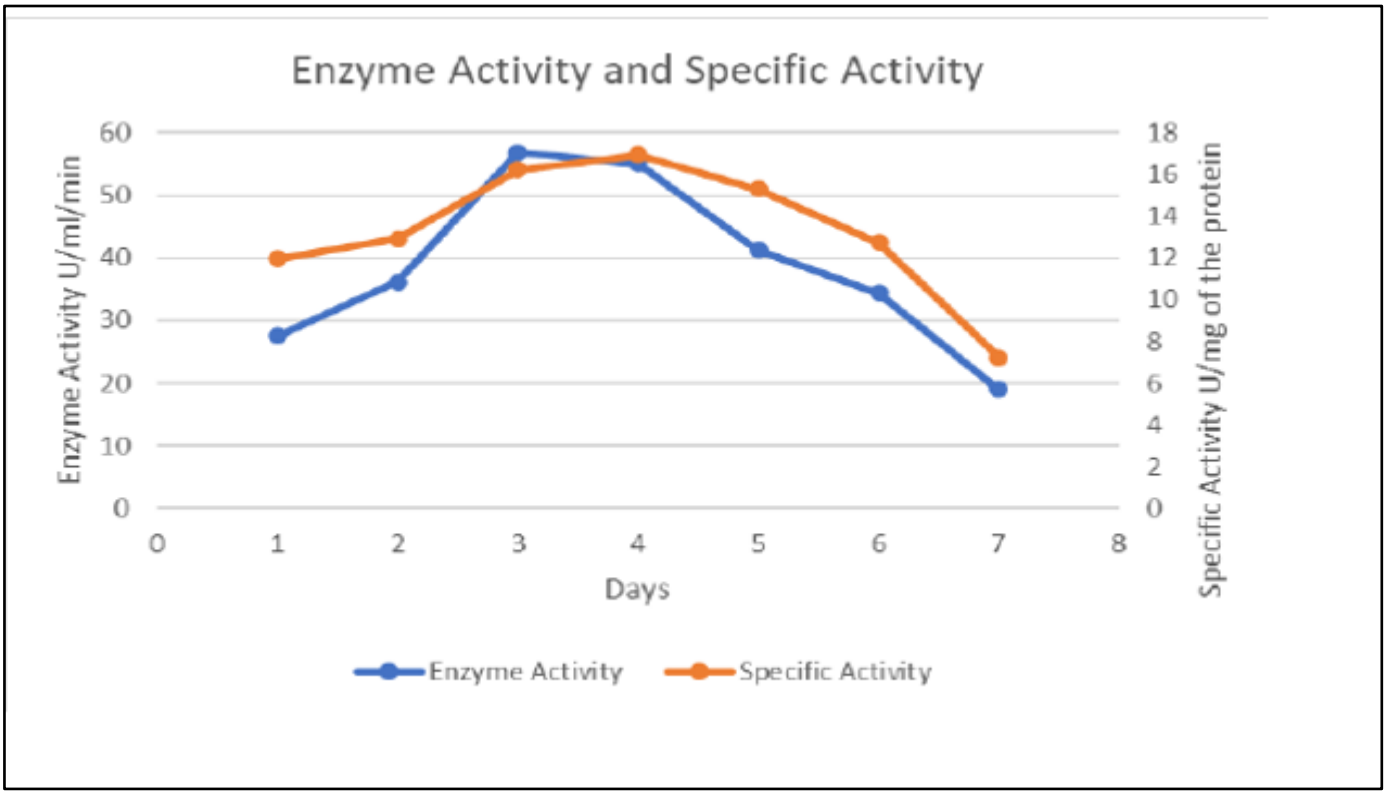


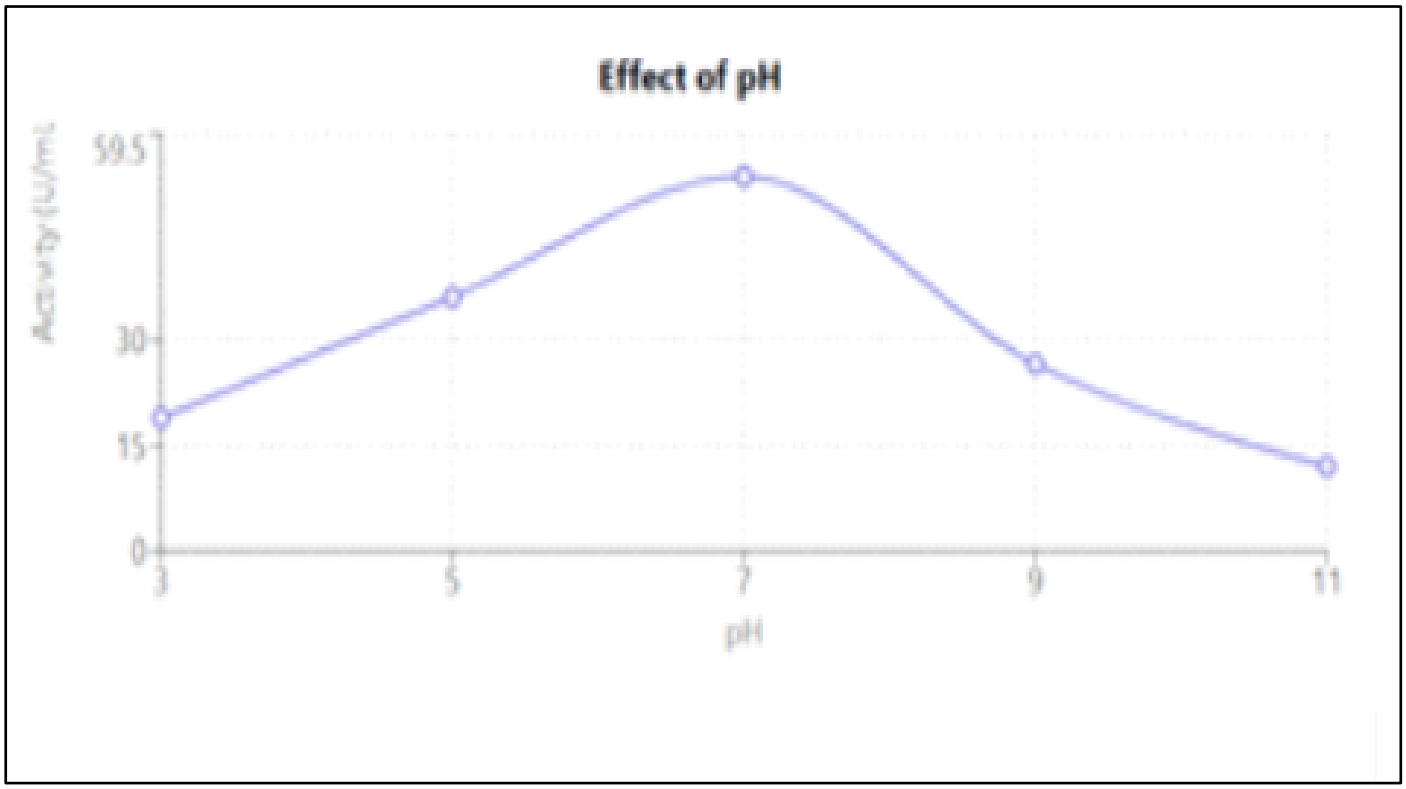

Fig 3 Activity vs Time and Conditions Tures. Kinetic constants confirmed moderate substrate affinity.

➢ *Identification:*

16S rRNA sequencing confirmed the isolate as *Brevundimonas diminuta* with 99% similarity.

➢ *Biotechnological Relevance:*

*B. diminuta* is recognized for resilience and diverse metabolic capacity, with extracellular tyrosinase opening opportunities for L-DOPA synthesis, pigment production, and phenol bioremediation.



ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25jul226

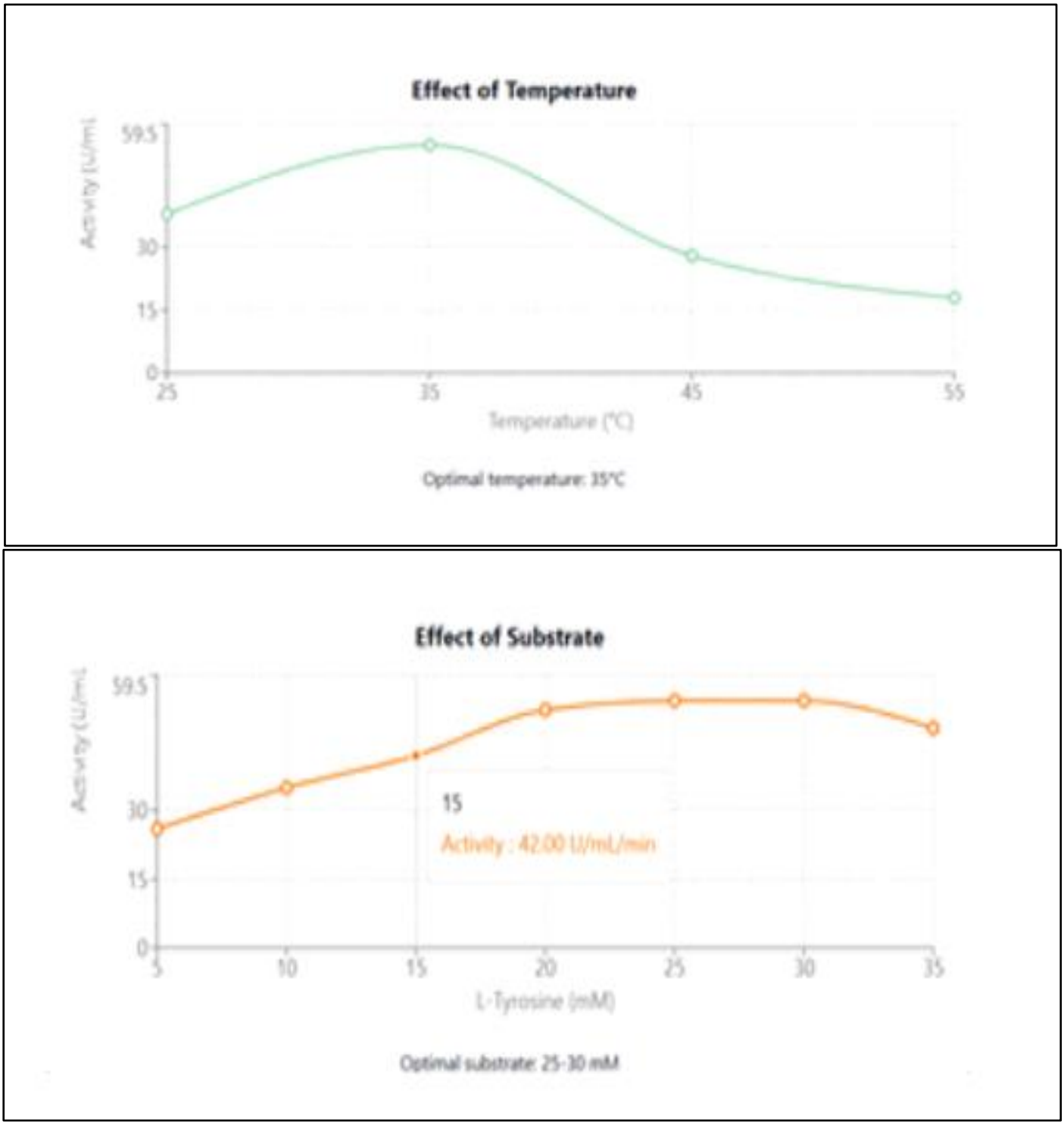



Fig 4 Activity vs Time and Conditions

## IV. CONCLUSION

This study demonstrates that freshwater environments harbor promising tyrosinase- producing bacteria. *B. diminuta* isolated from Kapurwadi mud showed high activity and robust growth under moderate conditions, making it a viable can- didate for industrial enzyme production and biotechnological exploitation.

## SUMMARY

The research isolated and characterized a potent tyrosinase-producing bac- terium from Ahmednagar freshwater samples. Optimized conditions and partial purification preserved high enzymatic activity. Future scale-up and immobi- lization studies could further enhance its applications in pharmaceuticals and environmental management.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25jul227

## ACKNOWLEDGMENT

The authors thank principal and the laboratory staff of KLE College of Pharmacy, as well as KLE Academy of Higher Education and Research, Belagavi, for their support.

## REFERENCES

[1]. Agarwal P et al. Biocatalysis and Agricultural Biotechnology 2016;8:185– 190.
[2]. Cordero RJB, Casadevall A. Fungal Biology Reviews 2017;31(2):99–112.
[3]. Faccio G et al. Process Biochemistry 2012;47(12):1749–1760.
[4]. Halaouli S et al. Journal of Applied Microbiology 2006;100(2):219–232.
[5]. Harir M et al. Journal of Biotechnology 2018;265:54–64.
[6]. Kang SY et al. Microbial Cell Factories 2018;17(1):54.
[7]. Liu H et al. Journal of Agricultural and Food Chemistry 2015;63(50):10858– 10866.
[8]. Moshtaghioun SM et al. International Journal of Environmental Science and Technology 2017;14(7):1519–1530.
[9]. Odeniyi OA et al. Biocatalysis and Agricultural Biotechnology 2019;18:101047.
[10]. Qadir F et al. Biocatalysis and Agricultural Biotechnology 2018;14:345– 351.
[11]. Roy S et al. Frontiers in Biology 2014;9(4):306–316.
[12]. Surwase SN et al. Microbial Biotechnology 2012;5(6):731–737.
[13]. Tungibar S, Dixit P. Applied Microbiology and Biotechnology 2024;108(1):1– 12.
[14]. Valipour E, Arikan B. Applied Biochemistry and Microbiology 2016;52(4):420– 427.
[15]. Zaidi KU et al. Biochemistry Research International 2014;2014:854687.