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RESEARCH ARTICLE

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Isolation, identification and mutants generation of isolate 04 for its improved bioconversion of ferulic acid to vanillin

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ABSTRACT: Vanillin is widely used as a food flavouring, scent ingredient in cosmetics and perfumes, as an intermediate in agrochemicals & medicines and also as a nutraceutical. Serial dilution of sediment sample yielded 32 strains, among which three isolates A2, A7, and 04 reported here were characterized at molecular level by 16S rDNA sequencing. They were identified and similar to *Pseudomonas aeruginosa* (94.97%), *Acinetobacter baumannii* (99.93%) and *Lysinibacillus macroides* (98.67%) respectively based on its 16S rDNA sequences. Among these three strains, isolate 04 was with high ferulic acid (FA) to vanillin bioconversion activity. Vanillin concentration was determined by the Thiobarbituric acid (TBA) method and analysed by UV-spectrophotometry, which showed 11.63 ± 0.05 mg/ml vanillin after optimization studies. HPLC with UV detector was also used for the characterization of the vanillin formed, which showed peak at 8.793 rt. To improve FA to vanillin bioconversion, physical mutagenesis was performed on isolate 04, which generated three mutants (1, 2, and 3). These three mutants were subject to FA to vanillin biotransformation study. TBA method and UV spectrophotometric analysis showed a higher conversion by mutant 2 than other two mutants, 1 and 3. Mutant 2 formed almost two-fold vanillin i.e. 19.78 ± 0.05 mg/ml when compared to its wild strain, isolate 04. Hence, this mutant may be further subjected to optimization studies and exploited for industrial biotransformation process.

KEYWORDS: Lysinibacillus macroides, Biotransformation, UV-spectrophotometry, HPLC, Physical mutagens

INTRODUCTION

One of the most widely used food flavourings is vanilla (4-hydroxy-3-methoxybenzaldehyde). It is an aromatic flavouring ingredient that is produced from *Vanilla planifolia* pods. It has a wide range of industrial uses, including in food (cakes, ice creams, chocolates, and cookies), drinks, pharmaceuticals, fragrances, and nutraceuticals [1, 2]. Several reports recommend its proposed antibacterial and antioxidant qualities.

Over fifteen thousand tons of vanillin are needed worldwide, only about two thousand tons of that are made from vanilla beans. Synthetic vanillin, which is made chemically from lignin and guaiacol, covers the remaining need. The production of natural vanillin, which is 300 times more expensive on the market than synthetic vanillin and accounts for less than 1% of the yearly demand [3, 4]. Natural vanillin is 1000–3000 USD/kg compared to 11 USD/kg for chemically synthesized vanillin, is another reason for its limited availability [5]. The ingestion of natural vanillin is preferred over its synthetic cousin, most likely because synthetic manufacture involves the use of a racemic combination. As a result, researchers are now looking at different ways to produce this flavour, like using microbes in biotechnological production. Eugenol, isoeugenol, and ferulic acid were among the substrates that *Bacillus subtilis* (MTCC 1427) was utilized to convert into vanillin.

Eugenol was used as a substrate in the culture medium, however *B. subtilis* did not demonstrate any growth there. Compared to isoeugenol, more vanillin synthesis was observed in the culture media using ferulic acid as substrate [6]. Eugenol, ferulic acid, and isoeugenol have demonstrated potential among the several precursors documented in the literature, because of their higher yields [7].

There are reports that several bacteria could convert ferulic acid to vanillin (g/L), such as, *Sporotrichum thermophile* [8], *Streptomyces halstedii* [9], *Streptomyces setonii* [10], *Streptomyces sannanensis* MTCC 6637 [11],

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Halomonas elongate DSM 2581 [12], *Bacillus licheniformis* SHL1 [5], and *Halomonas* sp. B15 [13].

A wild *Bacillus* strain with high tolerance to the substrate ferulic acid, isolated and screened from soil could produce 0.048 $g\,L^{-1}$, however, after optimization conditions its vanillin conversion was increased to 0.218 $g\,L^{-1}$ [14]. In another study, *Bacillus amyloliquefaciens* subsp. CICC 10025 produced vanillin of 386 mg/L in the presence of corn steep liquid (low-cost nitrogen source), ferulic acid concentration, and pH were 7.72 g/L, 2.33 g/L, and 9.34, respectively [15]. Though there are number of reports of microorganisms converting FA to vanillin, most of them are produced very less amounts of vanillin. There are no known reports about mutants generation and their possible increased potential of vanillin formation from FA by bacteria. Hence, the present study was to isolate, identify and generate mutants for their possible increase in FA to vanillin conversion.

MATERIALS AND METHODS

Chemicals

Vanillic acid, Ferulic Acid, Methoxy phenol and Vanillin were purchased from HiMedia Laboratories, India. 2- Thiobarbutric acid (TBA) was obtained from Sigma Aldrich, USA. Analytical grade Methylene chloride, Acetonitrile, Acetic Acid and Methanol were procured from SRL, Hyd., India.

Isolation of bacteria and media

The same methodology and media used for the isolation of nine strains (S1, S2, S3, S4, S5, O1, O2, O3 and O4) reported in Bathini et al. [16] was used here. This study was conducted at different time period by collecting sediment soil sample again for isolation of bacteria with high FA to vanillin conversion capacity. Isolates purified on Starch Casein Agar (SCA) and Oat meal agar (OMA) plates were sub-cultured on Enrichment agar medium with ferulic acid (485 mg/L) as the carbon source [17].

Enrichment Agar medium consists of: Minimal medium 990 ml [16]**;** Ferulic acid 485mg/10ml; Total volume 1000 ml**;** Agar 18g; pH 7.2**.** (Ferulic acid 485mg/l0ml distilled water was predissolved with 450mg of NaOH and filter sterilized using 0.2 µm syringe filters, before addition to the minimal medium).

Molecular characterization

Molecular characterization of the purified isolates was performed to identify them. The DNA of the isolates was extracted and subjected to PCR employing two primers, 8F and 1525R [16]. PCR products (1.5 kb size) were eluted from agarose gel, after they subjected to agarose gel electrophoresis. Later, they were purified and sequenced. The EZ Biocloud public portal for data and analytics (https://www.ezbiocloud.net/apps) was utilized for identification of the isolates.

Transformation studies

Erlenmeyer flasks (250 ml) containing 100 ml of bioconversion media containing 5% ($w\ v^{-1}$) of ferulic acid was inoculated with 1% ($v\ v^{-1}$) of each purified isolate separately. Bioconversion media composed of 0.5 g of carbon, 0.5 g of nitrogen and one ml of trace metal solution per liter ($g\ l^{-1}$: 0.37 $CaCl_2.2H_2O$; 0.62 $CuSO_4.5H_2O$; 0.60 $FeSO_4.7H_2O$; 0.59 $MnSO_4.4H_2O$; 0.42 $ZnSO_4.7H_2O$; 0.79 $CoCl_2.6H_2O$; 0.70 $NaMoO_4$) was included in the bioconversion medium [18]. Glucose was used as carbon source, whereas, yeast extract was used as nitrogen source. The bioconversion media were incubated in a rotary incubator (Remi, New Delhi, India) at 150 rpm, 30 °C, for 48 hours. The experiments were conducted in triplicate, and the pH of the bioconversion media was set at 7. The control experiment was also performed (without isolate).

Vanillin characterization by Thin layer chromatography and Spectrophotometric assay

The vanillin production by purified isolates was initially checked with thin later chromatography (TLC) after 48 hours of incubation. Methylene chloride, methanol, and anhydrous acetic acid (98.5:1:0.5, v/v/v) were used as the mobile phase for the chromatograms development in a vertical glass chamber [19]. TLC chamber saturation time of 30 minutes would be ideal at room temperature (25 ± 2°C) for the mobile phase. After TLC analysis, the isolate with high potential for the bioconversion process was selected for further analysis. The vanillin produced by the isolate (with high potential conversion) was determined using the thiobarbituric acid (TBA) method and spectrophotometric assay. Vanillin was analysed using spectrophotometry in accordance with the methodology previously outlined by He et al. [20]. 5 ml of 24% HCl, 2 ml of 1% thiobarbituric acid, and 0.5 ml of sample solution (analysed for determination of vanillin concentration) were added to distilled water to make 10ml solution. After 10 minutes of heating at 55 °C in a water bath (colour changes to yellow), the solution was allowed to cool to room temperature for 20 minutes. Using a Shimadzu UV-Vis spectrophotometer, the absorbance of the standard solution and unknown vanillin was measured at 434 nm. Using the vanillin standard (0.1-1 mg/ml) graph, the amount of vanillin in the liquid broth was estimated [6].

HPLC Analysis

The vanillin formed was also characterized using HPLC with UV detector [6]. Centrifugation (for ten minutes at 10,000 rpm) was performed to separate 500 µl of the liquid broth (containing vanillin) from biomass. The supernatant was lyophilized, diluted in 500 µl methanol, and filter sterilized using a 0.2 µm filter before injecting into the HPLC system with UV/Vis detector with baseline noice of +/- 1x10-5AU@t254nm (Waters India, New Delhi, India). Reversed Phase C18 Column with length-25 cm (4.6 × 250 mm) was used. The mobile phase consisted of acetonitrile and acetic acid (0.2%) at a 60:40 ratio at a flow rate of 1.0 ml per minute. injection volume was 20 µl and the maximum run pressure was 3000 psi. UV detection was performed at 280 nm.

Generation of Mutants by physical mutagenesis

Inoculation of 0.2 ml culture broth of isolate 04 on to each of three petri plates (with sterilized nutrient agar) and exposing them to UV light [21] for 10, 20 and 30min. separately (by keeping under UV light with distance of 10 cm to petri plates with culture from UV light) was performed. Petri plates were placed after removing the lids before placing them in the center of UV chamber. UV chamber was used after warming up the UV lamp for at least 30 minutes before each irradiation. After irradiation, the lids were replaced and immediately the plates were placed in dark.The plates were incubated (after UV light exposure) at 37˚C for 48h. Mutants developed were randomly selected and checked for their stable growth in nutrient broth for 24-72h. Highly growing mutants were selected for FA biotransformation to vanillin activity (experiments were conducted three times to ascertain the results), which was characterized by TLC, TBA method and UV spectrophotometric analysis. Vanillin concentration of wild strain was also compared with its potential mutant strain.

RESULTS AND DISCUSSION

Isolation and molecular characterization of bacteria

Serial dilution of the sediment soil sample and plating yielded 32 pure isolates on OMA and SCA. Among these, nine isolates reported elsewhere [16] and three isolates (A2, 04, A7) reported here, have the capacity to grow on ferulic acid as sole carbon source on Enrichment agar medium. Molecular characterization of the three isolates (A2, 04, A7) was shown in Table belonging to different genus with FA to vanillin conversion capacity.

Table. Molecular characterization of isolates with FA to vanillin bioconversion capacity

S. No.	Isolate name/number	bp	Top-hit taxon	Similarity (%)	Completeness (%)
1	A2 ([@] A2_new)	1,419	*Pseudomonas aeruginosa*	94.97	98.1
2	04 ([@] Full_4)	1,441	*Lysinibacillus macroides*	98.67	97.4
3	[@] A7	1,409	*Acinetobacter baumannii*	99.93	96.6

[@] = mentioned in https://www.ezbiocloud.net/identify

Transformation studies and characterization of vanillin

Three isolates (A2, 04, A7) were inoculated in bioconversion media and were incubated separately to study FA to vanillin conversion analysis. Figure 1 shows vanillin production by these three isolates (A2, 04, A7 sequentially) after 48 hours of incubation. When compared to two isolates A2 and A7, isolate 04 was showing more bioconversion (FA to vanillin) (Figure 1). Hence, isolate 04 was selected for further TBA method and spectrophotometric studies.

Microbial isolates have ability to convert eugenol or ferulic acid into vanillin. Ferulic acid, compared to the high toxic eugenol, was found to be an excellent precursor to vanillin conversion [22]. Bioconversion media with FA changes to yellow colour due to vanillin formation by isolate 04 with TBA method and control (without isolate 04) showed no colour change with the same method as vanillin was not formed in control (Figure 2). UV spectrophotometric analysis showed the concentration of vanillin formed from FA bioconversion was 11.63±0.05 mg/ml by isolate 04 after optimization studies. Similarly, *S. setonii* was forming 6.4g/L as per report of Muheim and Lerch [3]. Whereas, bioconversion of ferulic acid to vanillin (g/L) by other microorganisms after optimization studies was reported, *Streptomyces* sp. V-1 (19.2) [22], *Amycolatopsis* sp. DSM9991 or DSM9992 (11.5) [23]. HPLC was also performed on liquid broth (after filter sterilization) after bioconversion experiment involving isolate 04, to check the bioconversion of FA to vanillin by the isolate 04. It showed peak with retention time at 8.793 which confirmed the formation of vanillin from FA (Figure 3).

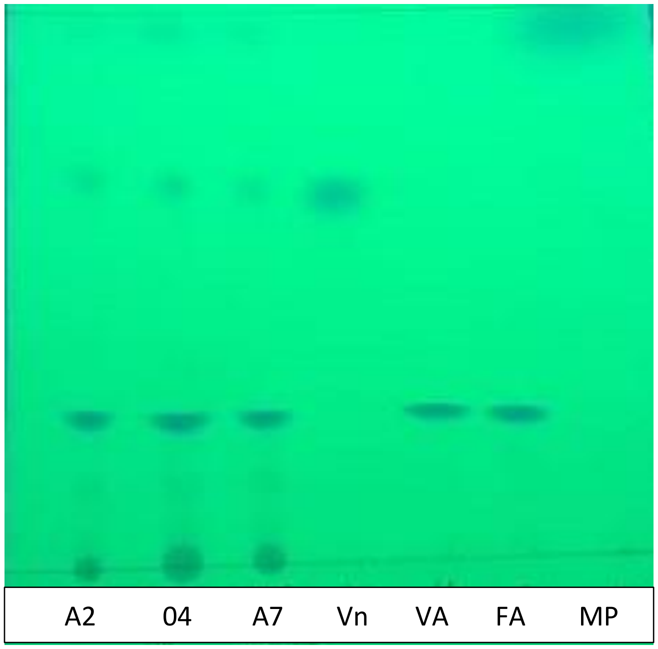

Figure 1. TLC of Vanillin formed by three isolates (A2, 04, A7) and standards. Note: Vn = standard vanillin; VA = standard vanillin acid; FA = standard ferulic acid; MP = standard Methoxy phenol.

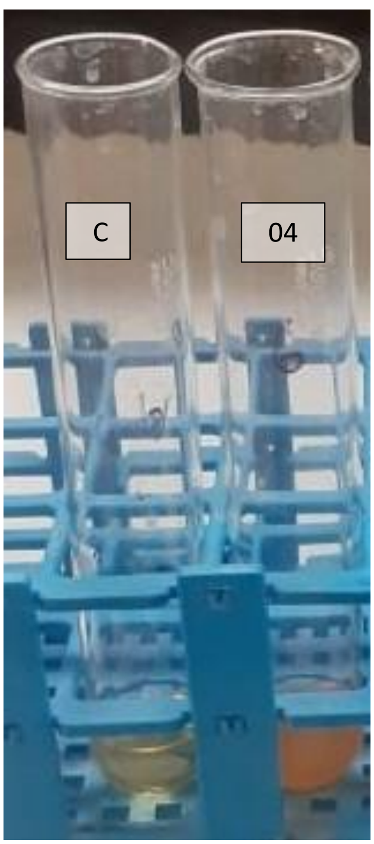

Figure 2. Analysis of Vanillin formed from FA biotransformation using TBA method. Note: C= control (bioconversion media without isolate 04); 04 = bioconversion media with isolate 04.

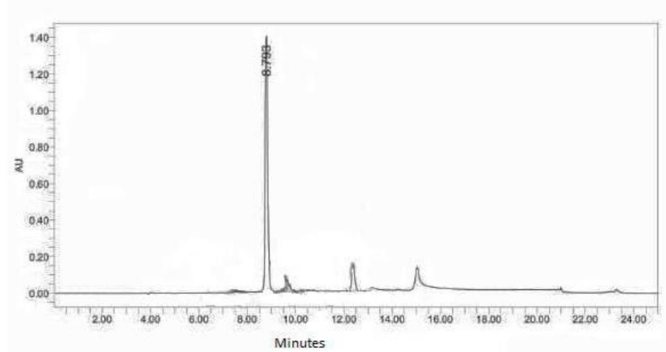

Figure 3. HPLC chromatogram of vanillin formed from FA by isolate 04.

Generation of Mutants by physical mutagenesis and their bioconversion analysis

The actinomycete *Amycolatopsis* sp. ATCC 39116 is capable of synthesizing large amounts of vanillin 13.9 g/liter with a molar yield of 75%. from the natural substrate ferulic acid [3]. This bacterium could be used for biotechnical production of natural vanillin. However, significant amounts of the desired product are lost due to inherent vanillin catabolism via vanillic acid [24]. Therefore, physical mutagenesis like UV light exposure may disrupt internal the vanillin catabolism and improve vanillin formation.

When exposed to UV light with different time periods (10, 20 and 30 min.), isolate 04 produced mutants. Number of mutants formed (on the petri plates) was reduced gradually with increase in UV light exposure time and after incubation at 37˚C for 48h (Figure 4). Stable and highly growing randomly picked three mutants (1,2 and 3) were subjected to FA to vanillin bioconversion study. Three mutants, 1,2 and 3 have potential of FA to vanillin bioconversion. Vanillin produced by these mutants was characterized by TLC (Figure 5). All three mutants were forming vanillin from FA after bioconversion process (mutant spots compared with the standard vanillin spot) (Figure 5). But, TBA method and UV spectrophotometric analysis showed a little higher conversion (high intensity of yellow colour) by mutant 2 than other two mutants, 1 and 3 (Figure 6). When compared vanillin concentration formed by mutant 2 with its wild strain (isolate 04), it was increased to almost two-fold i.e. 19.78 ± 0.05 mg/ml . This could be due to deletion or disruption of vanillin dehyhrogenase gene (vdh), which blocks the catabolism of vanillin which might have led to more concentration of vanillin formed by mutant 2.This was the first report that, UV mutant increasing ferulic acid bioconversion to vanillin, which should be explored further. Further optimization studies for vanillin production (by mutant 2), may find it suitable at industrial scale for improved vanillin production. In a report of Civolani et al.

[25], a transposon mutant of Ferulic-acid-degrading *Pseudomonas fluorescens* strain (BF13) retained the ability to bioconvert ferulic acid into vanillic acid but lost the ability to further catabolize and form vanillin.

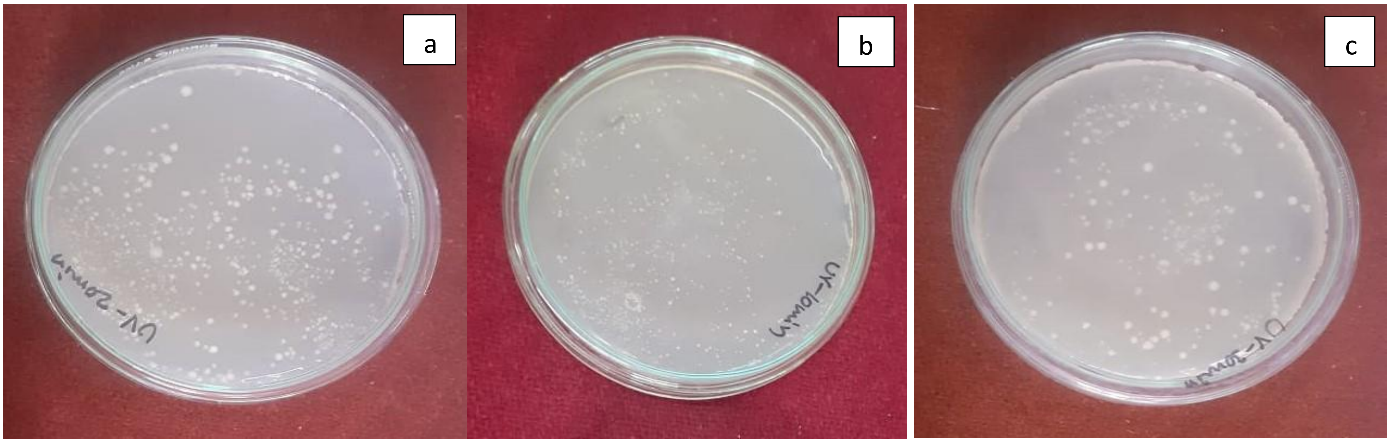

Figure 4. Mutants of isolate 04 generated after culture exposure to UV light for (a) 10min. (b) 20min. (c) 30min.

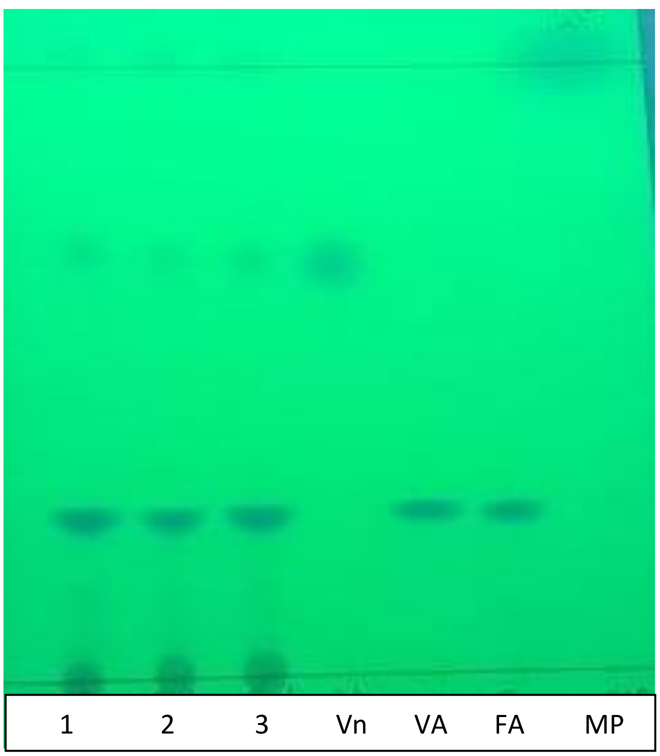

Figure 5. TLC of vanillin formed (from FA) by isolate 04 mutants (1,2 and 3) after 48h incubation period. Note: Vn = standard vanillin; VA = standard vanillin acid; FA = standard ferulic acid; MP= standard Methyl guiacol

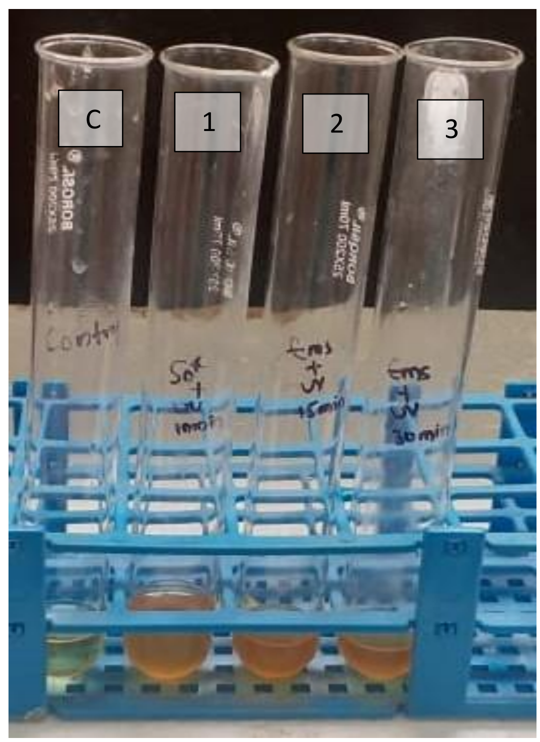

Figure 6. Analysis of Vanillin formed (from FA biotransformation) using TBA method by control (C) and three mutants (1,2, and 3) of isolate 04.

CONCLUSION

Three strains A2, 04 and A7 isolated from soil sediment sample have the capacity to grow on enrichment agar medium (ferulic acid as sole carbon source). Among these three strains, isolate 04 has high Ferulic acid (FA) to vanillin bioconversion activity, i.e. 11.63 ± 0.05 mg/ml vanillin. UV light exposure of isolate 04 yielded three UV mutants (1, 2 and 3). Vanillin formed by mutant 2 was increased to almost two-fold i.e. 19.78 ± 0.05 mg/ml than its wild strain (isolate

04). This was the first report that, UV mutant with increasing bioconversion activity from ferulic acid to vanillin, which can be further exploited for industrial scale vanillin production after optimization studies.

DECLARATION

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Authorship contributions

Concept: T.M., Design: T.M., Data Collection or Processing: N.B., Analysis or Interpretation: VR.S., Literature Search: N.B., Writing: T.M.

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Competing interests

The authors declared that there is no conflict of interest.

REFERENCES

[1] Karode, B., Patil, U., Aparna, J. (2013): Biotransformation of low-cost lignocellulosic substrates into vanillin by white rot fungus. *Phanerochaete chrysosporium*, Indian Journal of Biotechnology 12: 281-283.

[2] Cerruti, P., Alzamora, S. M., Vidales, S. L. (1997): Vanillin as an antimicrobial for producing shelf-stable strawberry puree. Journal of Food Science 62: 608–610. https://doi.org/10.1111/j.1365-2621.1997.tb04442.x

[3] Muheim, A. Lerch, K. (1999): Towards a high-yield bioconversion of ferulic acid to vanillin. Applied Microbiology and Biotechnology 51: 456–461. https://doi.org/10.1007/s002530051416

[4] Prince, R. C., Gunson, D. E. (1994): Just plain vanilla. Trends in Biochemical Sciences 19: 524. https://doi.org/10.1016/0968-0004(94)90049-3

[5] Ashengroph, M., Nahvi, I., Zarkesh-Esfahani, H., Momenbeik, F. (2012): Novel strain of *Bacillus licheniformis* SHL1 with potential converting ferulic acid into vanillic acid. Annals of Microbiology 62:553-558. https://doi.org/10.1007/s13213-011-0291-9

[6] Rana, R., Mathur, A., Jain, C.K., Sharma, S.K., Mathur, G. (2013): Microbial Production of Vanillin. International Journal of Biotechnology and Bioengineering Research (4)3: 227-234.

[7] Priefert, H., Rabenhorst, J., Steinbüchel, A. (2001): Biotechnological production of vanillin. Applied Microbiology and Biotechnology 56: 296–314. https://doi.org/10.1007/s002530100687

[8] Topakas, E., Kalogeris, E., Kekos, D., Macris, B. J., Christakopolos, P. (2003): Bioconversion of ferulic acid into vanillic acid by the thermophilic fungus *Sporotrichum thermophile*. Lebensmittel-Wissenschaft & Technologie 36(6): 561–565. https://doi.org/10.1016/S0023-6438(03)00060-4

[9] Brunati, M., Marinelli, F., Bertolini, C., Gandolfi, R., Daffonchio, D., Molinari, F. (2004): Biotransformations of cinnamic and ferulic acid with actinomycetes. Enzyme and Microbial Technology 34(1): 3–9. https://doi.org/10.1016/j.enzmictec.2003.04.001

[10] Longo, M. A., Sanroman, M. A. (2006): Production of food aroma compounds: microbial and enzymatic methodologies. Food Technology and Biotechnology 44(3): 335–353.

[11] Ghosh, S., Sachan, A., Sen, S. K., Mitra, A. (2007): Microbial transformation of ferulic acid to vanillic acid by *Streptomyces sannanensis* MTCC 6637. Journal of Industrial Microbiology and Biotechnology 34(2): 131–138. https://doi.org/10.1007/s10295-006-0177-1

[12] Abdelkafi, S., Labat, M., Gam, Z. B. A., Lorquin, J., Casalot, L., Sayadi, S. (2008): Optimized conditions for the synthesis of vanillic acid under hypersaline conditions by *Halomonas elongata* DSM 2581T resting cells. World Journal of Microbiology and Biotechnology 24(5): 675–680. https://doi.org/10.1007/s11274-007-9523-3

[13] Vyrides, I., Agathangelou, M., Dimitriou, R., Souroullas, K., Salamex, A., Ioannou, A., Koutinas, M. (2015): Novel *Halomonas* sp. B15 isolated from Larnaca Salt Lake in Cyprus that generates vanillin and vanillic acid from ferulic acid. World Journal of Microbiology and Biotechnology 31(8): 1291–1296. https://doi.org/10.1007/s11274-015-1876-4

[14] Gou, J., Guo, Y., Liu, H., Zhao, Y., Zhu, R., Dang, Y., Liu, N., Chen, M., Chen, X. (2022): Process optimization of vanillin production by conversion of ferulic acid by *Bacillus megaterium,* Journal of the science of food and agriculture 102 (13): 6047-6061. https://doi.org/10.1002/jsfa.11957

[15] Taiwo, A.E., Madzimbamuto, T.N., Ojumu, T.V. (2024): Process Optimization and Biotransformation of Ferulic Acid to Vanillin in a Low-Cost Nitrogen Source. ChemEngineering *8*, 68. https://doi.org/10.3390/chemengineering8040068

[16] Nagaraju, B., Sai Krishna, E., Premsagar, K., Vishnuvardhan Reddy, S., Thirumala, M. (2024): Isolation and identification of Isolate, S1 with high biotransformation potential of Ferulic acid to Vanillin. Journal of Pure and Applied Microbiology 18(3):1601-1609. https://doi.org/10.22207/JPAM.18.3.09

[17] Mishra, S., Kullu, M., Sachan, A. *et al.* (2016): Bioconversion of ferulic acid to vanillic acid by *Paenibacillus lactis* SAMS-2001. Annals of Microbiology 66: 875–882. https://doi.org/10.1007/s13213-015-1175-1

[18] Veena, P., Abhishek, D. T., Dinesh, C. R. (2021): Process Optimization and Characterization of Enhanced Vanillin Yield Using *Bacillus aryabhattai* NCIM 5503. Applied Food Biotechnology 8(2): 113-119.

[19] Magda, F., Melissa, S., Gustavo, C., Amelia, H., Luiz, A. L. S. (2018): Analysis of vanillin by TLC and HPLC-PDA in herbal material and tincture from Vanilla planifolia Jacks ex. Andrews. Drug Analytical Research 02:1-7. https://doi.org/10.22456/2527-2616.87008

[20] He, X.Y., Liu, J.X., Cao, X.H., Ye, Y.Z. (1999): The study and application of determination of vanillin by

spectrophotometry with thiobarbituric acid. Anal Lab Beijing, 18: 56-58.

[21] Winston, F. (2008): EMS and UV mutagenesis in yeast. Current Protocols in Molecular Biology Chapter 13: Unit 13.3B. PMID: 18425760. https://doi.org/10.1002/0471142727.mb1303bs82

[22] Hua, D., Ma, C., Lin, S., Song, L., Deng, Z., Maomy, Z., Zhang, Z., Yu, B., Xu, P. (2007): Biotransformation of isoeugenol to vanillin by a newly isolated *Bacillus pumilus* strain: Identification of major metabolites. Journal of Biotechnology 130(4): 463-470. https://doi.org/10.1016/j.jbiotec.2007.05.003

[23] Rabenhorst, J., Hopp, R. (1997): Verfahren zur Herstellung von Vanillin und dafür geeignete Mikroorganismen. German patent DE 195 32 317 A1.

[24] Sutherland, J.B., Crawford, D.L., Pometto, A.L. (1983): Metabolism of cinnamic, p-coumaric, and ferulic acids by *Streptomyces setonii*. Canadian Journal of Microbiology, 29:1253–1257. https://doi.org/10.1139/m83-195

[25] Civolani, C., Barghini, P., Roncetti, A. R., Ruzzi, M., Schiesser, A. (2000): Bioconversion of ferulic acid into vanillic acid by means of a vanillate-negative mutant of *Pseudomonas fluorescens* strain BF13. Applied Environmental Microbiology 66(6): 2311-7. https://doi.org/10.1128/AEM.66.6.2311-2317.2000