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Biotransformation of Geraniol to Geranic Acid Using Fungus *Mucor irregularis* IIIMF4011

Haseena Shafeeq, Bashir Ahmad Lone, Ananta Ganjoo, Nargis Ayoub, Hema Kumari, Sumeet Gairola, Prasoon Gupta, Vikash Babu,* and Zabeer Ahmed

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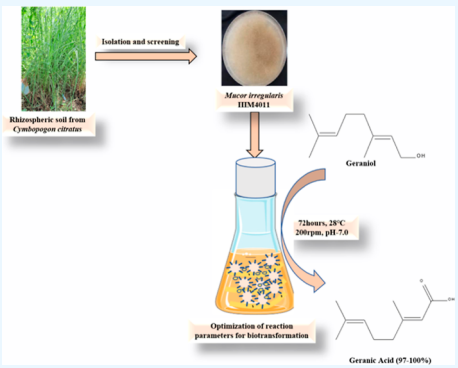
ABSTRACT: Geraniol is an important component in essential oils of aromatic plants such as lemongrass, rosa grass, and many others. It can be converted to different high-value products by using microbes/enzymes. The present study aims at the isolation and screening of microbes showing efficient production of geranic acid (a high-value product) from geraniol (a low-value monoterpene). *Mucor irregularis* IIIMF4011, isolated from the soil sample of *Cymbopogon citratus* (Lemongrass), showed biotransformation of geraniol to geranic acid. After optimization of reaction parameters, 97−100% conversion of geraniol to geranic acid was obtained after 72 h of incubation at 28 °C. Furthermore, the biotransformation reaction was also carried out in a 3 L fermentor (working volume 1.5 L), and 98.89% conversion was observed. Therefore, an efficient process of geranic acid production using *M. irregularis* IIIMF4011 was developed.



1. INTRODUCTION

One of the families of terpenes is monoterpenes, which are formed by the fusion of two isoprene units, belonging to the largest group of naturally occurring compounds.[1] They can be isolated from different parts of a plant like leaves, roots, bark, and fruits and also from some duramen by the method of steam distillation or solvent extraction.[2] These compounds have numberous applications as some of them have antitumor properties as they inhibit the formation of cancer and also lower the occurrence of cancer and are induced chemically in the different parts of the body, i.e., liver, lungs, skin, forestomach, and breast.[3] Additionally, they possess antimicrobial, antipruritic, antioxidant, hypotensive, anti-inflammatory, and analgesic pharmacological properties.[1] It is also reported that monoterpenes have antifungal properties against pathogenic fungi of plants; for instance, L-carvone inhibited the growth of postharvest fungi *Colletotrichum gloeosporioides*, *Colletotrichum musae*, etc.[4] These compounds, even though extracted from natural sources, are present in low amounts and are also limited by the availability of the raw material. Moreover, the chemical synthesis of a number of complex compounds like carveol, perillic acid, perillyl alcohol, geranic acid, vanillin, etc., is not economical. In order to overcome such kind of problems, these compounds can be synthesized through microbial transformation or biotransformation, as it can provide attractive substitutes, predominantly if regioselective or stereoselective reactions are involved.[5]

Among acyclic monoterpenes, geraniol, also known as (E)-3,7-dimethyl-2,6-octadien-1-ol, has a sweet and rose like fragrance. Over 250 essential oils contain geraniol as an important ingredient,[6] and geraniol can be extracted from many aromatic plants like palmarosa, citronella oil, etc.,[7,8] and also exhibit antitumor properties against melanoma, hepatoma, oral cancer, and leukemia.[6] As per earlier reports, many researchers have biotransformed geraniol into different types of products by using different strains of microorganisms.[9] *Polyporus brumalis* has been used to convert geraniol to *p*-menthane-3,8-diol.[10] *Saccharomyces cerevisiae* and *Saccharomyces pastorianus* showed the formation of citronellol ($C_{10}H_{20}O$), citronellyl acetate ($C_{12}H_{22}O_2$), and geranyl acetate ($C_{12}H_{20}O_2$).[11] Similarly, *Aspergillus niger* has been reported to convert geraniol to linalool ($C_{10}H_{18}O$) and α-terpineol ($C_{10}H_{18}O$).[12] Apart from that, *Brettanomyces anomalus* has been reported to convert geraniol to β-citronellol ($C_{10}H_{20}O$).[13] Furthermore, there is considerable interest to produce a single biotransformed product, which may be achieved by using a particular microbial strain. Studies showed that geraniol was transformed into a single biotransformed product, namely, geranic acid by the fungus *Rhodococcus* sp., which was isolated from soil.[9] One of the studies also reported the production of geranic acid by using two bacterial strains, namely, *Acetobacter* and *Gluconobacter*,[14] wherein *Acinetobacter* sp. is another reported strain that showed gas-phase

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bioproduction of geranic acid from geraniol.[15] It was also reported in the literature that *Pseudomonas putida* DSM 12,264 showed de novo biosynthesis of geranic acid by employing metabolic engineering techniques.[16] *Penicillium digitatum* is reported to transform geraniol to geranic acid via the novel biotransformation pathway. In the existing literature, geranic acid formation was reported by partial transformation of geranial from geraniol through NAD^+-dependent citral dehydrogenase activity.[17] NAD^+ in the cell is constantly synthesized, catabolized, and recycled to maintain stable intracellular NAD^+ levels[18] and it acts as a cofactor for the enzyme involved in the biotransformation process for the production of geranic acid. Generally, geranic acid is produced from geraniol via the action of the dehydrogenases. Initially, geraniol is transformed to geranial by geraniol dehydrogenase, and then geranial is converted to geranic acid by geranial dehydrogenase.[27]

Geranic acid, which is an open-chain monoterpenoid compound, has a number of applications in cosmetic, fragrance, flavor, and agro industries. Geranic acid also acts as an antifungal agent against two fungi of corn, namely, *Colletotrichum graminicola* and *Fusarium graminearum*.[16] Some studies also showed that geranic acid acts as a depigmenting agent by inhibiting tyrosinase activity.[19] Furthermore, geranic acid also has a food value as it acts as a flavor enhancer and is present in Sorachi Ace beer, American commercial beer, and mosaic beer.[20] Geranic acid can also be used as an artificially synthesized pheromones to attract honeybees.[21] As geranic acid has a number of applications, its demand is increasing progressively. The market value of geranic acid is Rs. 12000/kg (Indiamart), which is much higher than that of geraniol i.e., Rs. 1850−2200/kg (Indiamart). Therefore, the present study aims at screening of microorganisms for the efficient production of geranic acid.

2. MATERIALS AND METHODS

2.1. Chemicals and Media Components. All media components, geraniol, were purchased from Himedia (India), and geranic acid was purchased from Sigma-Aldrich, USA. The Wizard Genomic DNA purification kit was purchased from Promega. All reagents were of analytical grade. Silica gel 60 F_{254} for TLC was purchased from Merck & Co., Inc. All solvents that were used of HPLC grade and obtained from CDH, TCI, and Avra chemicals.

2.2. Isolation and Screening of Microbes for Biotransformation of Geraniol. The isolation of microbes was carried out from the rhizospheric soil samples of *Cymbopogon citratus* (Lemongrass), *Rosa damascene* (Damask rose), *Mentha spicata* (Mint), *Ocimum tenuiflorum* (Holey basil), and *Lavandula angustifolia* (Lavender), which were collected from experimental fields of CSIR—Indian Institute of Integrative Medicine, Jammu. For isolation and screening of microbes, 1 g of soil was added to 50 mL of the Sabouraud Dextrose Broth (SDB) medium in a 250 mL flask and incubated at 28 °C and 200 rpm.[22] After 5 days of incubation, 1.0 mL of broth was added to 50 mL of the same medium and incubated at 28 °C at 200 rpm for additional 5 days. Thereafter, 20 μL of liquid broth was taken and spread evenly over the Sabouraud Dextrose Agar (SDA) surface in order to purify each individual colony. Purification was followed by screening of the cultures for the biotransformation of geraniol to geranic acid. Further, the DNA of positive culture was extracted and then used for the amplification of its ITS (internal transcribed spacer) regions by adding the universal ITS primers in polymerase chain reaction (PCR); ITS 1 (5′-CTTGGTCATTTAGAGGAAGTA-3′) and ITS 4 (5′-TCCTCCGCTTATTGATATGC-3′). The amplified PCR product was sequenced and analyzed through bioinformatic tools MEGA 11 and BLAST.[23]

2.3. Reaction Preparation for Biotransformation of Geraniol to Geranic Acid. For the preparation of biotransformation reaction, initially, SDB media was prepared and autoclaved at 121 °C for 15 min. Thereafter, spores of isolated fungus were inoculated in the medium under laminar air flow and incubated at 28 °C and 200 rpm for 2 days. After 2 days of growth, the cultivation reaction and incubation reactions were prepared. For the cultivation reaction, culture media were supplemented with 20 mM substrate (geraniol). On the other hand, for incubation reaction, mycelium was separated from the broth using filter cloth and suspended in 0.1 M potassium phosphate buffer (pH7.0) containing 20 mM geraniol. Both the reactions were incubated at 28 °C and 200 rpm. Further, the reactions were terminated after 72 h by adding equal volume of ethyl acetate, and the crude extract was analyzed through thin-layer chromatography (TLC) and gas chromatography−mass spectrometry (GC−MS).

2.4. Gas Chromatography−Mass Spectrometry. The analysis was carried out by using a GC−MS-TQ8040 NX (Shimadzu, Japan) mass spectrometer and a gas chromatograph GC-2030 (Shimadzu, Japan) coupled with a Rtx-5MS column (30 m × 0.25 mm × 0.25 μm). The carrier gas, helium, was used at a flow rate of 1 mL/min with 36.3 cm/s linear velocity. The column temperature was first programmed at 50 °C for 1 min, then it was increased to 180 °C for 5 min, and then again increased to 290 °C for 5 min. The temperatures of the ion source, interface, and the injector were 220, 240, and 260 °C, respectively. Furthermore, using an autosampler, 2 μL of the sample was injected at a constant temperature of 240 °C after being diluted in 10:100 v/v acetone.

2.5. Nuclear Magnetic Resonance Spectroscopy. Spectroscopic data (NMR) of the biotransformed product was performed on ^{1}H NMR at 400 MHz and ^{13}C NMR at 100 and 125 MHz (Bruker Avance spectrometer). The chemical shift values were reported in δ (ppm) units and coupling constant values in hertz. Chemical shifts were referenced internally to the residual solvent peak ($CDCl_3$: ^{1}H-7.26 and ^{13}C-77.5 ppm). Tetramethyl silane (TMS) was used as an internal standard. The following abbreviations were used to explain multiplicities: *s* = singlet, *d* = doublet, *t* = triplet, *q* = quartet, *m* = multiplet, and *br* = broad.

2.6. Optimization of Media and Reaction Parameters. For media optimization, five different production media were taken (Table 1), and spores of isolated fungus was inoculated in a flask (500 mL) containing 100 mL of media and incubated at 28 °C and 200 rpm. Incubation reactions were prepared

Table 1. Composition of Media Used for Optimizing Geranic Acid Production

media	composition
SDB	glucose 20 g/L, peptone 5 g/L, and tryptone 5 g/L
GTS	glucose 20 g/L, tryptone 5 g/L, and soybean meal 5 g/L
G + S	glucose 20 g/L and soybean meal 10 g/L
G + T	glucose 20 g/L and tryptone 10 g/L
MS	molasses 20 g/L and soybean meal 10 g/L

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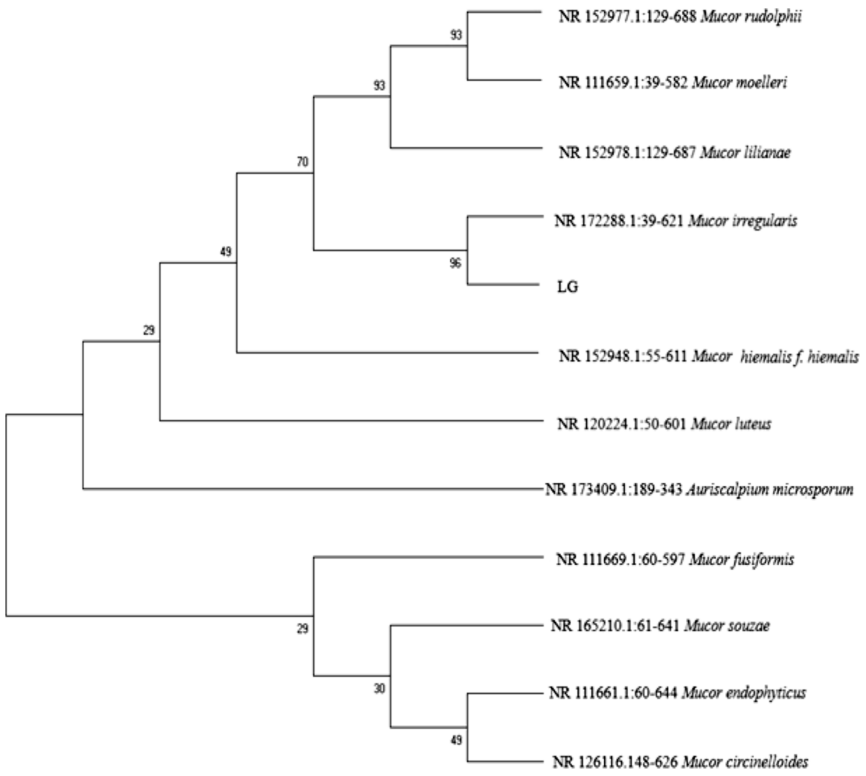

Figure 1. Phylogenetic tree showing evolutionary relationship of *Mucor irregularis* IIIMF4011 (LG) with closely related strains based on the ITS-based rDNA sequences. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) is shown next to the branches. The evolutionary distances were computed using the neighbor-joining method and are in the units of the number of base substitutions per site. Evolutionary analyses were conducted in MEGA 11.

after 48 h by separating the fungal mycelium from broth. Thereafter, reactions were terminated and extracted with equal volume of ethyl acetate. For temperature optimization, reactions were incubated at different temperatures, i.e., 20, 28, and 35 °C. Further, for the optimization of cell mass and substrate concentration, the biotransformation reactions were carried out using 10, 20, 30, 40, and 50 g/L biomass concentrations and different substrate concentrations, i.e., 10, 20, 30, and 40 mM, respectively. Thereafter, the reaction was terminated after 72 h, and the analysis of the biotransformed product was done by TLC and GC−MS.

2.7. Up-Scaling and Extraction of the Biotransformed Product. After optimization of reaction parameters, the biotransformation of geraniol to geranic acid was scaled up in a 3 L fermentor. SDB media (1.5 L) was prepared in a 3 L fermentor and autoclaved at 121 °C and 15 psi for 15 min. After autoclaving, 10 % seed culture was inoculated, and the fermentor's parameters were adjusted to 1.5 liters per minute (LPM), 200 rpm, and 28 °C. After 48 h of incubation, mycelium was separated by centrifugation at 5000 rpm. Thereafter, 1.5 L of reaction was prepared by adding 20 mM geraniol in 0.1 M potassium phosphate buffer (pH 7.0) and incubated at 28 °C, 200 rpm, and 1.5 LPM. After every 24 h, approximately 100 mL of sample was taken, and extraction was carried out by adding equal volume of ethyl acetate and stirring for about 5 min. After stirring, the solvent layer was separated by using a separating funnel. The extract was dried under reduced pressure on a rotary evaporator at 35−40 °C and redissolved in acetone. For analysis of product formation, the crude sample was applied to TLC plates and assessed using a solvent system consisting of 25:75 ethyl acetate and hexane. Furthermore, the TLC plate was developed using an iodine chamber containing powdered silica and iodine crystals to facilitate the visualization of product formation. To further purify the geranic acid, column chromatography was employed. The extract containing the biotransformed product was added into a normal phase glass column packed with silica gel (100−200 mesh size). A gradient elution using ethyl acetate−hexane (0:100−100:0) was performed, resulting in thirty-five distinct fractions based on TLC profiles. Fractions (17−22) eluted with 12.5% ethyl acetate/hexane mobile phase had a single consistent spot on the TLC and were hence pooled and concentrated. The concentrated fractions were again visualized through UV (265 or 365nm) and TLC by charring in an iodine chamber (containing powdered silica and iodine crystals), yielding 200 μL of a brownish oily pure compound. The purity of the compound was assessed using GC−MS and NMR data. A small quantity (10 μL) of the purified compound was dissolved in $CDCl_3$ for ^1H, ^{13}C, and DEPT NMR analysis.

2.8. Data Analysis. Data analysis was performed to understand the statistical significance of collected data using the excel data analysis tool. All of the parameters were tested with a 95% confidence level (alpha value as 0.05). All of the experiments were performed in triplicates.

Trendline analysis was carried out in order to understand the effect of the biomass concentration on geranic acid production.

The data that are providing on geranic acid synthesis are based on the NIST mass spectral library.

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3. RESULTS

3.1. Isolation and Screening of Microbes for Biotransformation of Geraniol. Soil samples were obtained from the rhizosphere of different plant species, including *M. spicata* (Mint), *Rosa damascene* (Damask rose), *O. tenuiflorum* (Holey basil), *C. citratus* (Lemongrass), and *L. angustifolia* (Lavender) which have geranic acid as an active compound[32] for the isolation of microbes. Thereafter, five microbes were isolated, out of which only one fungal culture, which was isolated from the soil of lemongrass, coded as LG showed the biotransformation of geraniol to geranic acid (Figure S1-A). The mycelium of isolated culture and its broth were used for the biotransformation of geraniol separately, but product formation was observed only with mycelium, which indicated that enzymes responsible for biotransformation are intracellular in nature. Analysis for the production of geranic acid was confirmed by comparing the GC−MS of standard geranic acid with that of the reaction product (Figure S1-B). Subsequently, the molecular characterization of LG revealed that the amplified sequence showed 96.93% similarity and 96% homology with fungus *Mucor irregularis* (NR172288.1). Therefore, the culture was identified as *M. irregularis* IIIMF4011, and its phylogenetic tree was constructed using the neighbor-joining method by using MEGA 11 (Figure 1). Identified culture was further submitted to Microbial Type Culture Collection, Chandigarh (accession number MTCC 25582).

3.2. Optimization of Media and Reaction Parameters. Five different media compositions were taken, having various carbon and nitrogen sources as shown in Table 1. It was observed that isolated fungal mycelium from SDB media containing 20 g/L glucose as the carbon source and 5 g/L peptone and 5g/L tryptone as nitrogen sources showed maximum production of geranic acid (98.89%) followed by MS (95.6%) and GT (95.42%) on comparison with other media compositions (Table 2).

Table 2. Effect of Different Media Compositions on Geranic Acid Production

media composition	geranic acid production (%)
SDB (glucose, peptone, and tryptone)	98.89
S (glucose, tryptone, and soybean meal)	94.61
G + S (glucose and soybean meal)	93.34
G + T (glucose and tryptone)	95.42
MS (molasses and soybean meal)	95.60

The effect of the temperature on geranic acid production was also studied. Reactions were incubated at different temperatures, i.e., 20, 28, and 35 °C and GC−MS showed maximum conversion of 99.20% at 28 °C in 72 h (Figures 2, S2, and S3) and approximately 60% conversion at 20 and 35 °C after 72 h.

Optimization of biomass concentration was carried out by preparing reactions with different concentrations. The concentrations of biomass used were 10, 20, 30, 40, and 50 (g/L), and its analysis was done by GC−MS. The results revealed that 30, 40, and 50 g/L biomass concentrations showed conversion of 97.43%, 92.99%, and 97.58%, respectively (Figure 3). However, 10 and 20 g/L showed 35.27% and 76.67% conversion respectively, at 28 °C in 72 h. Trendline analysis preformed revealed that the data points followed a second-order polynomial trend, which indicates that

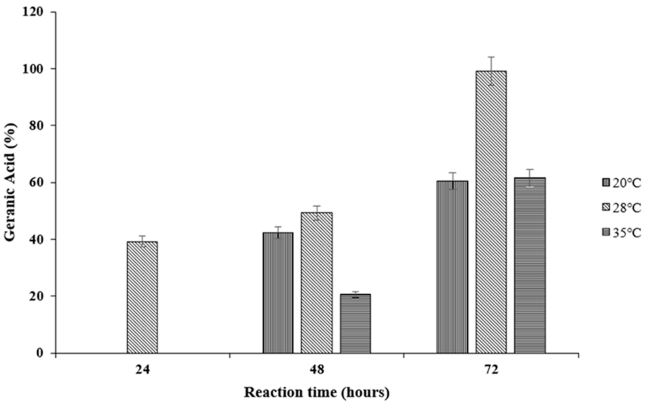

Figure 2. Effect of temperature on the production of geranic acid.

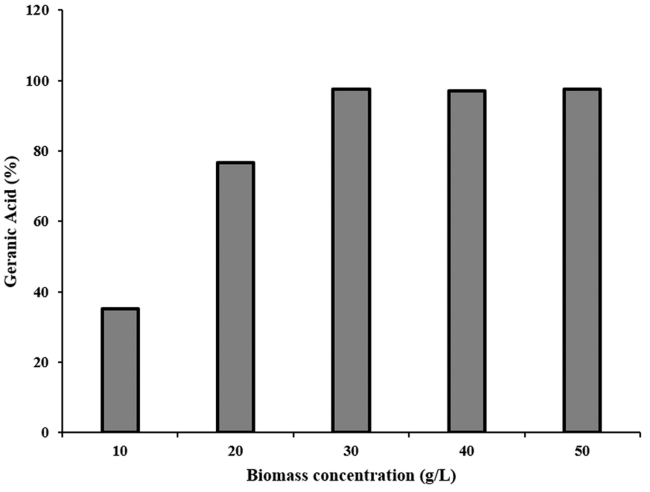

Figure 3. Effect of biomass concentration on geranic acid production.

there was a fluctuation in the data wherein with an increase in biomass concentration, geranic acid production also increased. However, after 30 g/L, the concentration showed little to no variation with the increase in biomass concentration. The R^2 for this trendline was 0.9812, which indicated that the curved trendline was a good fit for the data values.

After optimization of media, temperature, and biomass concentration, the optimization of substrate concentration was also carried out. For this, reactions were prepared with different geraniol concentrations, i.e., 10, 20, 30, and 40 mM. After analyzing the results, it was observed that 20 mM showed maximum conversion of 96.03%, followed by 10, 30, and 40 mM with 80.88%, 27.67%, and 17.15% conversion, respectively (Figure 4).

3.3. Up-Scaling and Extraction of the Biotransformed Product. The reaction was scaled up to 1.5 L in a 3 L fermentor using 2 day mycelium (30 g/L) of fungus *M. irregularis* IIIMF4011 (Figure 5A) for 3 days, and the samples were collected after every 24 h. Based on TLC and GC−MS analysis of the samples (Figures S4 and S5), it was observed that there was almost linear increase in the formation of geranic acid with a proportionate decrease in substrate concentration (geraniol) after 24 h. On the termination of the reaction, 98.89% conversion of geraniol into geranic acid was obtained after 72 h of reaction time (Figure 5B).

The purified compound was obtained as a brownish oil. Its molecular formula $C_{10}H_{16}O_2$ (Figure S6) was deduced by ESI-

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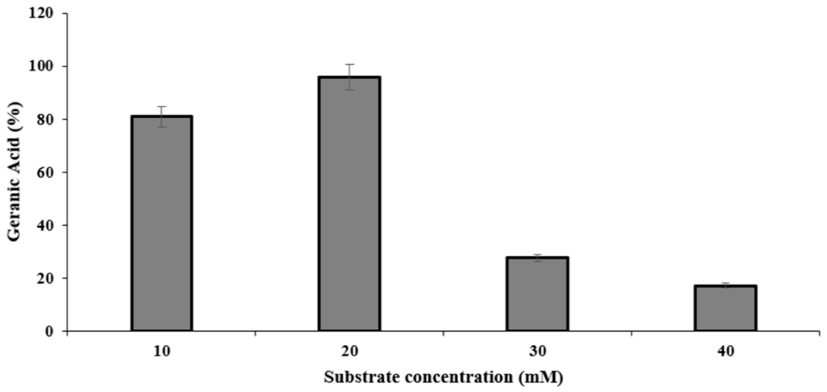

Figure 4. Effect of substrate (geraniol) concentration on the production of geranic acid.

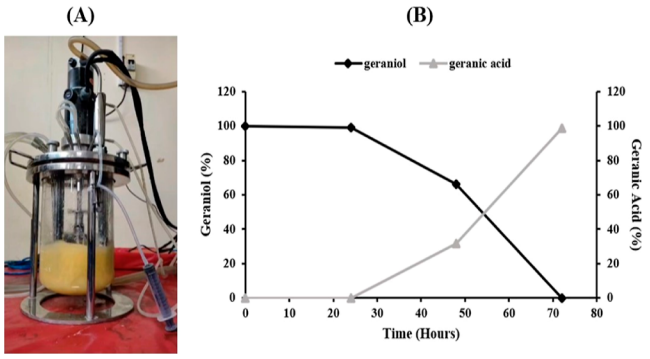

Figure 5. (A) Biotransformation reaction in a 3 L fermentor (1.5 L working volume) and (B) geranic acid production with respect to geraniol utilization in a fermentor.

MS data m/z 169.25 $[M + H]^+$ (calcd for $C_{10}H_{17}O_2$, 168.24), along with its ^1H and ^{13}C NMR data (Figures S7 and S8). ^1H NMR spectroscopy displayed a characteristic methyl signal in the downfield region for a methyl group attached to the sp^2-hybridized carbon (C-3), resonating at δ_H 2.12 (3H, s, H-10), along with two methyl singlets at 1.55 (3H, *s*, H-8) and 1.63 (3H, *s*, H-9), assigned to two methyl groups attached to sp^2-hybridized quaternary carbon (C-7). The olefinic protons at C-2 and C-6 were observed at 5.02 (1H, br *s*, H-6) and 5.63 (1H, br *s*, H-2), respectively. The remaining four aliphatic protons resonated at 2.13 (4H, *m*), attributed to H-4 and H-5. The ^{13}C NMR spectrum revealed ten signals, corroborated by DEPT 135, including a carboxylic acid carbon resonating at δ_C 172.5 (C-1). Four olefinic carbon resonances, encompassing two quaternary carbons, were identified at δ_C 162.8 (C-3), 132.6 (C-7), 122.8 (C-6), and 115.4 (C-2). Three methyl carbon signals appeared at δ_C 25.6 (C-8), 19.1 (C-10), and 17.7 (C-9). Additionally, two aliphatic methylene carbon signals were observed at δ_C 41.2 (C-4) and 26.0 (C-5) (Figure S9). The cumulative NMR data, consisting of ten carbon atoms, align with a monoterpenoid structure containing one acid group, leading to the identification of the compound as geranic acid (Figure 6).

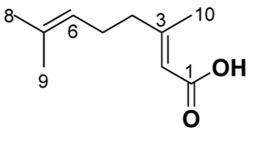

Figure 6. Chemical structure of geranic acid.

4. DISCUSSION

Numerous microorganisms have been employed in the literature to biotransform geraniol to geranic acid, for instance, *Acetobacter* sp. and *Gluconobacter* sp.;[14] *Acinetobacter* sp.;[15] *Rhodococcus* sp. GR3;[9] *P. putida*;[16] *C. maxima*;[24] *Acinetobacter* sp Tol5;[25] *S. cerevisiae*;[26] *P. digitatum*;[17] *C. defragrans*;[27] *P. aeruginosa*;[28] *G. roseus* (patent number: EP0289822B1), *Saccharomyces*, *Hansenula*, *Pichia*, *Candida*, and *Kluyveromyces* (patent number: EP0563346B1); *Escherichia*, *Enterococcus*, and *Enterobacter* (patent number: WO2020264400A2); and *T. versicolor*.[29] After molecular identification of the isolated culture, different parameters, including biomass concentration, media composition, substrate concentration, and other physical parameters such as reaction time and temperature, were optimized.

It has been reported that different carbon and nitrogen sources in the media may affect the production of enzymes involved in biotransformation reactions;[30,31] hence, the effect of different media compositions were assessed for efficient production of geranic acid. For instance, Ohashi et al. reported that YPD media-glucose (20 g/L), polypeptone (20 g/L), and yeast extract (10 g/L) and the minimal medium-maltose, 2 g/L; Leu/Trp/Ura DO supplement, 0.62 g/L; uracil, 0.2 g/L; leucine, 1 g/L; yeast nitrogen base, 1.7 g/L; and ammonium sulfate, 5 g/L) showed approximately 40 μg/mL production of geranic acid after 10 days.[26] Another group had used two different media, i.e., basal salt medium added with toluene, lactate, geraniol, or geranic acid (3.3×10^{-2} mol-carbon/L) and Luria−Bertani (LB) medium which resulted in 98.96% conversion on eighth day.[15] However, in our study, 98.89% conversion was observed only after 3 days using fungal mycelium grown in SDB medium.

Another factor, temperature is also responsible for affecting the production of geranic acid. This may be due to the lesser enzyme activity of biotransforming enzymes at lower and higher temperatures. As reported earlier, optimum temperature for maximum conversion of geraniol to geranic acid was 30 °C after 96 h.[9] Further, other studies have biotransformed geraniol to geranic acid at 24 °C,[24] 30 °C (patent number: EP0289822B1 and WO2020264400A2), 37 °C (patent number: EP0563346B1), and 25 °C,[17] while 99.20% conversion was observed at 28 °C after 72 h in our study.

Apart from media and temperature, substrate concentration may also affect the enzyme production and cell viability, as a high substrate concentration may have cell toxicity and prevent the efficient bioconversion of geranic acid. Based on a previous report, with the increase in the substrate concentration from 10

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to 80 mM, the production of geranic acid decreased. Results showed that the highest production of geranic acid (3 mM) was obtained when 10 mM geraniol was used; thereafter, the production decreased.[15] On the other hand, we observed that *M. irregularis* IIIMF4011 showed higher substrate tolerance (20 mM geraniol). As per the literature, the solubility of geraniol is 0.1 g/L, which is equivalent to 0.64 mM, but in this study, the biotransformation reaction using 20 mM geraniol concentration showed good geranic acid production. In the literature, it is also established that geraniol induces membrane disruption which increases fluidity and permeability, inducing disturbances in membrane function.[33] This may result in the exposure of intracellular enzymes to the substrate, i.e., geraniol. In our study, biotransformation reaction showed 97−100% geranic acid production after 72 h which concludes that with the increase in reaction time, the slow substrate solubility resulted in slow biotransformation reaction and conversion.

Further, in the existing literature, very limited work has been reported in scaling up of the biotransformation reaction of geraniol to geranic acid. Although >97% conversion was obtained by using *Acetobacter* sp. in 24 h, but the reaction was performed only at the flask level.[14] Therefore, this study is the first report of biocatalytic synthesis of geranic acid at fermentor scale with 98.89% conversion.

5. CONCLUSIONS

Based on the previous literature, it was observed that biotransformation of geraniol to geranic acid requires more optimization of reaction parameters for efficient conversion. Therefore, the present study aimed at developing an easy and efficient process that showed maximum conversion of geraniol, which is a low-value component of essential oil to a high-value product, i.e., geranic acid. The process basically involves the biocatalytic production of geranic acid using *M. irregularis* IIIMF4011. Thereafter, optimization of various reaction parameters like medium, temperature, biomass, and substrate concentration resulted in (99.28%) conversion of geraniol to geranic acid in a flask. Furthermore, the biotransformation reaction in 3 L fermentor (1.5 L working volume) also showed 98.89% conversion, making it a commercially viable process. Hence, an easy, economical, and efficient process is developed that may be utilized for large-scale production of geranic acid.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.4c03538.

GC−MS, images of TLC, structure of geranic acid, ^{1}H NMR,^{13}C NMR, and DEPT-135 (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Vikash Babu − *CSIR—Indian Institute of Integrative Medicine, Jammu 180001, India; Academy of Scientific and Innovative Research (AcSIR), Ghaziabad 201002, India;* orcid.org/0000-0001-9784-4135; Phone: +91 191-2584999 Ext. 374; Email: vikash@iiim.res.in; Fax: +91 191-2586333

Authors

Haseena Shafeeq − *CSIR—Indian Institute of Integrative Medicine, Jammu 180001, India; Academy of Scientific and Innovative Research (AcSIR), Ghaziabad 201002, India*

Bashir Ahmad Lone − *CSIR—Indian Institute of Integrative Medicine, Jammu 180001, India; Academy of Scientific and Innovative Research (AcSIR), Ghaziabad 201002, India*

Ananta Ganjoo − *CSIR—Indian Institute of Integrative Medicine, Jammu 180001, India; Academy of Scientific and Innovative Research (AcSIR), Ghaziabad 201002, India*

Nargis Ayoub − *CSIR—Indian Institute of Integrative Medicine, Jammu 180001, India; Academy of Scientific and Innovative Research (AcSIR), Ghaziabad 201002, India*

Hema Kumari − *CSIR—Indian Institute of Integrative Medicine, Jammu 180001, India; Academy of Scientific and Innovative Research (AcSIR), Ghaziabad 201002, India*

Sumeet Gairola − *CSIR—Indian Institute of Integrative Medicine, Jammu 180001, India; Academy of Scientific and Innovative Research (AcSIR), Ghaziabad 201002, India*

Prasoon Gupta − *CSIR—Indian Institute of Integrative Medicine, Jammu 180001, India; Academy of Scientific and Innovative Research (AcSIR), Ghaziabad 201002, India*

Zabeer Ahmed − *CSIR—Indian Institute of Integrative Medicine, Jammu 180001, India; Academy of Scientific and Innovative Research (AcSIR), Ghaziabad 201002, India*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.4c03538

Notes

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