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Bioconversion of D-limonene into value-added monoterpenes using *Paenibacillus popilliae* 1C and *Streptomyces rochei* AB1: Exploring bacterial biotransformation pathways and metabolite diversity

Madjeda Beichi[1], Fatiha Saidani[1], Nacera Riad[1], Fatima Elzahra Ferradji[1], Fatima Elzahra Mesbaiah[2], Mohamed Reda Zahi[1]*, Kamel Eddouaouda[1], Abdelmalek Badis[1] and Mohamed El Hattab[1]*

[1]Laboratory of Natural Products Chemistry and Biomolecules Faculty of Sciences - University Blida 1 Road of Soumaa - BP 270- Blida 09000, Algeria

[2]National Centre for Research and Development of Fisheries and Aquaculture (CNRDPA) 11, Colonel Amirouche Street, P.O. Box 67, Bou Ismaïl, 42415, Tipaza, Algeria

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The present study focuses on the bioconversion of D-limonene using two bacterial strains, *Streptomyces rochei* AB1 and *Paenibacillus popilliae* 1C, isolated and identified by our research team. Rich mediums (International *Streptomyces* Project 9 medium for strain AB1 and Luria–Bertani medium for strain 1C) were used for biotransformation experiments. Furthermore, biodegradation tests were conducted with D-limonene as the only carbon and energy source in a modified minimum medium. The minimum inhibitory concentration (MIC) of D-limonene was 10±0.09 mg/mL. The strains achieved a bioconversion yield of nearly 70% following 7-day of incubation period. Analysis by GC-MS revealed the formation of various hydrocarbons and oxygenated monoterpenes, including alcohols, ketones and terpene hydrocarbons. Some metabolites, such as cis-verbenol, isovaleric acid and 2-methylbutanoic acid, were identified in biotransformation assays using *Paenibacillus popilliae* 1C. To our knowledge, this genus has never before been known to produce these metabolites during the biotransformation of D-limonene. Carveol, carvone, limonene-1,2-diol, linalool, and α-terpinolene were among the other metabolites found. Similar biodegradation products were obtained with *Streptomyces rochei* AB1, suggesting the occurrence of common metabolic pathways involved in D-limonene bioconversion. These findings highlight the biotechnological potential of the studied strains for the production of value-added monoterpenes.

Keywords: Bioconversion, D-limonene, Monoterpenes, *Paenibacillus popilliae* 1C, *Streptomyces rochei* AB1

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Introduction

The development of new and inventive treatments based on small-molecule candidates remains an essential focus for the pharmaceutical sector. These compounds have repeatedly enabled significant medical advancements and addressed unmet healthcare needs, ultimately saving numerous lives. Additionally, tiny compounds are known to be essential in biomedical studies as chemical probes[1]. As a result, extensive research has been dedicated to biomolecules for therapeutic applications, and in the fields of flavour and fragrance as well[2]. In this context, the biotransformation process offers a wide range of opportunities for producing region-selective and stereo-selective molecules of industrial significance. Biotransformation can be defined as a process using microorganisms or enzymes to transform metabolites, leading to high-quality products in terms of region- and stereo-selectivity, as well as better performance[3]. Furthermore, it might be a useful tool for structurally altering both industrial and natural bioactive substances. The process of biotransformation is entirely in line with green chemistry, which explains the numerous studies carried out on the microbial conversion of several natural compounds, notably monoterpenes[4]. These later are made up of two isoprene units, which are produced in plants via the isoprenoid pathway. Due to their distinct scent and taste, monoterpenes are widely used in cosmetic products, food supplements and medicinal applications. Oxygenated monoterpenes, also known as monoterpenoids, are found abundantly in higher plant species, algae, fungi and certain types of insects[5,6]. D-limonene is a compound that can be derived directly from industrial food waste

*Correspondent author
Email: z-reda@hotmail.com, elhattabmohamed@gmail.com

or synthesised by microbial engineering. It can be utilised as a fragrance or further transformed into useful products for downstream use; it is one of the most common terpenes in nature and an inexpensive monocyclic monoterpene. It constitutes the primary element of essential oils derived from citrus peels (90–96%)[5] and serves as an important starting material for the production of pharmacologically active compounds, such as perillyl alcohol and perillic acid, which have been noted for their antimicrobial and anticancer activities[7]. Numerous studies have examined the biotransformation of D-limonene using different bacterial and fungal strains[7]. To contextualise the present study, a comprehensive review of the research reported in the literature on the bioconversion of D-limonene by bacteria and fungi is provided in Table 1 and Fig. 1. This study investigates the applicability of two aerobic bacterial strains (*Paenibacillus popilliae* 1C and *Streptomyces rochei* AB1) recently obtained from local ecological niches in Algeria by our research team[8,9], in the bioconversion of D-limonene. The primary goals of this research are to examine the capacity of the chosen strains to use D-limonene as a substrate, to identify and characterise the metabolites produced through suitable analytical methods, and to assess the region-selectivity and effectiveness of the bioconversion process.

Materials and Methods

Chemicals

The pure D-Limonene (≥97.0%) was purchased from Sigma Aldrich (France).

Bacterial strains

In our earlier investigation, the microbial strains were separated and identified. The strain *Paenibacillus popilliae* C1 was isolated from a sandy soil in May 2008, in Hassi-Messaoud at the south of Algeria[8]. In March 2007, the strain *Streptomyces rochei* was identified from damp soil in the Boufarik region, west of Algiers, Algeria[9].

Culture media

Luria-Bertani (LB) medium was prepared by dissolving 5 g yeast extract, 10 g peptone and 5 g sodium chloride in 1 L of distilled water. The International *Streptomyces* Project 2 (ISP2) medium consisted of 1 L of distilled water, 4 g yeast extract, 10 g malt extract and 4 g of glucose. 10 g glucose, 2.84 g $(NH_4)_2SO_4$, 2.38 g KH_2PO_4, 5.65 g $K_2HPO_4 \cdot 3H_2O$, 1 g $MgSO_4 \cdot 7H_2O$, 1 mL trace element solution (ETM) and 1 L of distilled water were used to form the International *Streptomyces* Project 9 (ISP9) rich medium. In addition, ISP9 poor medium: without glucose, had the same composition as ISP9 rich medium.

Minimum medium (MM): This mineral salt-based medium had the following contents (g/L): NH_4Cl (0.4), K_2HPO_4 (0.3), KH_2PO_4 (0.3), NaCl (10), $MgCl_2$ (0.33), $CaCl_2$ (0.05), yeast extract (0.1), and 1 mL trace element solution. Before sterilisation, the pH of all media was brought to 7.2 using 1N HCl or 30% (w/v) NaOH. They were than sterilized by autoclaving at 120°C for 20 minutes.

Cultivation of strains

A subculture from conservation media was made for the two strains using ISP2 and LB solid media. The cultures were incubated under fermentation conditions at 45°C for 48 h and at 30°C for 72 h for *Paenibacillus popiliae* 1C and *Streptomyces rochei* AB1, respectively, to obtain good, separated colonies.

Liquid medium (ISP2 or LB) was inoculated with colonies picked from the nutrient agar and incubated under the same conditions. The bacterial culture obtained in this step will serve as the necessary overnight culture for the bioconversion experiment.

The minimum inhibitory concentration

A series of diluted solutions (mg/mL, dissolved in methanol) at 0.05, 0.5, 1, 5, 10, 15, and 20 were prepared from a D-limonene stock solution (30 mg/mL) to determine the MIC of D-limonene. The disc diffusion assay was used for these experiments, and the results were calculated by measuring the inhibition zone diameter (mm).

Biotransformation experiments

The biotransformation experiments were assessed according to the method of Abolghasemi *et al.*[15] with minor modifications. About 2 mL of each bacterial suspension were added into an Erlenmeyer flask filled with 50 mL and adjusted to 1 McFarland turbidity standard, which is equal to 3×10^3 CFU/mL. They were then incubated in the dark under agitation (150 rpm) at 30°C for *Streptomyces rochei* and 45°C for *Paenibacillus popilliae* 1C for 3 days. After abundant growth, biotransformation was initiated by adding D-limonene directly at its MIC value, corresponding to a final volume of 595 µL culture in the culture flasks. A control test was prepared under the same conditions. All experiments were conducted in triplicate.

Table 1 — Conversion products of limonene by several bacteria, fungi, and yeast reported in the literature

S No	Conversion products	Bacteria, Fungi, and Yeast	References
01	Cis- Carveol	*Pseudomonas sp.PL*	10
	Trans-carveol	*Rhodococcus opacus PWD4*	11
	Cis-carveol	*Rhodococcus erythropolis dcl14*	11
	Carveol	*Pleurotus sapidus*	12
	Trans-carveol	*Escherichai coli BR388*	13
	Carveol	*Pseudomonas putidia S12*	14
	(Cis+trans)-carveol	*Synechococcus sp. PCC 7942*	15
	Carveol	*Cellilosimicrobium cellulans EB84*	15
02	Carvone	*Rhodococcus erythropolis PWD8*	11
		Pleurotus sapidus	12
		Escherichai coli BR388	10
		Pseudomonas sp.PL	10
03	α-Terpineol	*Armillareira mellae*	16
		Clodosporiumsp t12	17
		Escherichia coli	13
		Bacillus sterothermophilus BR388	18
		Pseudomonas sp. Ma.A toneka	19
		Mycrobacteriumsp HXN-1500	20
04	Perillyl alcohol	*Bacillus stearothermophilus BR388*	21
		Pseudomonas -putidia MTCC1072	22
		Echerichia coli (6.9kb)	23
05	p-Menth-1-ene-6,8-diol	*Peudomonas putida MTCC1072*	22
06	Perillyl aldehyde	*Bacillus stearothermophilus BR388*	21
		Escherichia coli BR 388	13
		Mycobacterium sp HXN-1500	20
07	Perillyl acid	*Pseudomonas incognita*	24
		Pseudomonas putida S12	14
		Pseudomonas gladioli	25
		Pseudomonas sp.PL	10
		Escherichia coli (cym genes from Pseudomonas putida F1)	26
		Mucrobaterium sp HXN-1500	20
08	Limonene 1,2- diol	*Rhodococcus erythropolis dcl14*	27
09	4-Hydroxy-mentha1,8-dien	*pseudomonas-sapidus*	28
10	Limonene 8,9-epoxide	*Xanthobacter sp.C20*	29
11	Cis-(-)-1,2-epoxy-p menth-8-ene	*Rhodococcus erythropolis dcl14*	27
12	Terpinen-1-ol	*Pseudomonas sp. Ma.A toneka*	19
13	linalool	*Pseudomonas sp. Ma.A toneka*	19
14	Isopepiritenol	*Hormonema sp.*	30
15	(Cis+trans)-carveol	*Aspergillus cellulosaeM77*	31
	Cis-carveol Trans-carveol (Cis+trans)-carveol	*Fusarium oxysporum Phomopsis sp.*	32
16	Carvone	*Fusarium oxysporum, Phomopsis sp.*	32
17	α-Terpineol	*Fusarium oxysporum*	32
		Aspergillus cellulosaeM77	31
18	Perillyl alcohol	*Aspergillus cellulose M-77*	31
19	Perillyl acid	*Chaetomium globosum*, *Yarrowia lipolytica*	33
20	Limonene 1,2- diol	*Aspergillus cellulosae M77*	31
21	p-Menth-2,8-dien-1-ol	*Yarrowia lipolytica*	33
22	p-Mentha-1,8(10)-diene	*Chaetomium globosum*	33

Biodegradation experiments

The tests were carried out in a MM for *Paenibacillus popilliae* and in ISP9-poor medium for *Streptomyces rochei* under the same experimental conditions as in the biotransformation experiments. In this case, D-Limonene is the only source of carbon and energy; therefore, it should be added to the medium at the start of the test (t_0). Every experiment was carried out in triplicate. Ultimately, the cells were extracted by centrifugation for 45 minutes at 6000 rpm. The biotransformation products were obtained by recovering the culture supernatant via

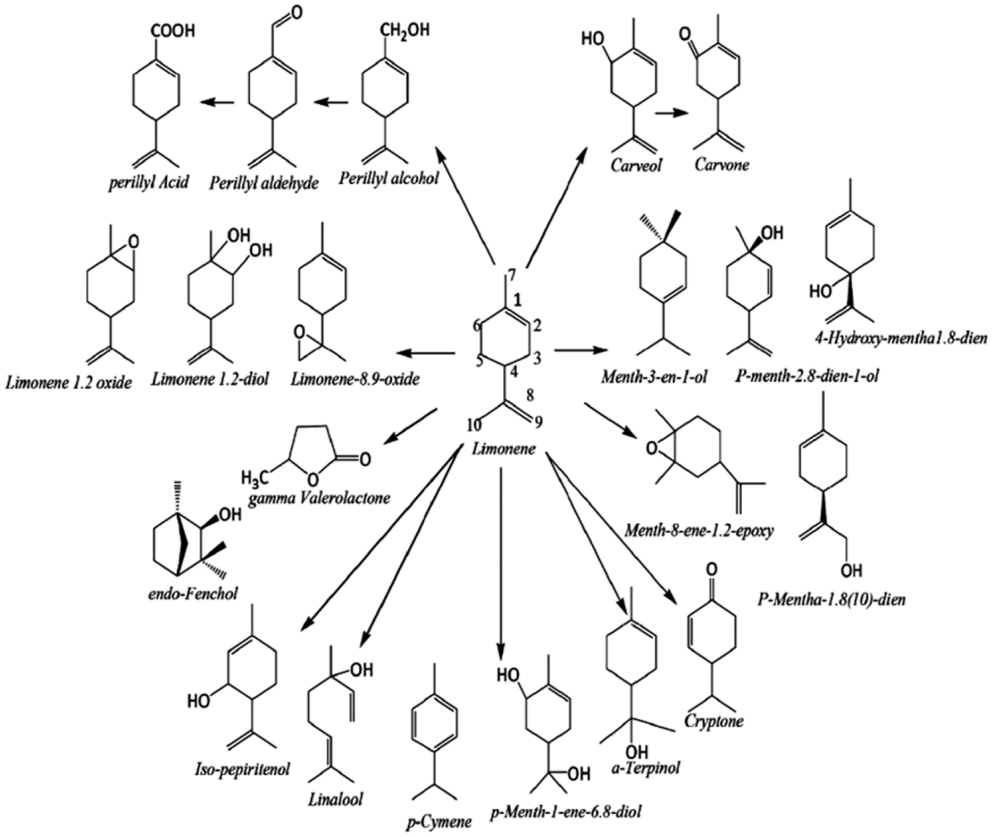

Fig. 1 — Main metabolic pathways of limonene described in the literature.

liquid-liquid extraction using diethyl ether. GC-MS analysis was performed on the latter.

GC-MS analysis

An HP 6800 gas chromatograph connected to an HP MSD 5973 mass spectrometer with a fused silica capillary column (HP5-MS, 30 m x 0.25 mm x 0.25 µm) was utilised for GC-MS analysis. After maintaining the oven temperature at 100°C, the temperature was gradually raised to 220°C at a rate of 5°C per minute. The temperature of the injector and detector was 250°C. The carrier gas was helium, which had a split ratio of 1/90 and a linear flow rate of 5 mL/min. With an iron source temperature of 230°C, ionisation energy of 70eV, and a mass range of m/z 40-500, the mass-selective detector was running in electron ionisation (EI) mode. Prior to injection, 1 mL of sample was diluted in 5 mL of ethyl acetate.

The following databases were used to characterise the various chemicals or metabolites from bio-conversion: NBS 75k (DB2) and Mass Finder 3 (DB3) (D.H. Hochmuth, www.massfinder.com); W11N17 (DB1) (Wiley11-Nist17, Wiley, Hoboken, USA). Two filters were used for identification: a linear retention index match computed using a C7-C30 saturated n-alkane homolog series (1000 mg/L) supplied by Merck KGaA (Darmstadt, Germany) with a filter window of ±10 IRL units and a spectral similarity match over 85%. The values of the computed linear retention index (LRI_{cal}) were compared with those found in the literature (LRI_{Lit}). Further identification was carried out using mass spectra published in the literature[34].

Results and Discussion

Determination of the minimum inhibitory concentration

In this test, no zones of inhibition were observed. These results imply that both bacterial strains could tolerate all tested D-limonene concentrations, suggesting that D-limonene did not exhibit any discernible toxicity towards the strains examined. The MIC for D-limonene was set at (10±0.09) mg/mL for technical convenience.

Biotransformation of D-limonene by *Paenibacillus popilliae* 1C

The biotransformation products of D-limonene by *Paenibacillus popilliae* C1, revealed the presence of 10 compounds (Table 2). Among these compounds,

Table 2 — Biotransformation products of limonene by *Paenibacillus popilliae* 1C

S No	Tr (min)	Compounds	LRI_{lit}	LRI_{Calc}	(%)	Database
1	2.34	Iso valeric acid (3-methylbutanoic acid)	834	830	5.9	DB1, DB2, DB3
2	2.38	2-Methybutanoic acid	858	850	9.1	DB1, DB2, DB3
3	5.03	Cis verbenol	1137	1130	9.2	DB1, DB2, DB3
4	5.24	Cis-p-mentha-2,8-dien-1-ol	1138	1137	11.5	DB1, DB2, DB3
5	5.94	1,8-Menthadien-4-ol	1182	1175	3.5	DB1, DB2, DB3
6	6.36	Unkown(*)	-	1201	4.9	
7	6.68	Trans carveol	1215	1210	20.5	DB1, DB3
8	6.91	Cis carveol	1229	1125	15.1	DB1DB3
9	7.16	Carvone	1239	1230	4.0	DB1, DB3
10	9.22	Limonene 1,2 diol	1321	1315	14.6	DB1
		Total			98.3	

(*): m/z: 41(20), 55(10), 69(15), 81(10), 84(100), 91(18), 108(10), 134(7), 152(2)

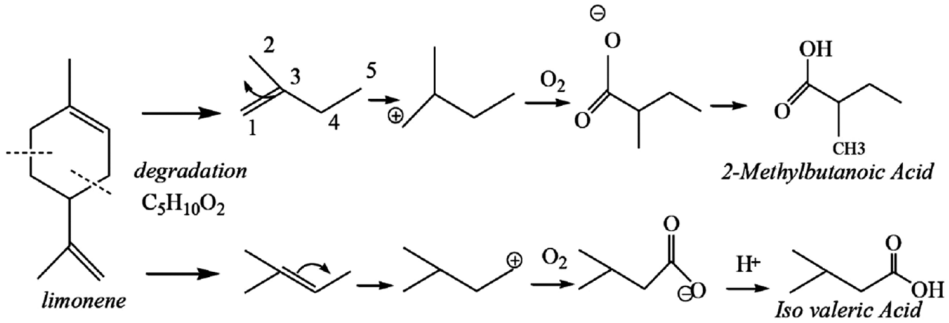

Fig. 2 — Plausible mechanism of bioconversion of limonene to Iso valeric Acid and 2-Methylbutanoic acid[35-36].

9 were correctly identified. One compound (t_r = 6.36 min) could not be identified from the mass spectra databases. Its retention index was estimated to be 1201. Its mass spectrum fragment (shown at the bottom of the table) revealed that it is most likely an oxygenated monoterpene with a molecular weight of 152 g/mol, corresponding to $C_{10}H_{16}O$. It has a peak at m/z = 91 and a base peak at m/z = 84, the latter is most likely the troppyllium ion, obtained by the rearrangement of a methylbenzene. All these structural data are in favour of an oxygenated monocyclic monoterpene.

Isovaleric acid, 2-methylbutanoic acid, and cis-verbenol have not previously been identified as metabolites produced during the microbial biotransformation of D-limonene[16,25-33], according to our examination of the reviewed sources. On the other hand, prior studies investigating the metabolism of D-limonene using various bacterial and fungal strains have reported the following compounds: trans-carveol, cis-carveol, carvone, 1,8-menthadien-4-ol, and cis-p-mentha-2,8-dien-1-ol, and limonene-1,2-diol[15,16-26].

Both isomers, isovaleric acid and 2-methylbutanoic acid, possess a branched-chain configuration, which aligns with the characteristics of isoprenoid-derived substances, including a carboxylic acid functional group. Their formation is probably a result of the biodegradation of D-limonene, which occurs through the breaking of the cyclic ring followed by β-oxidation, or it may arise from a reverse process (Fig. 2). Additionally, these metabolites may result from the breakdown of an acyclic monoterpene intermediate generated via D-limonene biotransformation.

Following the formation α-terpinyl-8 cation and then C2-C8 cyclization, α-pinene could obtained from D-limonene (Fig. 3). Cis-verbenol is newly reported in this study, along with metabolites featuring a pinane structure. To the best of our knowledge, no compounds corresponding to this structure have been previously documented in studies of D-limonene bio-transformation. The bacterial strain used in this research appears to have evolved a new metabolic pathway that leads to the formation of this monoterpene structure. It is most likely that D-limonene undergoes a C_2–C_8 cyclisation to form cis-verbenol, which is then subjected to further oxidation processes that could include reactions similar to the β-oxidation of α-pinene–type intermediate under H_2O/H^+ circumstances (Fig. 3).

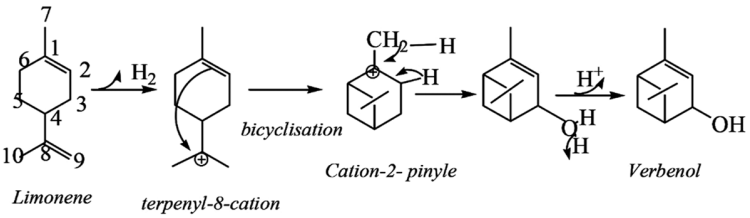

Fig. 3 — Plausible mechanism of bioconversion of limonene to Verbenol[35-36].

Table 3 — Conversion products of limonene by *Streptomyces rochei* AB1

S No	Tr (min)	Compounds	LRI_{lit}	LRI_{calc}	(%)	Data base
1	3.0	α-Pinene	932	930	t	DB1, DB2, DB3
2	3.29	Sabinene	973	970	t	DB1, DB2, DB3
3	3.36	β-Myrcene	988	980	0.5	DB1, DB2, DB3
4	3.83	Limonene	1024	1015	61	DB1, DB2, DB3
5	4.61	α-Terpinolene	1086	1089	1.3	DB1, DB2, DB3
6	5.02	Cis verbenol	1137	1130	3.0	DB1, DB2, DB3
7	5.24	Cis-p-mentha-2,8-dien-1-ol	1138	1137	4.0	DB1, DB2, DB3
8	5.86	Menthol	1161	1155	1.0	DB1, DB2, DB3
9	5.94	1,8-Menthadien-4-ol	1182	1175	1.1	DB1, DB2, DB3
10	6.35	Unknown	--	1201	2.1	
11	6.68	Trans carveol	1215	1210	6.0	DB1, DB3
12	6.91	Cis carveol	1229	1220	3.8	DB1, DB3
13	7.15	Carvone	1239	1230	2.7	DB1, DB3
14	9.22	Limonene 1,2 diol	1321	1315	11.4	DB1
		Total			97.9	

Table 4 — Biodegradation products of limonene by *Streptomyces rochei* AB1

S No	Tr (min)	Compounds	LRI_{lit}	LRI_{Calc}	(%)	Database
1	3.82	Limonene	1024	1016	23.9	DB1, DB2, DB3
2	4.61	Linalool	1095	1090	3.1	DB1, DB2, DB3
3	5.04	Cis verbenol	1137	1133	4.8	DB1, DB2, DB3
4	5.25	Cis-p-mentha-2,8-dien-1-ol	1138	1139	7.2	DB1, DB2, DB3
5	5.86	Menthol	1161	1160	2.9	DB1, DB2, DB3
6	5.95	1,8-Menthadien-4-ol	1182	1175	1.7	DB1, DB2, DB3
7	6.36	Unknown	-	1201	3.7	
8	6.69	Trans carveol	1215	1208	10.6	DB1, DB3
9	6.92	Cis carveol	1229	1218	7.2	DB1.DB3
10	7.16	Carvone	1239	1230	6.2	DB1.DB3
11	9.25	Limonene 1,2-diol-	1321	1320	25.3	DB1
		Total			96.6	

The compounds previously described in the literature (Table 1), namely 1,8-menthadien-4-ol, cis-p-mentha-2,8-dien-1-ol, trans-carveol, cis-carveol, limonene-1,2-diol and carvone, share the same monoterpene frameworks derived from D-limonene. Each of them is an oxygenated derivative that was probably produced by simple biotransformations, which could involve oxidation steps similar to those in β-oxidation.

In addition to the strain's inability to use D-limonene as a carbon and energy source, this is most likely due to the absence of a carbon source in contrast to the rich medium. The chromatographic profile, which only displayed the 3.4-minute D-limonene peak, verified this.

Biotransformation of D-limonene by *Streptomyces rochei* AB1

Streptomyces rochei AB1's biotransformation of D-limonene produced 14 compounds (Table 3), among which six metabolites have not been previously reported for this strain. They were 4 hydrocarbon monoterpenes (Sabinene, β-myrcene, α-pinene, α-terpinolene) and 2 oxygenated monoterpenes (Cis- verbenol; L- menthol), and Streptomyces AB1 produced 11 chemicals during the biodegradation of D-limonene (Table 4).

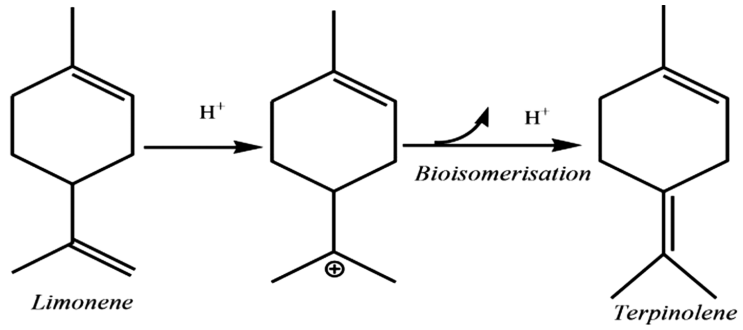

Fig. 4 — Plausible mechanism of bioconversion of limonene to α-Terpinolene[35-36].

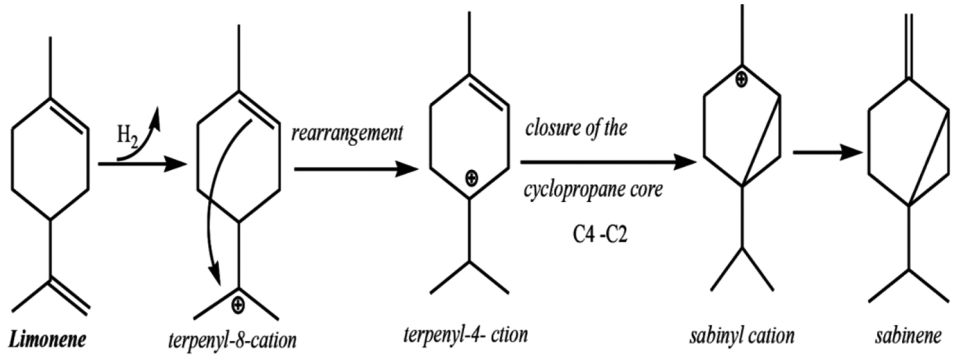

Fig. 5 — Plausible mechanism of bioconversion of limonene to α- Sabinene[35-36].

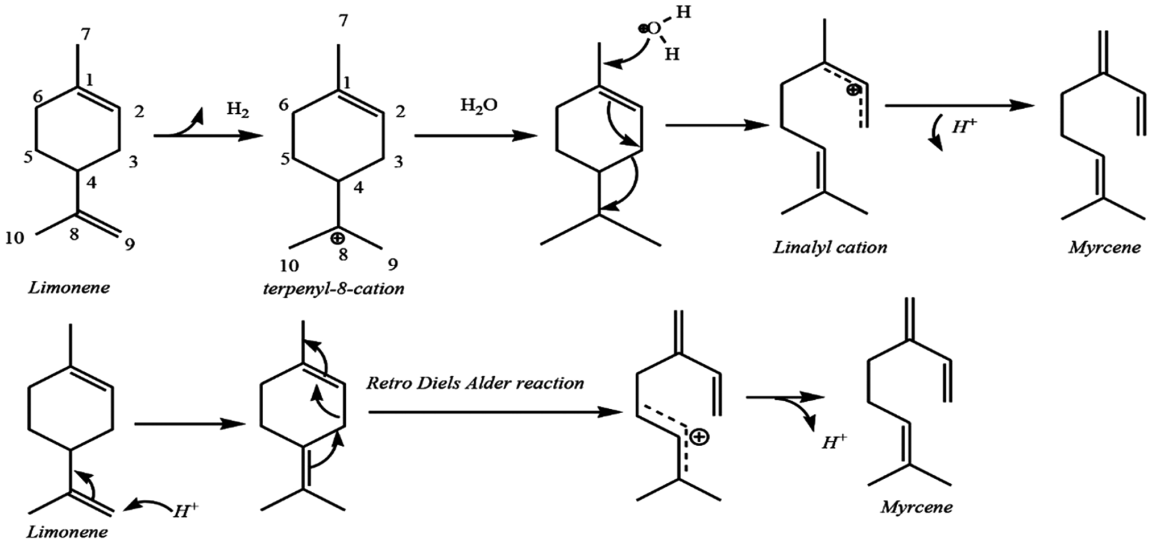

Fig. 6 — Plausible mechanism of bioconversion of limonene to Myrcene[35-36].

It is important to highlight that α-terpinolene serves as a structural isomer of D-limonene and may consequently arise from a straightforward bio-isomerization process in acidic environments (Fig. 4). In a similar manner, sabinene could be produced through a corresponding pathway that involves the generation of the α-terpinyl-8 cation, which is followed by a hydride shift from C_8 to C_4, resulting in the α-terpinyl-4 cation and the subsequent cyclization from C_4 to C_2 (Fig. 5). Furthermore, β-myrcene is likely formed from D-limonene through a retro-Diels-Alder reaction (Fig. 6). At the same time, menthol may be synthesized through oxidation at the C_3 position, followed by a hydrogenation step (Fig. 7).

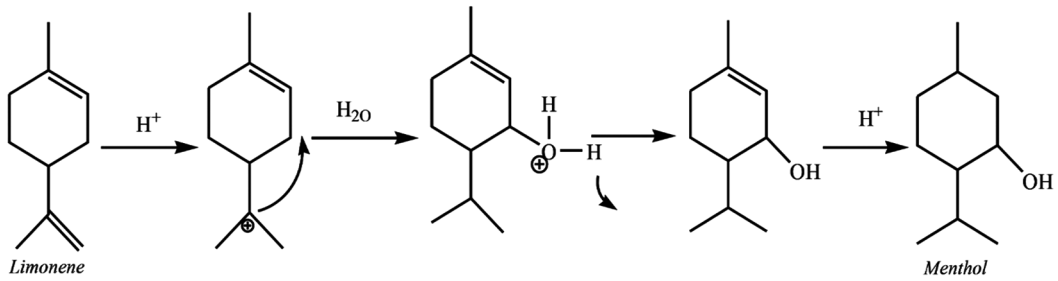

Fig. 7 — Plausible mechanism of bioconversion of limonene to Menthol[35-36].

Discussion

The *Streptomyces rochei* AB1 strain appears to possess intriguing capabilities not reported in other strains, as evidenced by the products obtained and the reactions implemented. It can perform biotransformations that yield new compounds due to its numerous metabolic pathways. The other compounds (7, 9, 11-14) have been previously reported in the biotransformation of D-limonene (Table 1); the unknown compound 10 is identical to that described in Table 2. The transformation of D-limonene by *Streptomyces rochei* AB1 in MM was found to be more similar to a biodegradation process, yielding particularly significant results. A total of 11 compounds were identified (Table 4), including cis-verbenol (3) and menthol (5). Furthermore, 7 additional monoterpenes (2, 4, 6, and 8–11), previously documented in studies on the biotransformation of D-limonene, were produced during the biodegradation process.

A noteworthy finding was that all products resulting from biodegradation, as identified by (GC-MS), except for the residual D-limonene (23.9%), consisted of oxygenated monoterpenes. Among these, the most abundant metabolite was limonene-1,2-diol, which accounted for (25.3%) of the total chromatographic area. This was followed by trans-carveol (10.6%), as well as cis-p-mentha-2,8-dien-1-ol and cis-carveol, both at (7.2%). Furthermore, carvone accounted for 6.2% and cis-verbenol for 4.8%. Linalool (3.1%), menthol (2.9%), and 1,8-menthadien-4-ol (1.7%) included an unidentified compound (3.7%). These findings strongly indicate that oxidative reactions are the primary metabolic process involved in the biodegradation of D-limonene by *Streptomyces rochei* AB1. All biodegradation products were cyclic, except for linalool, an acyclic monoterpene. The linalool was already reported in the biotransformation of D-limonene by Abolghasemi ***et al.***[15], and Duetz *et al.*[16]. The other monoterpenes could probably be obtained by a process similar to biotransformation. It should also be noted that the unknown product (7), described in the biotransformation of D-limonene by both strains (Table 3 and Table 4), was identified, which supports our hypothesis regarding the interaction between the two biotransformation and biodegradation processes. It is important to note that the biodegradation process did not result in complete consumption of D-limonene; an appreciable amount (23.9%) remained at the reaction completion.

The results demonstrate that the investigated bacterial strains have efficient enzymatic systems that transform D-limonene into different oxygenated derivatives by a sequence of oxidation and hydroxylation reactions. The prevalence of isomers of carveol, carvone and limonene-1,2-diol suggests that the conversion predominantly follows oxidative pathways typical of monoterpene metabolism. Furthermore, the presence of compounds such as isovaleric acid, 2-methylbutanoic acid and cis-verbenol suggests the existence of additional metabolic pathways specific to certain strains. The variations noted in the distribution of metabolites and the remaining levels of D-limonene also indicate differences in the bioconversion efficiency among the examined strains and the employed culture conditions. These results underscore the metabolic adaptability of the bacteria studied and reinforce their potential for microbial synthesis of oxygenated monoterpenes.

Conclusion

This study demonstrated the capacity of two microorganisms isolated from Algerian ecological habitats, *Paenibacillus popilliae* 1C and *Streptomyces rochei* AB1, to convert D-limonene into a variety of oxygenated byproducts. Analysis by GC–MS indicated that *Streptomyces rochei* AB1 displayed the highest bioconversion potential, yielding significant metabolites, including trans-carveol (20.5%), cis-

carveol (15.1%) and limonene-1,2-diol (14.6%). On the other hand, *Paenibacillus popilliae* 1C demonstrated reduced conversion effectiveness, retaining a considerable amount of D-limonene (61%). Studies on D-limonene biotransformation rarely mention certain metabolites, such as cis-verbenol, isovaleric acid, and 2-methylbutanoic acid, which may indicate particular metabolic pathways specific to the strains. These results emphasise the potential applications of the studied strains, especially *Streptomyces rochei* AB1, as promising biocatalysts for the sustainable synthesis of valuable monoterpene derivatives for use in pharmaceuticals, cosmetics, and fine chemicals.

Conflict of interest

The author (s) did not disclose any possible conflicts of interest.

AI use disclosure

No Generative AI tools were used for data analysis, image creation or visualization.

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