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MICROBIAL FERMENTATION OF LOVASTATIN AND OTHER BIOACTIVE SECONDARY METABOLITES USING *ASPERGILLUS TERREUS*

Rohit Raj, Sonu Kumar Gupta, Malkhey Verma*

Department of Biochemistry and Microbial Sciences, Central University of Punjab Bathinda, Punjab, India.

ABSTRACT: The fungus *Aspergillus terreus* has dominated the biological production of the "crackerjack" drugs known as statins, particularly lovastatin. The aim of this research was the production of lovastatin which is a known cholesterol-lowering drug, through microbial fermentation using *A. terreus*. Besides, it also aimed to analyze certain bioactive chemical products and evaluation of such antibacterial and antifungal products, if any produced. Bioactive chemical compounds often referred as secondary metabolites were analyzed using the Gas Chromatography-Mass Spectroscopy technique (GC-MS) technique. *A. terreus* is known to produce a vast variety of important secondary metabolites with high biological activities. The isolation of the natural statins such as lovastatin or mevastatin from *A. terreus* represents one of the great achievements of industrial Microbiology/Fermentation Technology. Here we report the *Aspergillus terreus* NBRC (IFO) 31217 (Strain I) and ATCC 11877 (Strain II) produce lovastatin and they also produce important bioactive compounds of high commercial value like isovaline ($C_5H_{11}NO_2$), 2-oxo-n-valeric acid and silane etc.

KEYWORDS: Lovastatin, Secondary metabolites, Fermentation, *Aspergillus terreus*, Bioactive, GC-MS, Industrial Microbiology.

Corresponding Author: Dr. Malkhey Verma* Ph.D.

Department of Biochemistry and Microbial Sciences, Central University of Punjab Bathinda, Punjab, India.

1. INTRODUCTION

Statins are the secondary metabolites which are produced by some fungal strains and are widely employed for reducing elevated levels of cholesterol in blood plasma [1]. So, they are considered

the most effective and suitable compounds in the treatment of hypercholesterolemia (one of the deadliest diseases in the world) to reduce the risk of cardiovascular disease [2]. Lovastatin, a naturally occurring secondary metabolite is commonly found in foods such as red yeast rice, oyster mushroom, and Pu-erh, although in low concentration, and is primarily used for the cure of dyslipidemia and the prevention of heart-associated diseases as well. Lovastatin is also produced by some specific higher fungi such as *Aspergillus terreus*, *Pleorotus ostreatus,* and closely associated *Pleorotus* species. Lovastatin was discovered in the 1970s and was employed in clinical development as potential drugs for the purpose of lowering LDL cholesterol.

Mechanism of Action

Lovastatin is a potent inhibitor of 3-hydroxy-3-methyl glutaryl-Coenzyme A reductase (HMG CoA reductase) which catalyzes the conversion of mevalonic acid from HMG CoA. Lovastatin thus blocks the cholesterol biosynthesis pathway acting as a reversible competitive inhibitor for HMG CoA, binding to HMG CoA reductase which interferes with mevalonate production. It is also known that cholesterol biosynthesis requires mevalonate as a building block [3,4]. A series of more than 25 enzymatic reactions catalyze the biosynthesis of cholesterol, of which, three successive acetyl-CoA condensation reactions are involved initially to form the six-carbon compound HMG-CoA. This is followed by further reductions to produce mevalonate which again gets converted to isoprene (building blocks of squalene) through a series of reactions. These are the immediate precursors to sterols which undergo subsequent conversions to lanosterol and finally metabolized to form cholesterol. A major rate-limiting step in this biosynthetic pathway mainly arises at the level of the microsomal enzyme which is involved in the conversion of HMG CoA to mevalonate. This property has been considered as a primary target for several years for pharmacological intervention. Lovastatin is thus a prodrug, which has a native form of an inactive lactone in the closed ring structure of gamma-lactone which is basically administered. This further gets hydrolyzed *in vivo* to its active form, β-hydroxy acid, an open ring structure. Lovastatin was also extracted from the fungus Aspergillus terreus and the chemical changes of this fermentation derived drug such as Simvastatin and its microbial modification lead to the invention of drugs such as Pravastatin. It is a general belief that the initial pH value of the medium for producing lovastatin by *Aspergillus terreus* should be somewhat near 6.3 which has been unarguably mentioned in the literature. The fact that the pH value of the medium may change the metabolism of any microorganism and so may in *Aspergillus terreus* which produces itaconic acid (another important metabolite of this fungus widely employed in the chemical industry) at a considerably low pH from glucose as the sole carbon source while lovastatin is biosynthesized at a neutral pH [5]. Several papers regarding the influence of the cultivation media composition for the lovastatin production have already been published and an optimum carbon source has been widely sought by many authors [5-11]. Almost all of them concluded a slowly utilized carbon source such as glycerol or lactose was better assimilated for the

mevalonate biosynthesis than glucose. A variety of nitrogen sources has also been tested regarding the optimization of mevalonate biosynthesis. And it was found that complex nitrogen sources such as yeast extract, soybean meal, and corn steep liquor were more suitable than single amino acids, e.g. sodium glutamate or salts containing ammonium ions. These single amino acid sources are generally useless because they acidify the medium. The cause of this acidification is the release of hydrogen ions from fungal cells during the transport of ammonium ions in order to sustain the electroneutrality of the cells. This phenomenon can be frequently met in filamentous fungi [12] and in such conditions, generally, no lovastatin is synthesized irrespective of the type of ammonium salt used [13]. Apart from the type and concentration of both carbon as well as nitrogen sources, the ratio of carbon to nitrogen is another key factor influencing the synthesis of lovastatin. Casas *et al*. [9] reported that an increase of the C/N ratio from 14.4 to 41.3 in the lactose fed culture led to the doubling of the lovastatin titer.

Importance of Fungi

The exploitation of fungi by human beings for antibiotic production, food processing, and other purposes are substantial and has long past. They have been deployed since long because of their capacity to produce a vast range of natural products having anticancer activity, anti-inflammatory activity, immuno-modulatory activity, inhibition of neurological and bone disorders etc [14-16]. Also, a recent study confirmed lovastatin as a candidate to inhibit methanogenic archaea present in ruminants [17,18]. Fungi are considered to be the most potent microorganism for statin production. Some other fungi involved in the production of statins include *Monascus perpureu, M. anka, Aspergillus terreus, A. flavipes, A. fischeri, A. umbrosus, A. parasiticum, Acremonium chrysogenum, Byssoclamys fulva, Fusarium fujikuroi, Trichoderma longibranchiatum, T. viridae* and *Penicillium funiculosum* etc. [19,20].

Aspergillus terreus

Aspergillus terreus, also known as *Aspergillus terrestris*, is a saprophytic fungus prevalent in warmer climates and now known to be capable of sexual reproduction as well. They are commonly used in the industry for the purpose of production of enzymes like xylanase as well as organic acids such as itaconic acid and cis-aconitic acid. They also serve as an initial source for the drug lovastatin, a drug commonly used for lowering serum cholesterol. In filamentous fungi, one such as *Aspergillus terreus* the secondary metabolites are often produced following the phase of rapid growth (tropophase) during a subsequent production stage. Secondary metabolism, in this case, starts when one of the key nutrients like carbon (glucose), nitrogen or phosphate gets exhausted which initiates a stage with low or nil growth rate but high production rate [21-23]. Certain complex regulatory mechanisms are responsible for governing the gene functions in these species, such as induction by different environmental stimuli, carbon catabolite regulation, feedback regulation and others. Some of the broad domain transcription factors that mediate these regulatory mechanisms include AreA,

nitrogen regulation [24] and CreA, Carbon catabolite repressor [25]. In addition to these factors, many secondary metabolites, including lovastatin, are regulated through the global regulator of secondary metabolism Lae A [26]. Talking at the molecular level, the lovastatin biosynthetic gene cluster consists of 18 putative open reading frames (ORFs), among which lovE was annotated to encode a regulatory protein. The lovE, regulating lovastatin biosynthetic genes encodes a Zn2Cys6 type transcription factor and it is assumed to regulate the production of lovastatin at the transcriptional level. The biosynthesis cluster includes two types 'l' polyketide synthase genes: lovB and lovF. lovF encodes the lovastatin diketide synthase, enzyme that specifies the formation of 2-methyl butyrate and interacts closely with an additional transesterase (lovD) responsible for assembling lovastatin from this polyketide and monacolin J [27].

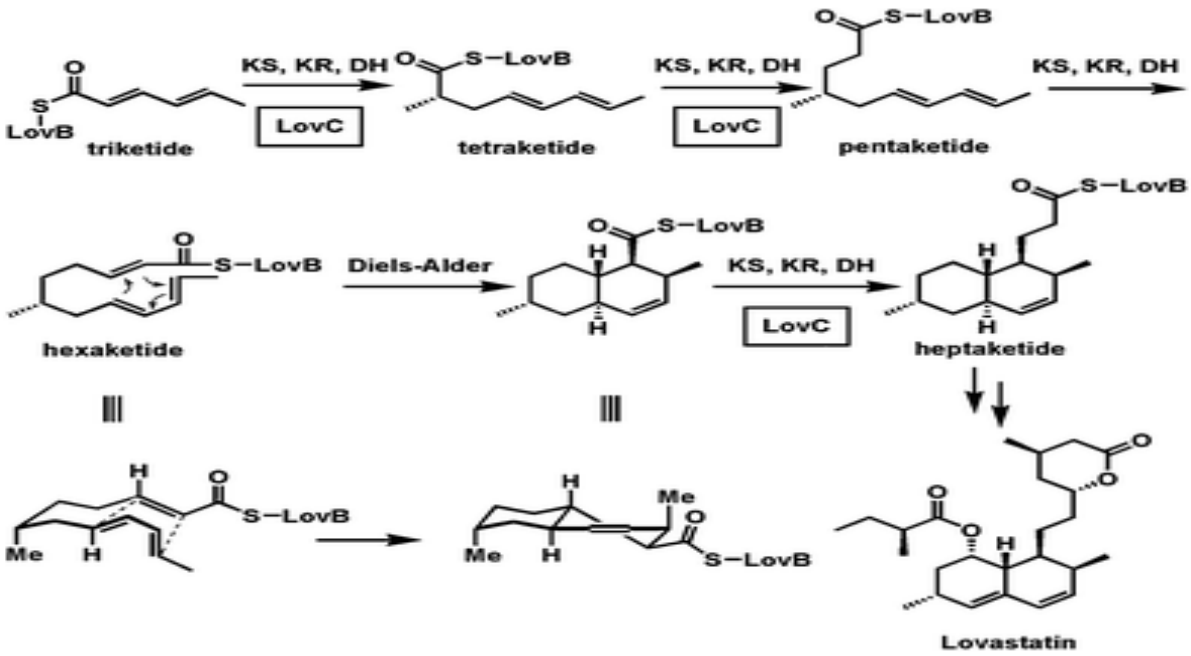

Figure 1: Lovastatin biosynthesis pathway

Despite having plenty of knowledge regarding the genes and the enzymes involved in the biosynthetic pathway, very little effort has been directed towards studying physiology and regulation of the lovastatin biosynthesis. However, from the optimization studies of the production medium, it is quite evident that lovastatin is carbon catabolite regulated, probably mediated by CreAp [13]. Hence, the onset of lovastatin biosynthesis, after the exhaustion of glucose can be attributed to relief from carbon catabolite repression and the change to lactose consumption during idiophase. In addition, the same evidence also indicates that lovastatin biosynthesis in *A. terreus* undergoes negative feedback regulation i.e. lovastatin inhibits its own biosynthesis [28]. However, certain uncharacterized factors or stimuli influencing the lovastatin biosynthetic genes were also revealed during studies on lovastatin biosynthesis in solid state fermentation (SSF). It was found that in SSF, the yield of the secondary metabolites was significantly higher as compared to that in submerged

fermentation (SmF) and it is thought to be due to different physiology displayed by the fungus in SSF. Searching for the environmental stimuli responsible for this higher lovastatin production rates, it was found that direct contact with the air was very important stimulus inducing the higher production, and considered that it's stimulating effect could be through oxidative stress or reactive oxygen species (ROS) formation [29].

2. MATERIALS AND METHODS

Microorganisms: The fungal strains of *Aspergillus terreus* NBRC (IFO) 31217 (Strain I) and *A. terreus* ATCC 11877 (Strain II) were bought from Microbial Type Culture Collection and Gene Bank (MTCC) housed at IMTECH Chandigarh, Punjab. The strains were supplied in the lyophilized powdered form.

Growth Medium: Czapek Yeast Extract Agar was recommended for the re-culturing and maintenance of *Aspergillus terreus*. The composition of the media was (g/l); Sucrose, 30; Yeast extract, 5; Dipotassium hydrogen phosphate (K_2HPO_4), 1; Sodium nitrate($NaNO_3$), 0.3; Potassium chloride (KCl), 0.050; Magnesium Sulphate ($MgSO_4$), 0.050; Ferrous Sulphate ($FeSO_4$), 0.001; Zinc Sulphate ($ZnSO_4$), 0.001; Copper Sulphate ($CuSO_4$), 0.0005; Agar, 15 and water to 1 litre. 51.40 grams of the above-mentioned trace elements was suspended in 1000 ml distilled water and heated to boiling to dissolve the medium completely. The pH was adjusted to 6.3 with 1 N HCl. It was sterilized by autoclaving at 15 lbs pressure (121ºC) for 15 minutes and then cooled down. It was mixed well and poured into sterile Petri plates. The lyophilized *A. terreus* cultures were revived by spreading after serial dilution and incubated at 25ºC which was the suggested optimum growth temperature for the strains. After 5 days of incubation (suggested incubation time for that particular strain), we obtained a clear growth of the fungus on the plates.

Study of Growth Curve

We took twelve 50 ml Erlenmeyer flasks which were properly sterilized. Then we poured 25 ml of the prepared growth media i.e. Czapek yeast extract media into those sterilized flasks. Following pouring of the medium, each of them was inoculated with an equal volume of seed culture of *A. terreus* which were already grown in the shake flask. Each of the flasks was properly marked for an incubation period interval of eight hours as 8, 16, 24, 32……..96 hrs respectively. Then the flasks were placed in the shaker incubator at 150 rpm and the temperature of 27 ºC. After incubation of 8 hrs, the first flask was taken out and its content was poured into a 50 ml falcon tube. It was centrifuged at 14,000rpm for 15 minutes. The same process was repeated for each of the flasks taken out at a regular interval of eight hours. The supernatant was discarded and the pellets were washed with milli-Q water and dried in the hot air oven for 24 hrs at 40-50 ºC. The dry cell biomass of each of the flasks was weighed and dry biomass were recorded, values were plotted on an excel sheet to obtain the growth profile to understand the different phases of growth and further use this

information during fermentation for the production of lovastatin. The whole process was repeated with the other strain of *A. terreus* as well. Growth curve experiments were repeated thrice.

Fermentation: The fermentation experiments were carried out in 1 litre Erlenmeyer flasks containing 250 ml of the fermentation medium consisting of (g/l); Sucrose, 30; Yeast extract, 5; Dipotassium hydrogen phosphate (K_2HPO_4), 1; Sodium nitrate (NaNO3), 0.3; Potassium chloride (KCl), 0.050; Magnesium Sulphate ($MgSO_4$), 0.050; Ferrous Sulphate ($FeSO_4$), 0.001; Zinc Sulphate ($ZnSO_4$), 0.001; Copper Sulphate ($CuSO_4$), 0.0005; Agar, 15 and water to 1 litre. 51.40 grams of the above-mentioned trace elements was suspended in 1000 ml distilled water and heated to boiling to dissolve the medium completely [30]. The pH was adjusted to 6.3 [31] with 1 N dilute HCl. It was sterilized by autoclaving at 15 psi pressure (121ºC) for 15 minutes. In addition to this, 2.5% corn steep liquor, as a source of nitrogen as well certain important amino acids was also added to the fermentation medium. The fermentation process was carried out in two different 1 litre Erlenmeyer flasks for the two separate strains of *A. terreus*. The fermentation medium was cooled after autoclaving and was inoculated with seed culture of 48 hrs old. Fermentation flasks were then incubated in a rotary shaker incubator at 200 rpm at 27 ºC for 7 days. Apart from these two flasks, another fermentation batches were set up in a 1 litre Erlenmeyer flask with the same fermentation medium composition containing 225 ml of fermentation medium. But this time instead of corn steep liquor, 25 ml of fully toned milk was added to the fermentation medium making the volume up to 250 ml. Flasks were inoculated with seed culture of both the strains used earlier (strain I & II), it was also placed in the rotary shaker incubator provided the same conditions. After completion of the fermentation, the fermentation broth and fungal mycelium were separated by centrifugation. These fermentation experiments were repeated multiple times on different days.

Extraction of statin from fermentation broth

The fermentation broth along with the fungal mycelium was centrifuged at 10,000 rpm for 10 mins and supernatant was separated which was further used for the extraction of statin in ethyl acetate: water mixture (1:1, v/v) in a 500ml Erlenmeyer flasks keeping the flask in a rotary shaker at 200 rpm for 2 hrs. After 2 hrs of shaking, the flasks were kept static for some time and we observed the formation of two separate layers. The upper layer that is the ethyl acetate containing the desired secondary metabolite was separated in a 500 ml round bottom flask. The samples collected were further evaporated to dryness in a rotary evaporator keeping the temperature to 45ºC. The residue was dissolved in 1 ml methanol and filtered through a 0.2 µm syringe filter. It was stored at 4ºC for 24h before being used for GC-MS. The fungal mycelium left as debris after centrifugation was oven dried at 40ºC for 24h and the dry cell biomass were weighed for all the flasks.

Spectral analysis of lovastatin as well as other bioactive chemical compounds using gas chromatography-mass spectrometry (GC/MS) technique

The analysis was conducted using GC-MS (Agilent 789 A) equipped with a DB-5MS column (30 mm×0.25 mm i.e., 0.25 µm film thickness, J&W Scientific, Folsom, CA). The column oven temperature was programmed at 40ºC. Helium was used as the carrier gas at the rate of 1.0 mL/min. The effluent of the GC column was introduced directly into the source of the MS via a transfer line (260ºC). Ionization voltage was 70eV and ion source temperature was 200ºC. Scan range was 50-800 amu. After GC-MS separation, all the peaks were equated with the structural library of the secondary metabolite compounds to determine the expected compounds. The identification of the components was also based on a comparison of their mass spectra with those of NIST mass spectral library as well as on comparison of their retention indices either with those of authentic compounds or with literature values for the purpose of estimation of any such bioactive chemical compounds produced.

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3. RESULTS AND DISCUSSION

GC-MS Analyses of fermentation broth extract

The observations of the GC-MS analysis of the methanolic extract of given strains of the fungus *A. terreus* showed the production of lovastatin found in the broth extract of both wild-type strains used. Although few other important bioactive and commercially-valuable compounds were found in the GC-MS analysis (shown in Figure 2, 3 and 4). Few of them are described below:

Isovaline ($C_5H_{11}NO_2$): Isovaline, which is similar in structure to the chief inhibitory neurotransmitters (GABA and Glycine) in the mammalian CNS, is one of the rare amino acids which was brought to the earth by the Murchison meteorite in 1969 landing in Australia. Isovaline acts as an analgesic in mice by the virtue of its capability to activate peripheral $GABA_B$ receptors. In a study with a mouse model of osteoarthritis, isovaline was found to restore mobility. This novel compound has the ability to treat acute and chronic pain, without any negative side effects which are generally found with other commonly used analgesics.

Silane: Silane is a colourless inorganic compound having general formula SiH_4. It is a pyrophoric gas with a sharp repulsive smell. It has got several medical as well as industrial applications: it is commonly used in dentistry as a tooth-coloured substance for the filling of teeth; it acts as a coupling agent used to adhere carbon fibers and glass fibers to some kind of polymer matrices. It is also deployed in supersonic combustion ramjets to start-up the combustion process in the compressed air stream. Apart from all these, some of its other applications include water-repellent, masonry protection, control of graffiti etc.

2-oxo-n-valeric acid: 2-oxo-N-valeric acid having general formula $C_5H_8O_3$ is a keto-acid that is usually found in human blood serum and urine. But unlike some of the other keto-acids, this metabolite is not an intermediate or associated with amino acids. Its origin is still unknown.

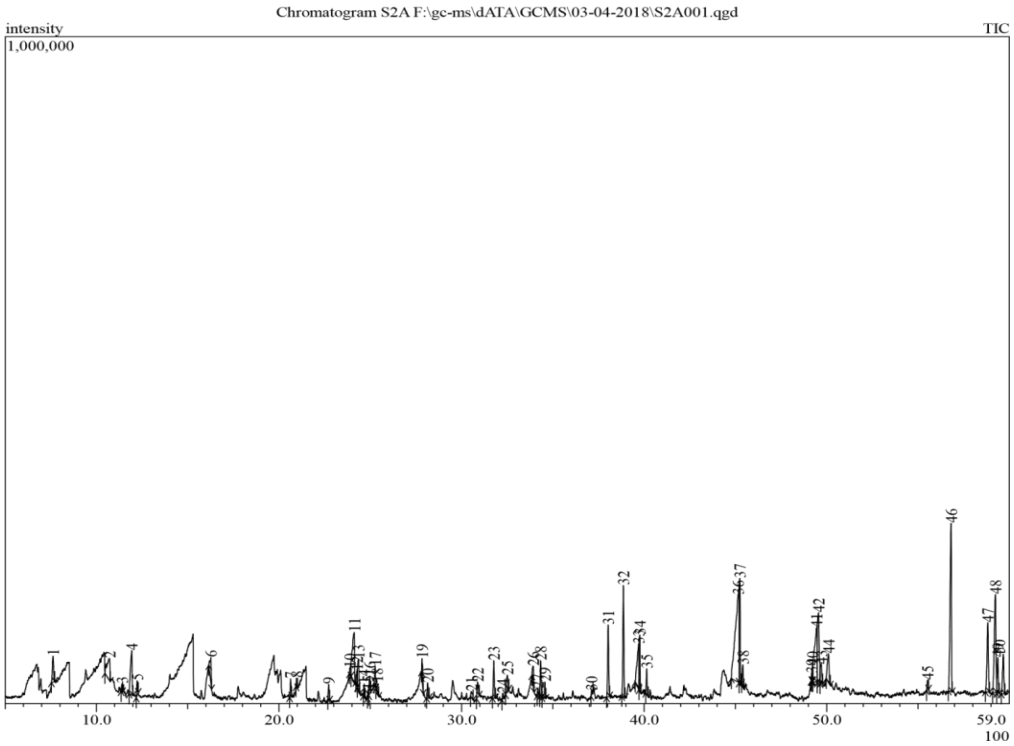

Figure 2: GC-Chromatogram showing retention times of different components of sample mixture (SIIA) of *A. terreus* strain II.

Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	7.617	7.533	7.717	190930	1.36	38768	1.53	4.92		2,2-Dimethylthiirane
2	10.714	10.458	10.758	325669	2.32	33215	1.31	9.80		Butanoic acid, 2-methyl-
3	11.398	11.350	11.425	32135	0.23	12049	0.48	2.67		Pentanoic acid
4	11.927	11.783	12.000	423335	3.02	66424	2.62	6.37		Isovaline, 3-hydroxy-
5	12.229	12.175	12.283	78338	0.56	21623	0.85	3.62		1,2,4-Trithiolane, 3,5-bis(1-methyleth
6	16.260	16.150	16.300	158189	1.13	37941	1.50	4.17		Butanoic acid, 2-hydroxy-3-methyl-
7	20.630	20.575	20.683	78175	0.56	27228	1.08	2.87		4-Dodecene, (E)-
8	20.960	20.925	21.008	29306	0.21	13284	0.52	2.21		Decane
9	22.718	22.675	22.775	66080	0.47	23116	0.91	2.86		Benzene, 1,3-bis(1,1-dimethylethyl)-
10	23.875	23.850	23.933	57292	0.41	9352	0.37	6.13		1,5-Hexadien-3-yne, 2-methyl-
11	24.115	23.933	24.158	650844	4.64	75986	3.00	8.57	V	Cycloheptatrienylium, iodide
12	24.267	24.200	24.292	89767	0.64	22088	0.87	4.06		Butyric acid, 2,2-dimethyl-, vinyl este
13	24.345	24.292	24.417	207036	1.48	46479	1.84	4.45	V	Acetic acid, 3,4-dihydroxy-3-methyl-l
14	24.654	24.608	24.708	52219	0.37	19689	0.78	2.65		5-Tridecene, (Z)-
15	24.875	24.842	24.917	29834	0.21	10072	0.40	2.96		Silane, tetramethyl-
16	24.959	24.917	25.025	93816	0.67	26785	1.06	3.50	V	5-Tridecene, (Z)-
17	25.259	25.208	25.308	98623	0.70	28996	1.15	3.40		5-Tridecene, (Z)-

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
18	25.367	25.308	25.458	62120	0.44	13404	0.53	4.63	V	Methylmalonic acid
19	27.830	27.792	27.900	117262	0.84	30984	1.22	3.78		4-Tridecene, (Z)-
20	28.137	28.083	28.217	78171	0.56	22710	0.90	3.44		Tridecane
21	30.523	30.492	30.633	51816	0.37	8477	0.33	6.11		2-Methyl-2,4-dimethoxybutane
22	30.878	30.808	30.967	113291	0.81	22441	0.89	5.05		n-Heptyl hexanoate
23	31.760	31.692	31.850	207404	1.48	56160	2.22	3.69		Phenol, 3,5-bis(1,1-dimethylethyl)-
24	32.242	32.208	32.408	24851	0.18	8198	0.32	3.03		5-Tridecene, (Z)-
25	32.500	32.408	32.583	152909	1.09	24777	0.98	6.17	V	Imidazole, 1-benzyl-2-(4-nitrophenyl]
26	33.906	33.842	33.967	113439	0.81	26956	1.06	4.21		n-Hexadecanoic acid
27	34.167	34.133	34.275	49573	0.35	10758	0.42	4.61		2-Oxo-n-valeric acid
28	34.325	34.275	34.400	202202	1.44	54174	2.14	3.73	V	2-Tridecene, (E)-
29	34.558	34.400	34.617	78243	0.56	24511	0.97	3.19	V	Octadecane, 6-methyl-
30	37.108	37.083	37.200	19326	0.14	6700	0.26	2.88		Dodecane, 1-fluoro-
31	38.027	37.942	38.108	417505	2.98	108043	4.27	3.86		Cyclopropylphenylmethane
32	38.861	38.767	38.950	651111	4.64	167501	6.61	3.89		Styrene
33	39.692	39.508	39.708	455846	3.25	67105	2.65	6.79		1,3-Pentanediol, 4-methyl-2-nitro-
34	39.755	39.708	39.825	309643	2.21	84195	3.32	3.68	V	n-Hexadecanoic acid
35	40.135	40.075	40.225	128414	0.92	38404	1.52	3.34		2-Tridecene, (Z)-
36	45.142	44.767	45.192	1861554	13.27	130849	5.17	14.23		t-Butyl-(1,2-dimethylpent-3-enyloxy)
37	45.238	45.192	45.325	614046	4.38	157170	6.21	3.91	V	n-Hexadecanoic acid
38	45.401	45.342	45.458	107676	0.77	31500	1.24	3.42		Pentadecanoic acid, 2,6,10,14-tetram
39	49.167	49.142	49.183	20837	0.15	11383	0.45	1.83		Chloroacetic acid, 4-octyl ester
40	49.208	49.183	49.242	78207	0.56	22792	0.90	3.43	V	Methyl 12,13-octadecadienoate
41	49.433	49.242	49.475	839810	5.99	81921	3.24	10.25	V	Cyclohexanebutanoic acid
42	49.543	49.475	49.633	685759	4.89	105028	4.15	6.53	V	Hexadecenoic acid, Z-11-
43	49.708	49.633	49.775	155914	1.11	28815	1.14	5.41	V	E-11-Hexadecenoic acid, ethyl ester
44	50.094	49.967	50.192	267414	1.91	42659	1.68	6.27		n-Hexadecanoic acid
45	55.516	55.458	55.575	51793	0.37	14376	0.57	3.60		1,4-Methano-1H-cyclopenta[d]pyrida
46	56.805	56.658	56.875	1384087	9.87	252530	9.97	5.48		Benzene, (5-iodopentyl)-
47	58.820	58.692	58.908	570338	4.07	104366	4.12	5.46		Benzene, (2-iodoethyl)-
48	59.237	59.083	59.308	955112	6.81	147328	5.82	6.48		Benzene, (2-iodoethyl)-
49	59.363	59.308	59.450	231353	1.65	53399	2.11	4.33	V	Benzene, (2-iodoethyl)-
50	59.666	59.567	59.758	307141	2.19	59584	2.35	5.15		Benzene, (2-iodoethyl)-
				14025755	100.00	2532293	100.00			

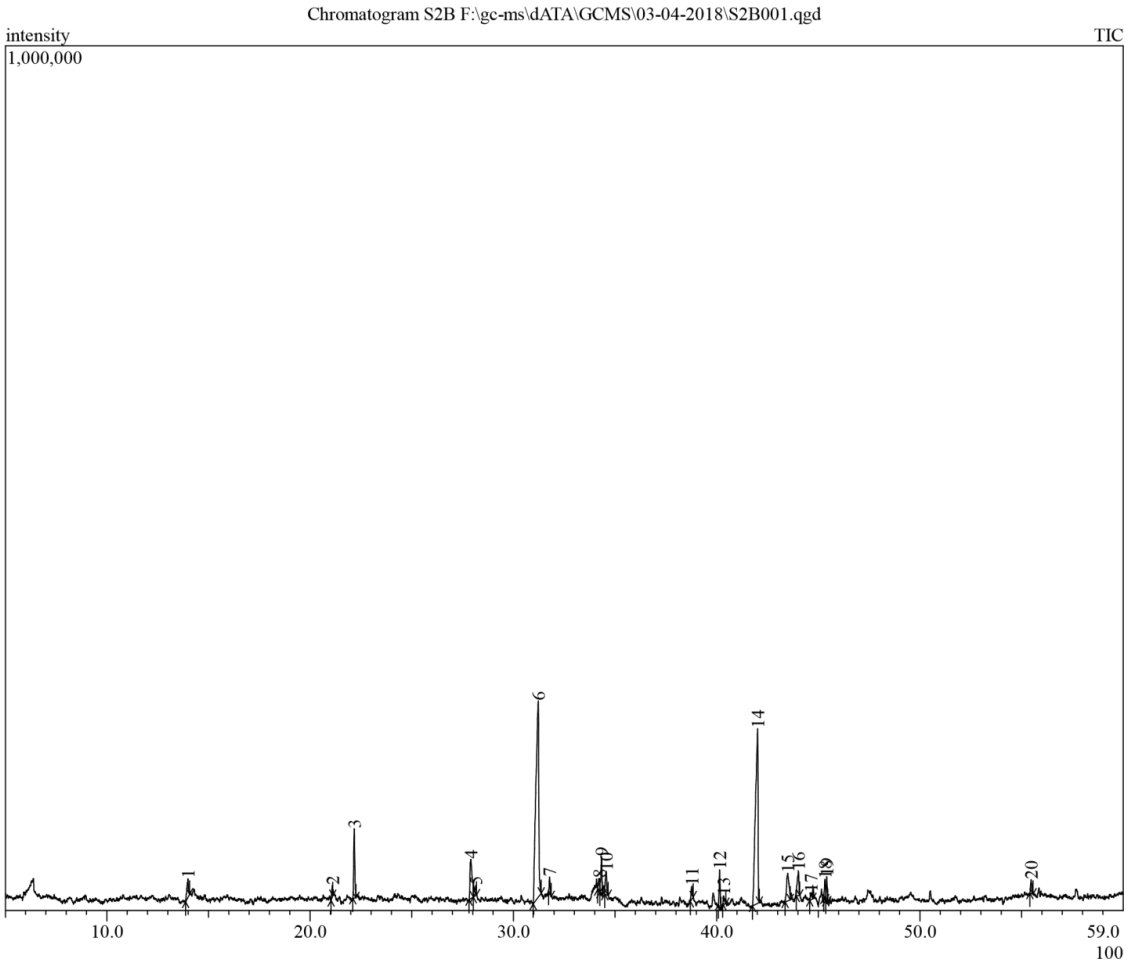

Figure 3: GC-Chromatogram showing retention times of different components of sample mixture (SIIB) of A. terreus strain II.

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Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	13.986	13.867	14.058	116706	1.86	21178	2.33	5.51		3,4-Dimethyldihydrofuran-2,5-dione
2	21.089	21.017	21.108	43581	0.69	12947	1.42	3.37		2(3H)-Furanone, 5-ethoxydihydro-
3	22.163	22.092	22.250	304196	4.84	80242	8.83	3.79		2,4,6-Cycloheptatrien-1-one
4	27.901	27.817	28.017	328815	5.24	45543	5.01	7.22		1,3-Benzenediol, 2-methyl-
5	28.142	28.017	28.183	84209	1.34	11724	1.29	7.18	V	Dodecane
6	31.219	30.967	31.367	2249588	35.82	226932	24.96	9.91		2,3-Dimethylhydroquinone
7	31.774	31.725	31.858	57290	0.91	18640	2.05	3.07		Phenol, 3,5-bis(1,1-dimethylethyl)-
8	34.221	34.133	34.250	72617	1.16	15285	1.68	4.75	V	Cyclohexanone, 2-(2-propenyl)-
9	34.331	34.250	34.383	160163	2.55	43616	4.80	3.67	V	2-Tridecene, (E)-
10	34.559	34.492	34.658	131694	2.10	27344	3.01	4.82	V	Decane
11	38.769	38.700	38.842	62567	1.00	14987	1.65	4.17		Benzoic acid, 2,4-dihydroxy-6-methy
12	40.143	40.075	40.208	149084	2.37	43652	4.80	3.42		2-Tridecene, (E)-
13	40.334	40.292	40.450	47099	0.75	9322	1.03	5.05		Heptadecane, 2,6,10,14-tetramethyl-
14	42.011	41.758	42.100	1753754	27.93	201833	22.20	8.69		2H-Benzocyclohepten-2-one, 3,4,4a,5
15	43.493	43.358	43.617	269180	4.29	31786	3.50	8.47		2-Heptyn-1-ol
16	44.017	43.917	44.083	152008	2.42	28594	3.14	5.32	V	Pyrrolo[1,2-a]pyrazine-1,4-dione, hex
17	44.625	44.575	44.725	53982	0.86	8240	0.91	6.55		Bicyclo[4.1.0]heptane,-3-cyclopropyl

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
18	45.323	45.258	45.367	88639	1.41	23133	2.54	3.83		7-Octen-2-ol, 2-methyl-6-methylene-
19	45.411	45.367	45.467	89446	1.42	28049	3.08	3.19	V	2-Tetradecene, (E)-
20	55.465	55.408	55.558	64859	1.03	16171	1.78	4.01		Cyclopenta[e]-1,4-benzoazepine, 1-[2
				6279477	100.00	909218	100.00			

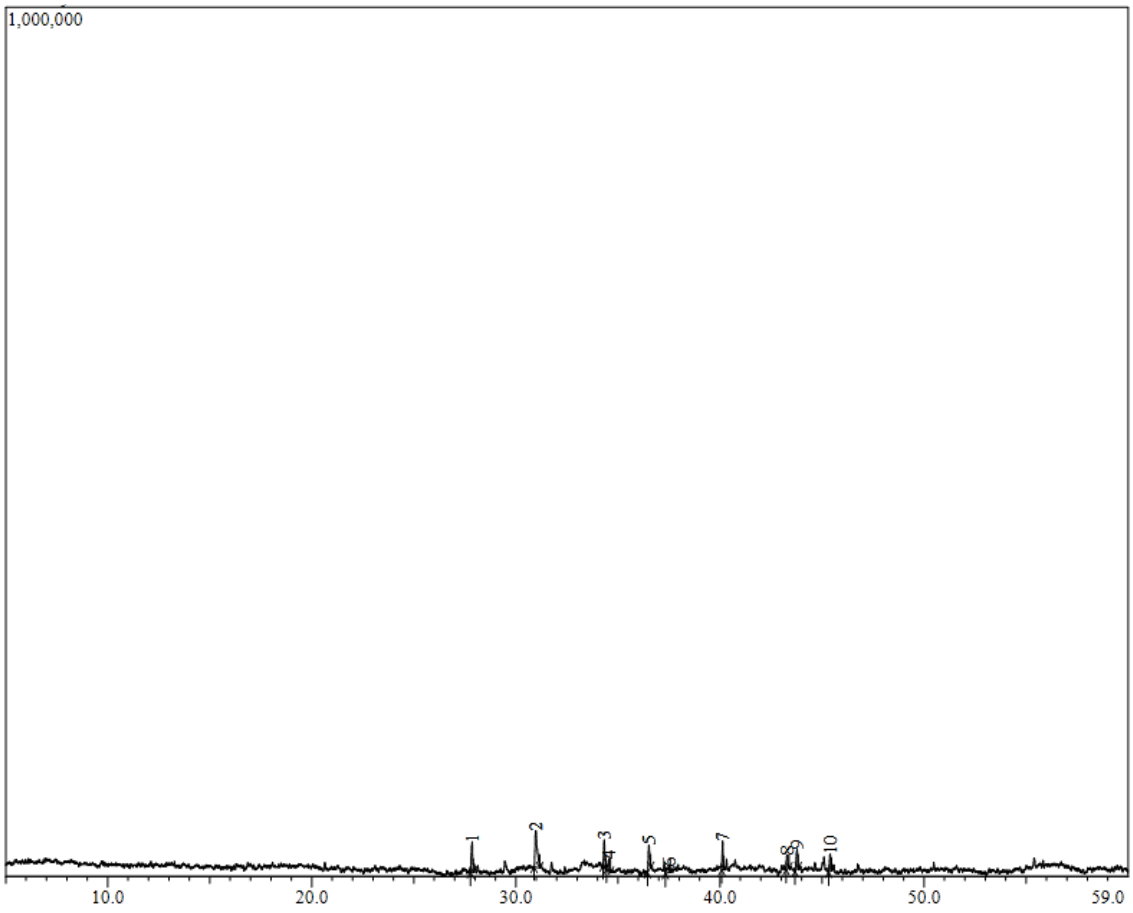

Figure 4: GC-Chromatogram showing retention times of different components of sample mixture of *A. terreus* strain I.

Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	27.851	27.767	27.908	162614	13.04	34929	13.97	4.66		4-Trifluoroacetoxytetradecane
2	30.969	30.883	31.167	324780	26.05	42112	16.84	7.71		2,5-Cyclohexadiene-1,4-dione, 3-hyd
3	34.322	34.258	34.392	124618	9.99	30790	12.31	4.05		2-Tridecene, (Z)-
4	34.547	34.433	34.625	62132	4.98	14062	5.62	4.42	V	Heptadecane, 2,6,10,14-tetramethyl-
5	36.511	36.450	36.608	135401	10.86	29068	11.62	4.66		2,5-Cyclohexadiene-1,4-dione, 3-hyd
6	37.508	37.317	37.567	63032	5.06	7237	2.89	8.71		Decane, 2,9-dimethyl-
7	40.123	40.075	40.192	109459	8.78	33048	13.22	3.31	V	2-Tetradecene, (E)-
8	43.286	43.233	43.375	77640	6.23	15985	6.39	4.86	V	Pyrrolo[1,2-a]pyrazine-1,4-dione, hex
9	43.774	43.675	43.833	109024	8.74	22933	9.17	4.75		Pyrrolo[1,2-a]pyrazine-1,4-dione, hex
10	45.397	45.317	45.458	78113	6.27	19888	7.95	3.93		2-Tetradecene, (E)-
				1246813	100.00	250052	100.00			

4. CONCLUSION

In the current study, two trains of *Aspergillus terreus* ATCC 11877 and *A. terreus* NBRC(IFO) 31217 were used for the production of lovastatin; a cholesterol-lowering drug. Although the lovastatin production was observed, the present study also provides new insight into the production of other metabolites of pharmaceutical importance of high-value secondary metabolites. Isovaline ($C_5H_{11}NO_2$) and silane production observed in the current study are novel fermentation products and not reported in the literature before using *Aspergillus terreus*. The production of lovastatin, isovaline, 2-oxo-n-valeric acid and silane production could further be optimized using statistical optimization methods for fermentation, genome-scale metabolic modelling and metabolic engineering for strains improvement.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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