Production and Optimization of Lovastatin by Microbial Fermentation using *Aspergillus terreus*

Project submitted to
Central University Of Punjab

For the award of
Master of Science

in

Biochemistry and Microbial Sciences

By

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Supervisor

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May 2018

DECLARATION

I declare that the project report entitled "**Production and Optimization of Lovastatin by Microbial Fermentation using *Aspergillus terreus*"** has been prepared by me under the guidance of Dr. Malkhey Verma, Associate Professor, Department of Biochemistry and Microbial Sciences, School of Basic and Applied Sciences, Central University of Punjab. No part of this project has formed the basis for the award of any degree or fellowship previously.

Rohit Raj

Department of Biochemistry and Microbial Sciences,

School of Basic and Applied Sciences

Central University Of Punjab, Bathinda-151001

Date:

CERTIFICATE

I certify that Rohit Raj has prepared his project report entitled **"Production and Optimization of Lovastatin by Microbial Fermentation using *Aspergillus terreus*",** for the award of M.Sc. degree of the Central University of Punjab, under my guidance. He has carried out this work at Department of Biochemistry and Microbial Sciences, School of Basic and Applied Sciences, Central University of Punjab.

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ABSTRACT

Production and Optimization of Lovastatin by Microbial Fermentation using *Aspergillus terreus.*

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In the recent times, the fungus *Aspergillus (A.) terreus* has been highly popularized regarding its domination for the 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. Bioactives (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 extraction of the natural statins such as lovastatin or mevastatin from *A. terreus* is seen as one of the major breakthrough in the field of Industrial Microbiology/Fermentation Technology. Here we report the *Aspergillus terreus* NBRC (IFO) 31217 (Strain I) and ATCC 11877 (Strain II) don’t produce lovastatin but they produce important bioactive compounds of high commercial value like Isovaline ($C_5H_{11}NO_2$) and Silane etc.

Rohit Raj Dr. Malkhey Verma

ACKNOWLEDGEMENT

I at this moment declare that the work I have done would not have been possible without the keen assistance of my supervisor Dr. Malkhey Verma, Associate Professor, Department of Biochemistry and Microbial Sciences. He guided me through every step of my project with full interest.

I also desire to express my sincere gratitude to Prof. Ramakrishna Wusirika, HoD, Department of Biochemistry and Microbial Sciences, for providing me with the lab facility. I express my heartfelt thanks to Mr. Sonu Gupta (Ph.D. Scholar) and other Ph.D. Scholars for their valuable guidance and timely suggestions during the entire period of my project work. Mr. Deepak Kumar (Lab Assistant) was also very helpful for providing necessary requirements without which this work would not have been completed. And at last, I would like to extend my gratitude to Gaurav Chambyal and my other batchmates for helping me throughout my dissertation work.

Rohit Raj

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INTRODUCTION

Statins are the secondary metabolites which are produced by some fungal strains and are widely employed for reducing the raised cholesterol levels in blood plasma (Alberts,1988). So, they are considered the most efficacious and appropriate compounds for the cure of hypercholesterolemia (one of the deadliest diseases in the world) to reduce the risk of cardiovascular disease (Goldstein and Brown,1984). 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.

Structure of Lovastatin:

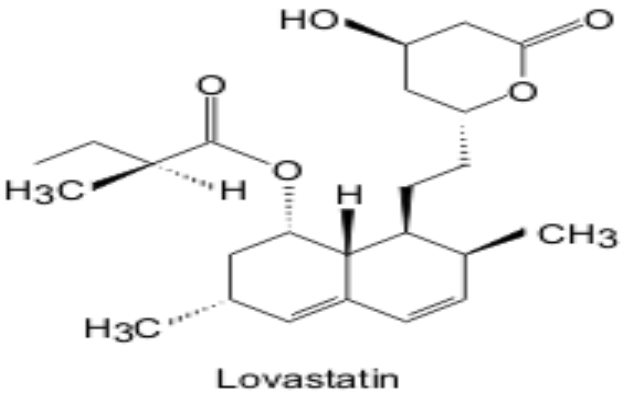

Fig 1.1: Chemical structure of Lovastatin

1.1 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 functioning as a reversible competitive inhibitor for HMG CoA, which binds itself to HMG CoA reductase and thus interferes with mevalonate production. It is also known that cholesterol biosynthesis requires mevalonate as a building block.

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 isoprenes (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 modification of this fermentation based drug yielded certain other such as Simvastatin and its further microbial modification lead to the invention of drugs such as Pravastatin.

Knowledge gaps:

A limited number of strains of *Aspergillus terreus* are known for the production of lovastatin. Also, the statistical methods used for the optimization, of lovastatin production are still, lacking for the predictable production.

Objective:

1. Production and optimization of lovastatin by microbial fermentation using *Aspergillus terreus.*
2. Analyze the bioactive chemical products, if any produced, through GC-MS technique.

Hypothesis:

Although, not all of the wild strains of *A. terreus* are capable of producing lovastatin, few wild strains of the fungus have been reported (ATCC 20542 and KMO17963) regarding the production of lovastatin which is an important secondary metabolite as well as a highly potent cholesterol-lowering drug. Apart from this, certain essential bioactive chemical products which have antibacterial and antifungal capabilities can also be produced by some of the wild strains of *A. terreus*. In the current study, we are trying to find new strains. Further, to make medium components selection and optimization predictable for the production of lovastatin by using existing genome-scale metabolic model of *Aspergillus terreus.*

REVIEW OF LITERATURE

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 (Lai *et al*., 2007).

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 (Sitaram Kumar *et al*.,2000; Casas Lopez *et al*.,2003, Lai *et al*.,2003). 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 (Nielsen,1992). And in such conditions, generally, no lovastatin is synthesized irrespective of the type of ammonium salt used (Hajjaj *et al*.,2011).

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 Lopez *et al*.(2003) reported that an increase of the C/N ratio from 14.4 to 41.3 in the lactose fed culture led to doubling of the lovastatin titer.

Importance of Fungi:

The exploitation of fungi by human beings for antibiotic production, food preparation, and other purposes is substantial and has a long past. Because of their capacity to produce a vast range of natural products with an antibiotic, antifungal or other biological activities, they have been deployed since long and are being modified for industrial production of vitamins, antibiotics, anti-cancer and cholesterol-lowering drugs.

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, Penicillium funiculosum* etc.

***Aspergillus terreus*:**

Aspergillus terreus, which is also popular by the name *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 xylanose 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(Barrios-Gonzalez *et al*.,2005).

Certain complex regulatory mechanisms are responsible for governing the gene functions in these species. These mechanisms include 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; Marzluf 1997), CreA (Carbon catabolite repressor; Espeso and Penalva 1992). In addition to these factors, lovastatin as well as many other secondary are regulated through the global regulator of secondary metabolism Lae A (Bok & Keller, 2004).

Talking at the molecular level, the biosynthetic gene cluster of lovastatin comprises of 18 apparent open reading frames (ORFs), among which lovE was designated to encode a regulatory protein. The lovE, regulating lovastatin biosynthetic genes encodes a Zn2Cys6 type transcription factor and it is believed to regulate the production of lovastatin at the transcriptional level. Apart from this, the lovastatin biosynthesis cluster mainly comprises of two polyketide synthase genes: lovB and lovF. Among which lovF is known to encode the lovastatin diketide synthase which is an enzyme that specifies the genesis of 2-methyl butyrate and intercommunicates closely with an additional transesterase (lovD) which facilitates the assembling of lovastatin from this polyketide and monacolin J (Kennedy et al.,1999).

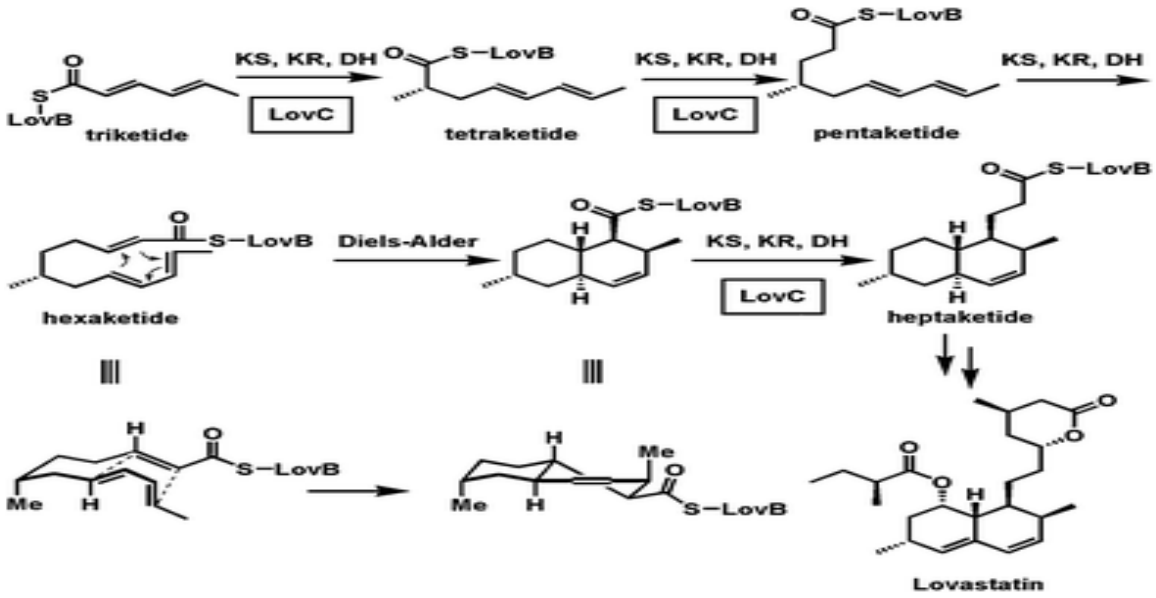

Figure 2.1: Lovastatin biosynthesis pathway

*__Source of figure__- Walsh, C.T. and Wencewicz, T.A. (2013). Flavoenzymes: Versatile catalysts in biosynthetic pathways. *Natural Product Reports*-Issue 1.

Despite having plenty of knowledge regarding the genes and the enzymes involved in the biosynthetic pathway, the efforts that has been made towards studying physiology and regulation of the lovastatin biosynthesis is not appreciable. However, from the optimization studies of the production medium, it is quite obvious that lovastatin is regulated by a carbon catabolite, which is probably mediated by CreAp (Hajjaj *et al*.,2001). Hence, the onset of lovastatin biosynthesis, after the exhaustion of glucose, can be accredited to relief from carbon catabolite repression, and the transformation to lactose consumption during idiophase. Apart from this, some evidence also suggest that the process of lovastatin biosynthesis in *A. terreus* under the control of negative feedback regulation i.e. lovastatin inhibits its own biosynthesis (Jia *et al*.,2010).

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 presumed to be like this by the virtue of 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 a highly significant stimulus inducing the higher yields, and considered that oxidative stress or reactive oxygen species (ROS) formation could be the possible factors responsible for its stimulating effect.

MATERIALS AND METHODS

3.1 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.

3.2 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 dilute 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.

3.3 Revival of the Culture:

Six 2 ml Eppendorf tubes were taken and 900 µl of distilled water was pipetted to each of them. Now we took a small quantity of the powdered strain of *A. terreus* with the help of an inoculating loop and added it to the first tube and it was vortexed well. Now each of the following tubes was serially diluted (100 µl) and

vortexed properly. Then we took Petri plates in which the media was poured and streaked each of the plates separately with the prepared culture from the Eppendorf tubes. Then they were placed in the incubator and temperature was set to 25ºC which was the suggested optimum growth temperature for that particular strain. The same process was repeated with the other strain of fungus as well.

3.4 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 flasks. Following pouring activity, each of them was inoculated with the live culture of *A. terreus* which were already grown on the Petri plates. Each of the flasks was properly marked for an incubation period interval of eight hours as 8h, 16h, 24h, 32h……..96h. Then the flasks were placed in the shaker incubator at 150 rpm and the given temperature was 25 ºC. After an incubation of 8hrs, the first flask was taken out and its content was poured in 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 regular interval of eight hours. The supernatant was discarded and the pellets were oven dried for 24h at 40 ºC. The dry cell biomass of each of the flasks was weighed and its values were plotted on an excel sheet to obtain a graph for further study of the growth curve obtained. The whole process was repeated with the other strain of *A. terreus* as well.

3.5 Shake flask fermentation

Fermentation batch: The fermentation process was performed in 500 ml 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. The pH was adjusted to 6.3 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 500 ml Erlenmeyer flasks for the two separate strains of *A. terreus*. The fermentation medium was cooled after autoclaving and was inoculated with the different strains of *A. terreus* grown earlier on Petri plates with the help of an inoculating loop. Fermentation flasks were further kept at incubation in a rotary shaker incubator for 7 days provided the conditions, 200 rpm at 27 ºC.

Apart from these two flasks, another fermentation batch was set up in a 250 ml Erlenmeyer flask with the same fermentation medium composition containing 90 ml of fermentation medium. But this time instead of corn steep liquor, 10 ml of fully toned milk was added to the fermentation medium making the volume up to 100 ml. Then after inoculation with one of two strains used earlier (strain II was used), it was also placed in the rotary shaker incubator provided the same conditions. After the accomplishment of the of the fermentation process, the fermentation broth as well as fungal mycelium were separated by centrifugation.

3.6 Extraction of statin from fermentation broth:

The fermentation broth along with the fungal mycelium was centrifuged for 10 minutes at 10,000 rpm following which, the supernatant was separated which was further utilized for the isolation of statin in ethyl acetate: water mixture (1:1, v/v) in a 500 ml Erlenmeyer flask keeping the flask in a rotary shaker at 200 rpm for 2 hrs. After 2 hrs of shaking, the flasks were kept constant for some time and we could observe 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 45 ºC. The residue was dissolved in 1 ml methanol and filtered through a 0.2 µm syringe filter. It was stored at 4 ºC for 24h before being used for GC-MS. The fungal mycelium left as debris after centrifugation was oven dried at 40 ºC for 24h and the dry cell biomass was weighed.

3.7 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 40 ºC. 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 (260 ºC). Ionization voltage was 70 eV and ion source temperature was 200 ºC. 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 compound. 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.

RESULT

4.1 After 5 days of incubation, we obtained a clear growth of the fungus on the petri plates as shown in the figure below:

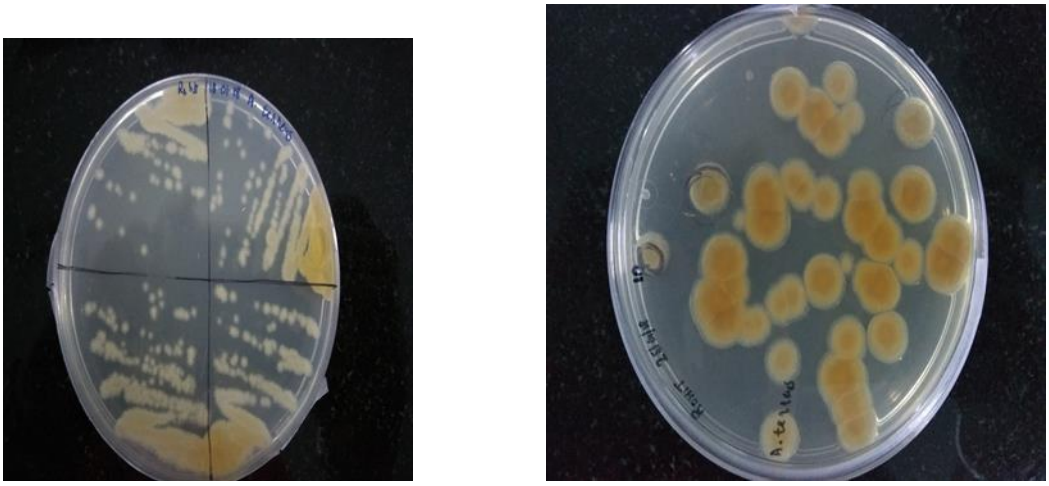

Figure 4.1: Revived culture of *A. terreus* after incubation of five days.

4.2. The dry cell biomass obtained as a fermentation byproduct was centrifuged and oven dried.

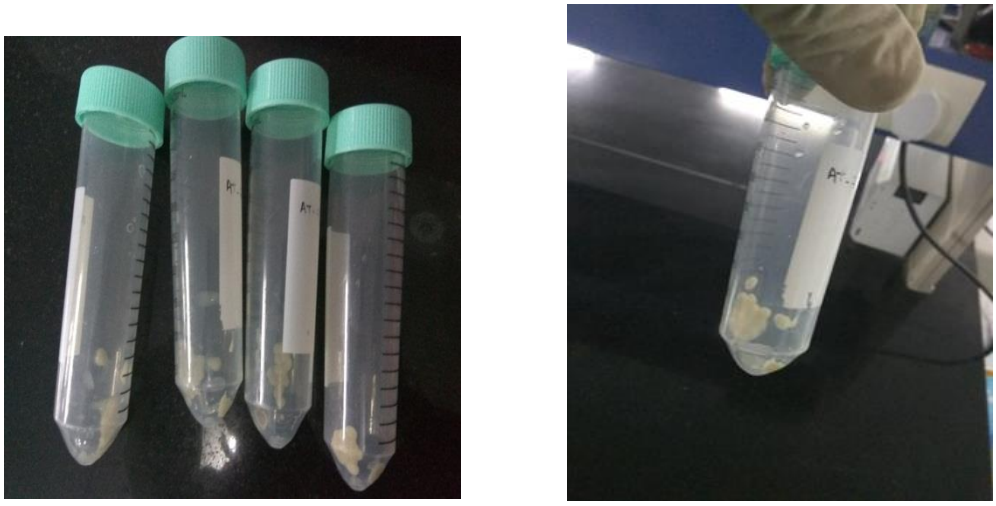

Figure 4.2: Dry cell biomass for the study of the growth curve

4.3 The growth curve of the fungus was studied and plotted on an excel sheet to obtain the following table and graph:

Incubation Time (Hrs)	Total Weight (Dry Cell Biomass)
0	0.0134
8	0.0242
16	0.0624
24	0.0483
32	0.0625
40	0.0062
48	0.1059
56	0.2019
64	0.0228
72	0.0223

Table 1: Time course measurement of the dry cell mass of *A. terreus*

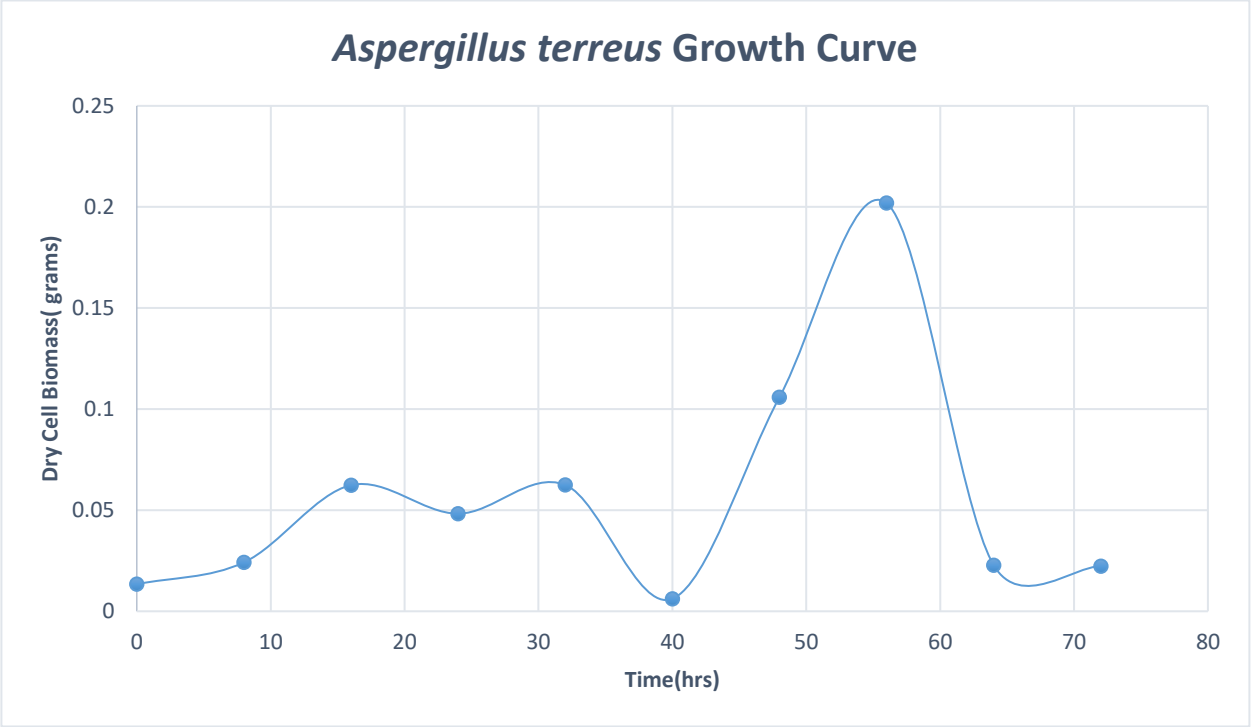

Figure 4.3: Growth-curve of *A. terreus*

4.4 Result of the GC-MS analysis:

The observations of the GC-MS analysis of the methanolic extract of given strains of the fungus *A. terreus* showed that there was no production of lovastatin found in the broth extract samples of the two used wild-type strains. Although a few other important bioactive compounds and certain other commercially-valuable compounds were found in the GC-MS analysis (shown in the figure 5, 6 and 7). Few of them are discussed below:

Isovaline (C5H11NO2): 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 colorless 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.

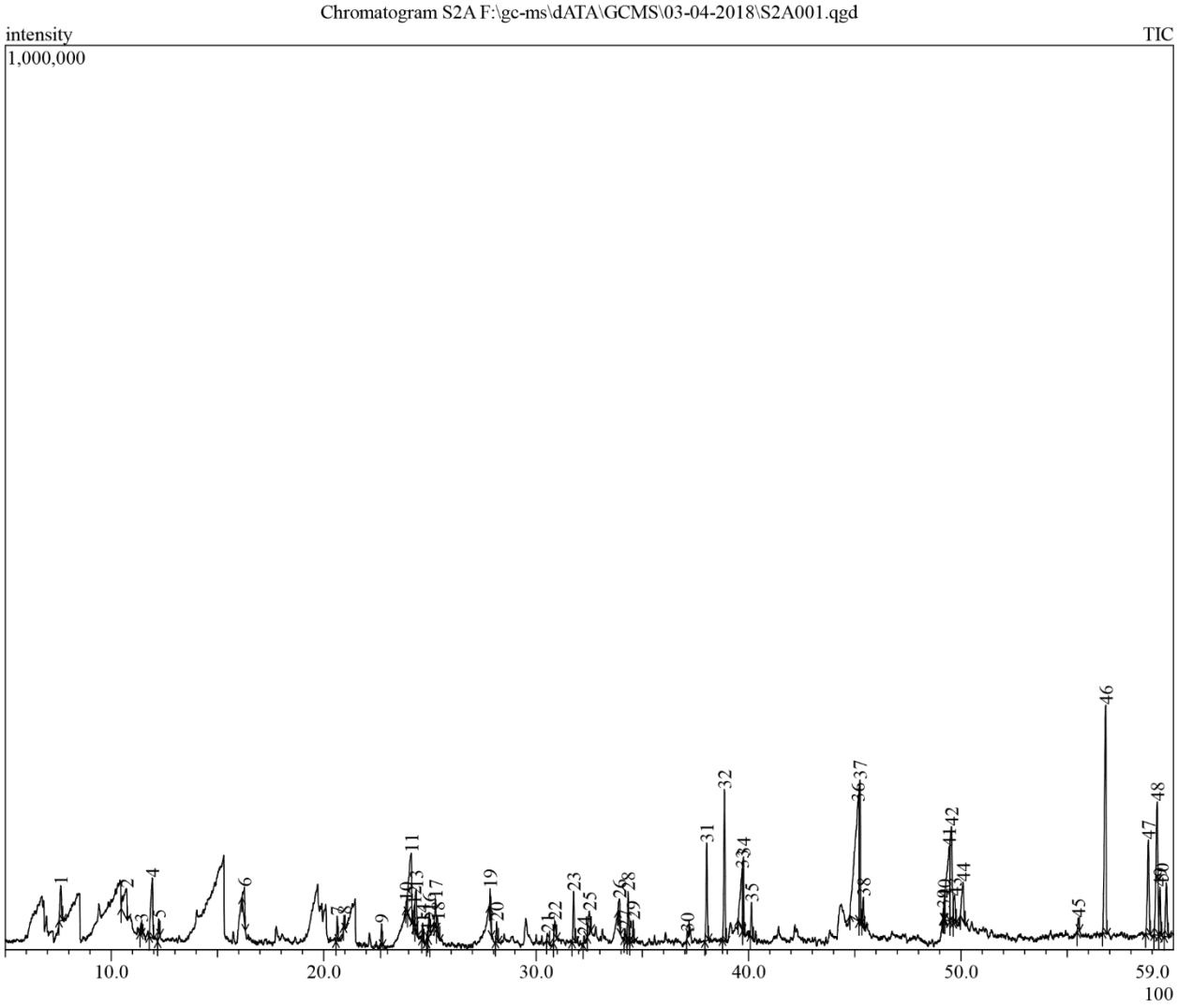

Figure 4.4: GC-Chromatogram showing retention times of different components of sample mixture (SIIA) of *A. terreus* strain II.

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			

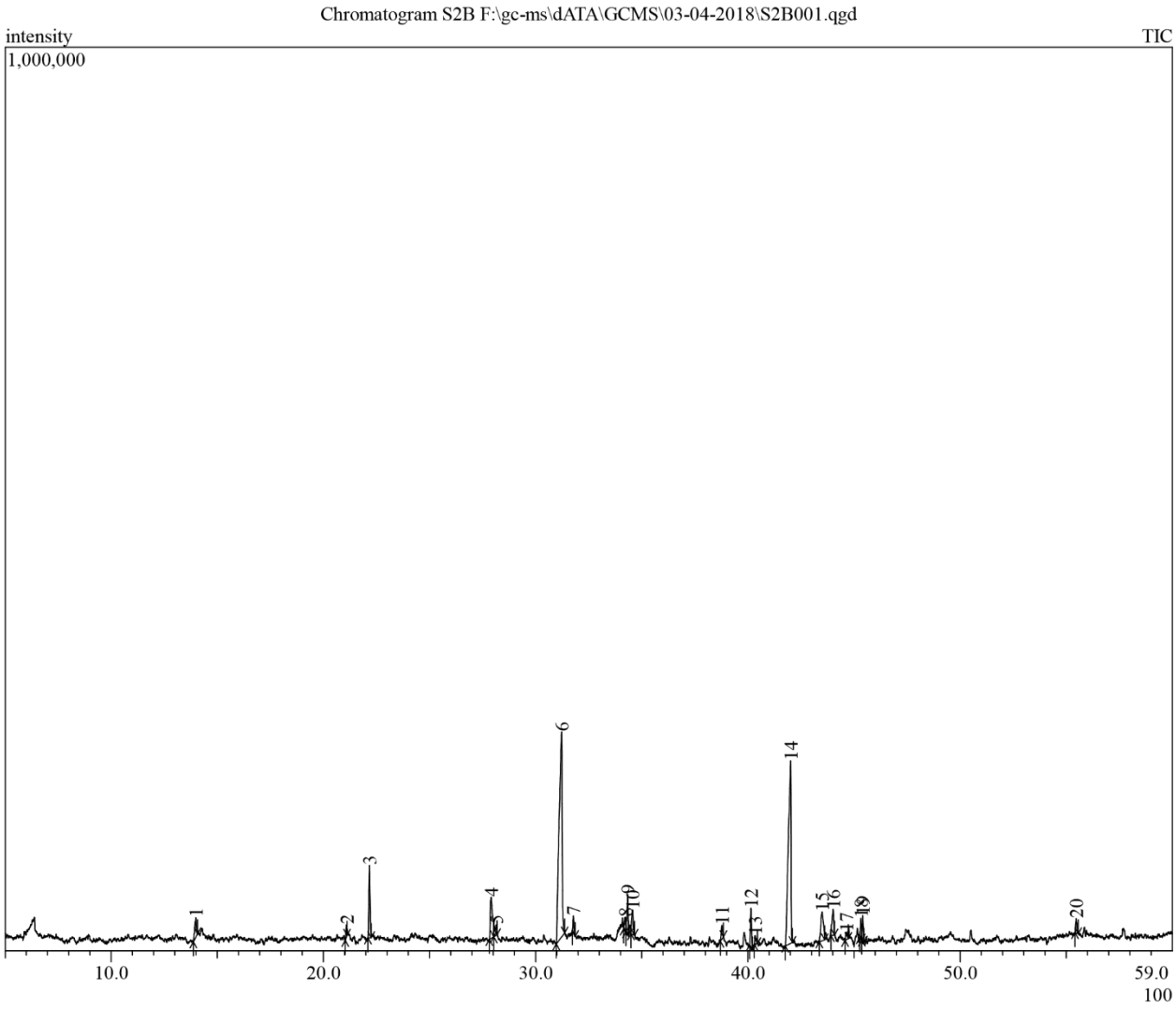

Figure 4.5: GC-Chromatogram showing retention times of different components of sample mixture (SIIB) of *A. terreus* strain II.

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, he
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			

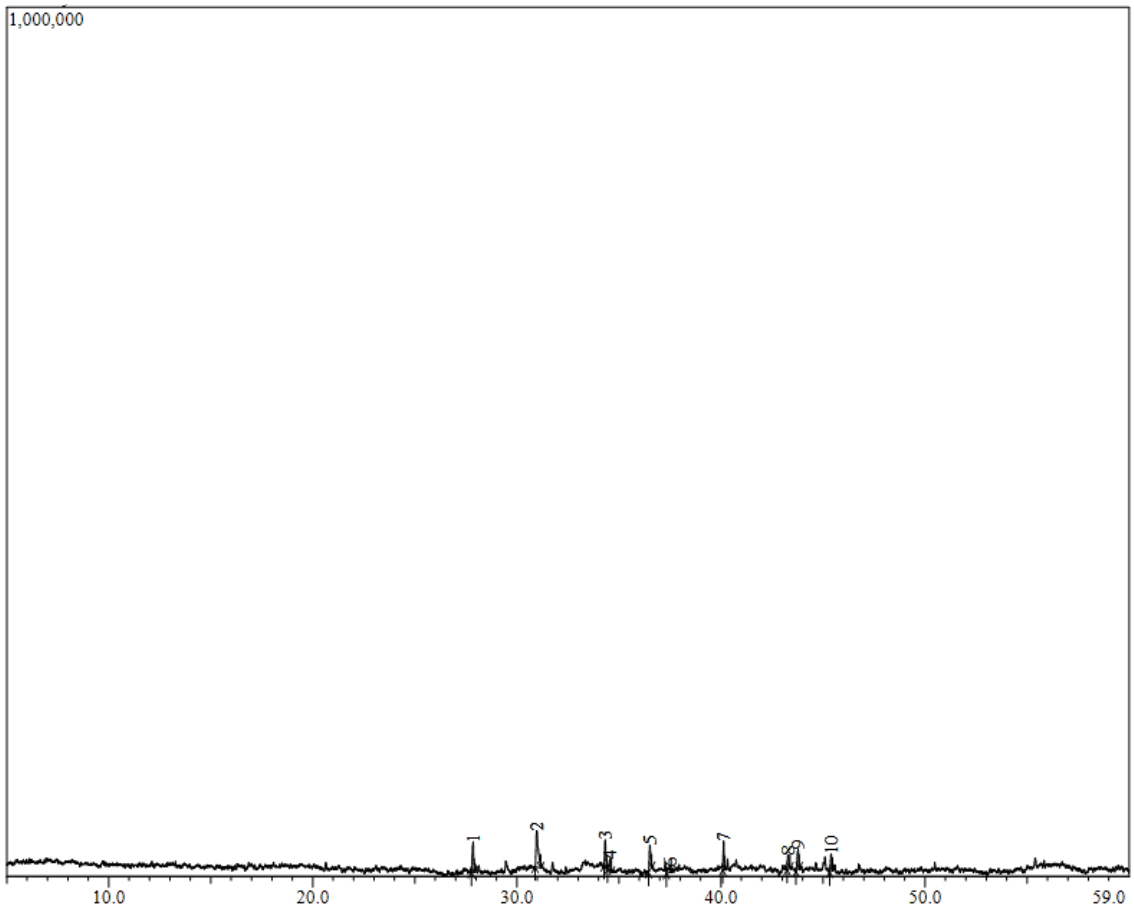

Figure 4.6: GC-Chromatogram showing retention times of different components of sample mixture of *A. terreus* strain I.

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			

DISCUSSION

As per the observed analysis of the GC-MS study, there was no production of lovastatin seen in the fermentation product. A possible reason or assumption behind this failure of production of lovastatin could be that the used wild-type strains of *A. terreus* are not capable of producing lovastatin.

CONCLUSION

In the present project report, *Aspergillus terreus* ATCC 11877 and *A. terreus* NBRC(IFO) 31217 were deployed for the lab-scale synthesis of lovastatin; a highly renowned cholesterol lowering drug. Although, the results for lovastatin production were not obtained as expected but the present study provides a new insight for the production of other important biomaterials of high value.

Novelty of Current Study:

Isovaline ($C_5H_{11}NO_2$) and Silane (SiH_4) production observed in the current study is novel and is not reported in the literature using *Aspergillus terreus.*

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