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Isolation, Screening and Identification of Indole Acetic Acid (IAA) Producing Bacteria from Rhizospheric Soil

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Abstract: ***A total of 14 bacterial strains were isolated from rhizospheric soil collected from Koparkhairane, Navi Mumbai, India. All isolates were screened primarily for their potential to produce indole acetic acid (IAA) using L-tryptophan as a substrate. Subsequently, in secondary screening, strain P2C11 was found to be a potent IAA producer based on the IAA concentration produced by all the isolates. Further, the isolate P2C11 was analyzed by MALDI-TOF & 16S rRNA sequencing and it was identified as Microbacterium hydrocarbonoxydans. It produced 290 µg/ml IAA which is higher than the reported IAA production by this genus. L-tryptophan and IAA analysis was done using High Performance Liquid Chromatography (HPLC). Confirmation of IAA production in fermentation broth was done by Gas Chromatography-Mass Spectrometry (GC-MS). The isolate P2C11 was found to have the potential to be used as an efficient bio-fertilizer inoculant to promote growth of economically and agriculturally valuable plants.***

Keywords: Indole acetic acid, L-tryptophan, PGPR, *Microbacterium hydrocarboxydans*, Microbial characterization.

1. Introduction

Plant growth hormones, also known as phytohormones, regulate plant development and evoke a specific physiological response in plants, at very low concentration. Auxins were the first class of plant growth regulators to be identified [1]. Auxins have many regulatory functions in plants, including stimulating cell enlargement, cambium cell division, differentiation of phloem and xylem, rapid establishment of roots, and lateral root formation. Auxins also help in regulating flowering and fruiting. Auxins mediate the tropism response to gravity and light [2], [29]. Many studies have demonstrated that, in addition to plants, some plant-associated fungi and bacteria also produce auxins [18].

The great array of root– microbe interactions results in the development of a dynamic environment known as the rhizosphere where microbial communities interact [4]. The rhizosphere, representing a thin layer of soil surrounding plant roots and the soil occupied by the roots, supports large active groups of bacteria [5]. Innumerable metabolic activities occur in rhizosphere due to presence of highly versatile microorganisms [6]. Rhizobacteria inhabit plant roots and exert a positive effect ranging from direct influence mechanisms to an indirect effect and are termed as Plant Growth Promoting Rhizobacteria (PGPR) [4]. In recent years, scientists have focused their attention towards the potential of beneficial microbes such as PGPR for sustainable agriculture. PGPR are known to produce phytohormones which are responsible for enhancing plant growth. PGPR can stimulate plant growth regulators such as indole acetic acid (IAA) or cytokinins or both, to increase host growth. Interactions between plants and microbes in rhizosphere are responsible for the improvement of plant health and soil fertility [7]. Microorganisms from rhizospheres of various plants synthesize and release auxins as secondary metabolites because of rich substrates exuded from the roots in rhizosphere as compared with non-rhizospheric soil [8]. Indole acetic acid (IAA) is one of the most physiologically active auxins, produced by PGPR through the L-tryptophan (trp) metabolism pathway [9]. IAA is primarily a product of secondary metabolism and L-tryptophan apparently becomes more available for IAA synthesis once primary metabolism has slowed [26]. IAA accumulation occurs mainly in the stationary phase of growth, indicating that in addition to the supplemented L-tryptophan, various proteins released during stationary phase and dead cells also contribute to the tryptophan pool for cells [6]. Despite progress in identifying enzymes in tryptophan dependent IAA biosynthesis, no single IAA biosynthetic pathway is yet defined to the level that all of the relevant genes, enzymes, and intermediates are identified [10]. Although PGPR are reported to synthesize a whole variety of phytohormones, auxins and more so IAA have been studied extensively [6].

Diverse groups of micro-organisms, including soil, epiphytic and endophytic bacteria and some cyanobacteria were found to synthesize IAA in the presence of L–tryptophan [8]. The earlier studies showed that plant growth–promoting bacteria from different genera *(Azospirillum, Enterobacter, Azotobacter, Burkholderia, Klebshiella, Alcaligenes, Pseudomonas, Xanthomonas, Pantoea, Rhizobium, Micrococcus, Microbacterium, Lactobacillus and Bacillus),* actinomycetes and various fungi enhanced plant growth by the synthesis of IAA [8], [16], [19], [27], [28], [32]. Few filamentous fungi such as *Aspergillus niger* and *Rhizopus* are known to produce IAA [11]. Red yeast *Rhodosporidium paludigenum* has been reported to produce IAA [12]. IAA is the principle and first auxin isolated from plants [13]. It is a commercially important and one of the most widely studied plant growth promoting hormone [14]. IAA is known to control organogenesis, tropic responses, cellular responses such as cell enlargement, division, and differentiation, gene regulation and responses to light and gravity [15]. IAA

produced by rhizobacteria helps in increasing the absorption of nutrients by increasing the production of root hair by the plant body [4]. IAA stimulates cell elongation by modifying certain conditions like, increase in osmotic contents of the cell, increase in permeability of water into cell, decrease in wall pressure and increase in cell wall synthesis [16]. IAA can have inhibitory effect on root elongation at high concentration [8]. Microorganisms synthesize IAA for their own benefit [17]. IAA can have a direct effect on bacterial survival and its resistance to plant defense. Evidence has been accumulating that some microorganisms, independent of their ability to produce IAA, make use of auxins as a signaling molecule steering microbial behavior [18]. IAA is a metabolite derived from its precursor L-tryptophan by many trp-dependant and trp-independent pathways in plants and bacteria. More than one pathway could be present in a microorganism [16]. Root exudates and decaying cells are natural source of tryptophan for PGPR for IAA biosynthesis [19]. The indole pyruvate pathway which is believed to be the main route for IAA production in plants has also been reported in bacteria. IAA production by microorganisms varies according to different strain dependant as well as external parameters. Bacteria can produce IAA by various other pathways such as, Indole-3-acetamide pathway, tryptamine pathway, tryptophan side-chain oxidase pathway and Indole-3-acetonitrile pathway [18]. Compounds which serve as IAA precursors in these pathways may also have auxin activity (e.g., indoleacetaldehyde) [9]. Few microorganisms such as *Azospirillum brasilense, Azotobacter*, *Pseudomonas* have ability to produce IAA in absence of tryptophan [20], [21]. Chorismate is a principle precursor of tryptophan synthesis pathway which is used by these microorganisms in absence of exogenous tryptophan [22], [23]. Prevalence of this tryptophan independent route of IAA biosynthesis has not been conclusively proven in bacteria [6].

The world population keeps increasing and this maintains the demand for high crop yield to provide food for all. For better crop yield, usage of chemical fertilizers and pesticides also has been high. Though satisfactory crop yield is obtained by the application of chemical fertilizers and pesticides, their use brings out certain disadvantages. These include pollution of large water resources, destruction of microorganisms, acidity of the soil, reduction in soil fertility etc. [15]. Perhaps a better strategy to increase crop yields would be to encourage the natural, beneficial interactions between crop plants and associated microbes. In recent years considerable attention has been paid to PGPR to replace agrochemicals (fertilizers and pesticides) for the plant growth promotion [24]. Commercial applications of PGPR are being tested and are frequently successful [27]. A better understanding of the microbial interactions responsible for enhanced plant growth is necessary for successful application of IAA in fields.

The objective of the present study was to isolate a high IAA yielding microorganism from rhizospheric soil and to study its IAA production efficiency in presence and absence of L-tryptophan.

2. Materials and Methods

2.1. Materials

L-tryptophan and indole acetic acid standards of analytical grade were procured from Spectrochem Pvt. Ltd. and Loba chemie respectively. Dehydrated media (nutrient broth, trypticase soya broth), yeast extract, malt extract, agar, sugars such as sucrose, glucose, xylose, arabinose, mannose, lactose, mannitol and chemicals such as Kovac's oxidase reagent, Kovac's indole reagent, Alpha-napthol, sulphanilic acid, Alpha napthylamine, Nessler's reagent, phenol red indicator were bought from Himedia Laboratories Pvt. Ltd. Hydrogen peroxide was procured from Merck. Methanol and Phosphoric acid of analytical grade were used for HPLC analysis and bought from Rankem and Thomas Baker (chemicals) Pvt. Ltd. respectively. Water used for experiments was of RO grade from a Millipore ultrapure water system. Membrane filters of 0.2 micron size were procured from Sartorius stedim.

2.2. Methods

2.2.1 Sampling of soil from rhizosphere

Soil sample was collected from the rhizosphere (narrow zone of soil surrounding plant roots) of Brinjal (*Solanum melongena)* plant in Koparkhairane area of Navi Mumbai, India. Intact root system was dug out and the rhizosphere soil sample was collected in plastic Ziploc bag. Soil sample was stored at room temperature until further use.

2.2.2 Isolation of IAA producing microorganisms from rhizospheric soil

The plant growth promoting rhizobacteria were isolated from the rhizospheric soil by serial dilution plate method. One gram of soil was added in 9 ml sterile distilled water and serially diluted up to 10^{-5} dilution. 0.1 ml suspension from appropriate dilutions was plated on sterile trypticase soya agar (TSA) medium plates and incubated at 30°C for 72 hours. Isolated and morphologically distinct colonies were selected from 2 plates (P1 and P2) and streaked on sterile TSA medium plates to obtain pure colonies. These pure colonies were maintained on sterile nutrient agar plates and stored at 4°C for further use. All bacterial isolates were coded to facilitate their future identification. Isolates from plate 1 were coded as P1C1, P1C2, P1C3, and P1C4 whereas from plate 2 as P2C1, P2C2, P2C3, P2C4, P2C5, P2C6, P2C7, P2C8, P2C10 and P2C11. They were further assessed for their morphology, physiology, characterization and also evaluated for IAA production.

2.2.3 Screening of IAA producing isolates

All the test isolates were grown in sterile 250 ml Erlenmeyer flasks containing sterile 50 ml liquid medium composed of 0.9% sucrose, 0.9% yeast extract and 0.2% (w/v) L-tryptophan [12]. Flasks were incubated on rotary orbital shaker, at 30°C and 200 RPM for 10 days. After incubation, 5 ml broth was centrifuged at 5000 RPM for 10-15 minutes. Culture supernatant was analyzed for IAA production by High Performance Liquid Chromatography (HPLC) as described in method section 2.2.6.1. IAA producing isolate

of highest efficacy was selected on the basis of concentration of IAA produced.

2.2.4 Identification of isolate

2.2.4.1 Morphological characterization

All the 14 microbial isolates were examined on TSA medium plate for morphological characterization. Macroscopic colony characteristics of all isolates were recorded after 72 hours of incubation. Gram straining and motility test was also done for all the isolates.

2.2.4.2 Biochemical characterization

Selected isolate (P2C11) was biochemically characterized by carbohydrate fermentation (glucose, xylose, arabinose, lactose, mannose, galactose, ribose, sucrose and fructose), indole test, methyl red test (MR), Voges-Proskauer test (VP), citrate utilization test, catalase test, oxidase test, nitrate reduction test, urease test etc. by standard methods explained in Bergey's manual of systematic bacteriology volume five, The *Actinobacteria*, Part A [30].

2.2.4.3 Molecular characterization

Selected isolate (P2C11) was sent to Microbial Type Culture Collection (MTCC) for MALDI-TOF (Matrix Assisted Laser Desorption/Ionization- Time of Flight mass spectrometry) analysis and 16S rRNA sequencing. According to the MTCC report, MALDI-TOF was done by using Bruker Daltonics flex analysis. For 16S rRNA sequencing, genomic DNA was isolated from pure culture using ZR bacterial DNA MiniPrep kit. 16S rRNA gene was PCR amplified using universal primers, 27F (AGAGTTTGATCCTGGCTCAG) and 1492R (TACGGYTACCTTGTTACGACTT). PCR product was visualized on 1% agarose gel. Purified PCR product was sequenced using Sanger DNA sequencing method. Obtained sequences were visualized and analyzed using Finch TV software version 1.4. Assembled nucleotide sequences of 16S rRNA gene were subjected to similarity search using BLAST (Basic Local Alignment Search Tool) tool in EzBiocloud portal.

2.2.5 IAA production by P2C11 isolate by fermentation

IAA production by P2C11 isolate was studied in both presence and absence of L-tryptophan. Approximately $2x10^8$ cells were inoculated in sterile 50 ml liquid medium composed of 0.9% sucrose and 0.9% yeast extract, in 2 separate 250 ml Erlenmeyer flasks. One of the flasks was supplemented with 0.2% (w/v) L-tryptophan. In another experiment, high cell density fermentation was done using this isolate. In this mode of fermentation, more than $2x10^{11}$ cells were inoculated in sterile 50 ml medium composed of 0.9% sucrose, 0.9% yeast extract and 0.2% (w/v) L-tryptophan. All the flasks were incubated on rotary orbital shaker at 30°C and 200 RPM for 10 days. 5 ml broth from each flask was centrifuged and the supernatant was analyzed for IAA production by HPLC.

2.2.6 Analytical Methods

2.2.6.1 High Performance Liquid Chromatography (HPLC)

Analysis of fermentation broth samples by HPLC was carried out on Phenomenex C18 column (250 X 4.6 mm, 5 micron). Mobile phase used for elution comprised of 0.01M phosphoric acid and methanol in the ratio of 70:30. Elution was done at the flow rate of 1 ml /minute. Ultra Violet (UV) detector was used for analysis at 220 nm wavelength. The average run time was 30 minutes [8], [28]. Sample preparation of both, the standard IAA and IAA produced in fermentation broth was done in HPLC-grade methanol. The concentration of IAA produced was estimated against the standard calibration curve of IAA.

2.2.6.2 Extraction of IAA and analysis by GC-MS

Confirmation of IAA production in broth was done by Gas Chromatography- Mass Spectroscopy (GC-MS). Fermentation broth containing IAA was extracted twice with equal volume of ethyl acetate. Ethyl acetate extract containing IAA was evaporated at 45˚C under vacuum and the concentrated residue was re-dissolved in 1 ml methanol. The methanol diluted sample was analyzed on non-polar HP-5 column (30 m X 0.25 mm ID, 0.25 mm film thickness). Injector and detector temperatures were both set to 280˚C. The oven temperature was held at 80˚C for 2 minutes, then programmed to rise from 80˚C to 200˚C at 10˚C / minutes and then finally from 200˚C to 280˚C at 20˚C/ minutes rate, held for 7 minutes [8]. IAA was identified by using the NIST (National Institute of Standards and Technology) library on the basis of mass spectrum.

3. Results and Discussion

3.1. Isolation and screening of IAA producing strains

A total of 14 bacterial strains were successfully isolated from rhizospheric soil (Figure 1). All the isolates were coded for their future study. Data presented in graph (Figure 2) indicates that out of 14 soil isolates, 12 were selected in primary screening based on their IAA production ability. IAA production was not observed in 2 isolates coded as P2C8 and P2C10. Isolate P2C9 could not grow when subcultured on fresh medium; hence it was not taken into the consideration during this study. Relatively higher content of IAA (36.32 µg/ml) was found in the fermentation broth of bacterial isolate P2C11 as compared to other isolates (Figure 2). P2C11 isolate was selected for further study based on the high production of IAA.

3.2. Identification of the selected isolate

3.2.1 Morphological characterization

Colony characteristics of all the 14 isolates on TSA plate were recorded after 72 hours and are shown in Table 1. Most of the isolates were motile gram positive short rods. All the colonies were circular in shape. The colony of selected isolate, P2C11, was small, yellow, circular and translucent. Microscopic observations showed that the isolate is gram positive motile short rod.

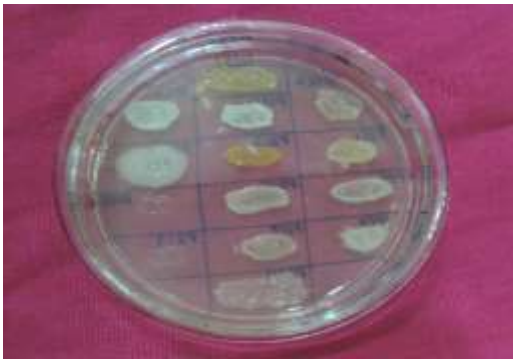

Figure 1: IAA producing soil isolates grown on nutrient agar plate

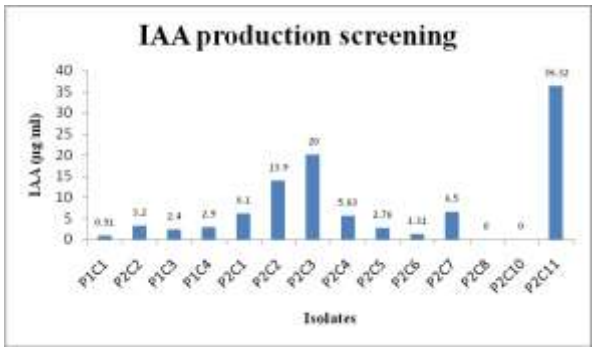

Figure 2: IAA production by various soil isolates

Table 1: Colony characteristics of selected isolates

Sr. No.	*Colony*	*Size*	*Color*	*Consistency*
Gram positive motile short rods				
1	P1C1	4 mm	Yellow	Translucent
2	P1C2	4 mm	Off-white	Mucoid
3	P2C2	2 mm	Orange	Translucent
4	P2C4	1 mm	Off-white	Translucent
5	P2C6	Pin-point	Colorless	Translucent
6	P2C7	3 mm	White	Translucent
7	P2C11	3 mm	Yellow	Translucent
8	P2C8	3 mm	White	Translucent
Gram positive non-motile short rods				
9	P2C5	4 mm	Off-white	Mucoid
10	P1C3	3 mm	White	Dry
11	P2C1	2 mm	Yellow	Mucoid
Gram positive motile cocci				
12	P1C4	3 mm	Off-white	Dry
13	P2C10	1 mm	Off-white	Translucent
Gram negative motile short rods				
14	P2C3	1 mm	Off-white	Mucoid

3.2.2 *Biochemical characterization of P2C11 isolate*

P2C11 isolate (potent IAA producer) was further identified based on biochemical characteristics. Bergey's manual of systematic bacteriology was used as a reference. Biochemical characteristics of isolate are explained in Table 2. Based on morphological, microscopic and biochemical results, this isolate could be from one of the following genera: *Arthrobacter* (though typical rod-cocci cycle was not observed), *Brevibacterium*, *Microbacterium* or *Agreia* [30], [31]. Very low IAA production by *Microbacterium*, *Arthrobacter* and *Brevibacterium* genera has been reported [32], [33], [34]. IAA production by *Agreia* genus has not been reported in literature.

Table 2: Biochemical characterization of P2C11 strain

Sr. No.	*Biochemical test*	*Results*
1	Indole production	Negative
2	Methyl red test (MR)	Negative
3	Voges-Proskaeur test (VP)	Negative
4	Citrate utilization	Negative
5	Nitrate reduction	Negative
6	Urease test	Negative
7	Catalase test	Positive
8	Oxidase test	Negative
9	Acid Production in	
a	Glucose	Negative
b	Xylose	Positive
c	Arabinose	Negative
d	Lactose	Positive
e	Mannose	Negative
f	Fructose	Negative
g	Sucrose	Positive
h	Galactose	Negative
i	Ribose	Negative

3.2.3 Molecular characterization of P2C11 isolate: 16s rRNA sequencing

In MTCC MALDI library, no reliable match was found for P2C11 isolate as their library is largely restricted to clinical isolates identity. Therefore, this strain was identified by 16S rRNA gene sequence analysis. The BLAST results of the 16S rRNA gene sequences allowed classifying the isolated strain from soil into the family Microbacteriaceae. The evaluated strain was aligned against sequences available from GenBank data. As per 16s rRNA gene sequencing of the P2C11 isolate, base pair sequence is as follows:

CAGTCGACGGTGACACGGAGCTTGCTCTGTGGGATCA
GTGGCGAACGGGTGAGTAACACGTGAGCAACCTGCCC
CTGACTCTGGGATAAGCGCTGGAAACGGCGTCTAATA
CTGGATACGAACCGCGAAGGCATCTTCAGTGGTTGGA
AAGAATTTCGGTTGGGGATGGGCTCGCGGCCTATCAG
CTTGTTGGTGAGGTAATGGCTCACCAAGGCGTCGACG
GGTAGCCGGCCTGAAAAGGTGAACGGGCACCCTGGG
GACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCA
GTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCA
GCAACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTA
AACCTCTTTTAGCAGGGAAGAAGCGAAAGTGACGGTA
CCTGCAGAAAAAGCGCCGGCTAACTACGTGCCAGCAG
CCGCGGTAATACGTAGGGCGCAAGCGTTATCCGGAAT
TATTGGGCGTAAAGAGCTCGTAGGCGGTTTGTCGCGT
CTGCTGTGAAATCCCGAGGCTCAACCTCGGGCCTGCA
GTGGGTACGGGCAGACTAGAGTGCGGTAGGGGGAGA
TTGGAATTCCTGGTGTAGCGGTGGAATGCGCAGATATC
AGGAGGAACACCGATGGCGAAGGCAGATCTCTGGGC
CGTAACTGACGCTGAGGAGCGAAAGGGTGGGGAGCA
AACAGGCTTAGATACCCTGGTAGTCCACCCCGTAAACG
TTGGGAACTAGTTGTGGGGTCCATTCCACGGATTCCGT
GACGCAGCTAACGCATTAAGTTCCCCGCCTGGGGAGT
ACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGG
ACCCGCACAAGCGGCGGAGCATGCGGATTAATTCGAT
GCAACGCGAAGAACCTTACCAAGGCTTGACATATACGA
GAACGGGCCAGAAATGGTCAACTCTTTGGACACTCGTA
AACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTG
AGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTC
GTTCTATGTTGCCAGCACGTAATGGTGGGAACTCATGG
GATACTGCCGGGGTCAACTCGGAGGAAGGTGGGGAT

GACGTCAAATCATCATGCCCCTTATGTCTTGGGCTTCA
CGCATGCTACAATGGCCGGTACAAAGGGCTGCAATAC
CGCGAGGTGGAGCGAATCCCAAAAAGCCGGTCCCAGT
TCGGATTGAGGTCTGCAACTCGACCTCATGAAGTCGGA
GTCGCTAGTAATCGCAGATCAGCAACGCTGCGGTGAA
TACGTTCCCGGGTCTTGTACACACCGCCCGTCAAGTCA
TGAAAGTCGGTAACACCTGAAGCCGGTGGCCTAACCC
TTGTGGAGGGAGCCGTCGAAGGTGGGATCGGTAATTA
GGACTAAGTCGT.

After NCBI BLAST analysis this sequence matched with base sequence of *Microbacterium hydrocarbonoxydans* with 99.08% similarity. Conclusively, on the basis of morphological, biochemical and molecular characterization, the isolate under study, i.e. P2C11, was identified and confirmed as *Microbacterium hydrocarbonoxydans.*

3.3. Analytical results

3.3.1 HPLC Analysis

The HPLC chromatogram of standard IAA showed major peak at retention time 24.9 minutes, while chromatogram of the fermentation broth also resulted in similar peak at retention time 24.9 minutes. Presence of IAA was confirmed by spiking the culture broth sample with standard IAA. Figure 3 shows chromatogram of both fermentation broth and spiked broth sample in overlaid form.

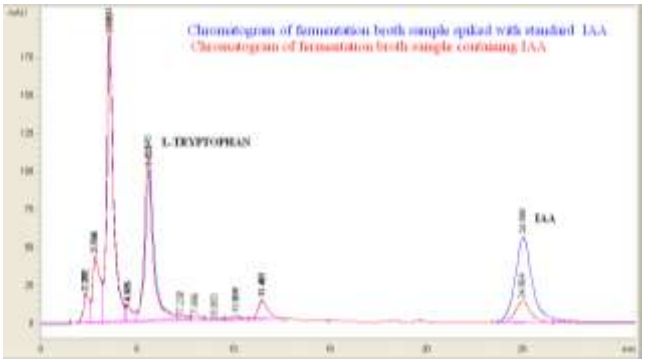

Figure 3: Overlaid chromatogram of fermentation broth and spiked sample

3.3.2 GC-MS Analysis

GC–MS analysis of the extracted samples confirmed the presence of IAA by its mass fragmentation pattern. The gas chromatogram of standard IAA showed presence of a major peak at retention time 16.3 minutes, with the major molecular ion of m⁄z 130. Similar results were obtained for fermentation broth sample where the major peak was seen at 16.3 minutes retention time with the molecular ion of m/z 130. Both the results were confirmed from the database of NIST/Wiley library available in the MS software with matching probability of 96%.

3.4. IAA production by *Microbacterium hydrocarbonoxydans* by fermentation

IAA production capability of *Microbacterium hydrocarbonoxydans* strain was checked in presence and absence of L-tryptophan. This strain was found to produce very low concentration of IAA i.e. 8 µg/ml in L-tryptophan free medium against 40 µg/ml in L-tryptophan supplemented medium. L-tryptophan is reported as the primary precursor for the formation of IAA in plants and microorganisms [35]. However, work with tryptophan-auxotrophic mutants and isotope labeling has established that IAA biosynthesis can also occur via a tryptophan independent route [25], [27]. Few microorganisms have capability to produce IAA in absence of L-tryptophan [20], [21]. Such organisms use a five step reaction encoded by the trp genes for biosynthesis of tryptophan from chorismate which is synthesized from phosphenolpyruvate and erythrose-4-phosphate in the shikimate pathway [2]. The low concentration of IAA (8 µg/ml) produced by *Microbacterium hydrocarbonoxydans* in L-tryptophan free medium suggests that in the absence of exogenous tryptophan, most of the tryptophan produced by cells through shikimate pathways is diverted into primary metabolic processes and very less amount of tryptophan is available for cells for IAA production [26]. In high cell density fermentation, 290 µg/ml IAA was produced within 10 days. Increase in IAA production can be attributed to high cell count in the broth. IAA production in very low concentration (14.3 µg/ml) has been reported in the literature by *Microbacterium hydrocarbonoxydans* [32]. Relatively higher IAA production was observed in this study using *Microbacterium hydrocarbonoxydans* isolated from soil. Further optimization of growth and production parameters may facilitate the IAA production by this strain and thus it will prove very helpful in the field of agriculture.

4. Conclusion

The main objective of this study was to isolate strain with maximum IAA producing capability, from soil. A total 14 bacterial strains were isolated from rhizospheric soil collected from Koparkhairane area in Navi Mumbai, India. Out of 14 isolates, 12 were observed to be IAA producers. P2C11 was the only isolate capable of producing high concentration i.e. 290 µg/ml of IAA by utilizing L-tryptophan as a precursor. IAA production was analyzed by HPLC and confirmed by GC-MS. Based on morphological, biochemical and molecular characterization, this isolate was identified to be *Microbacterium hydrocarbonoxydans*. High cell density fermentation was found to be the effective mode of IAA production for this isolate. In conclusion, the finding of the present study highlighted that IAA producing bacteria *Microbacterium hydrocarbonoxydans* isolated from rhizospheric soil may be used for agricultural use as plant growth promoting rhizobacteria. It will also help in preventing environmental pollution by avoiding excessive application of synthetically produced fertilizers. With increasing awareness about the chemical-fertilizers-based agricultural practices, it is important to search for region-specific microbial strains which can be used as potential plant growth promoters to achieve desired product.

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