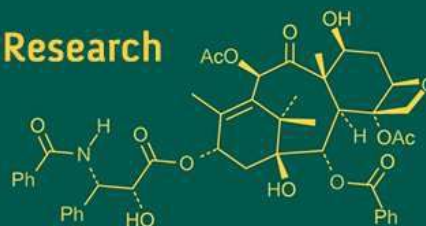


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Multiple plant growth promoting activities of *Burkholderia lata* I-38, a bacterium from sugarcane field

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Abstract

Sugarcane is one of the most productive plant systems in the tropical and subtropical areas of different nations. It accounts for 80% part of the total sugar produced in the world. The aim of the present study was obtain a most potential bacterium that can be used as a biofertilizer for sugarcane growth promotion in different types of soils. Total 6 soil samples were taken from the South Gujarat sugarcane production regions (Navsari, Bardoli, and Vyara). Total of 38 isolates were isolated from the collected samples, out of that 34 isolates showed N-fixation, 4 isolates showed P solubilization, 6 isolates showed K mobilization, 3 isolates showed Zn solubilization, 8 isolates showed siderophores production and 4 showed IAA production. Screening was done based on multiple plant growth promoting properties. Isolate I-38 was reported to have maximum number of plant growth promoting properties. Its identification was done based on morphological, biochemical and molecular biological characteristics and it was identified as *Burkholderia lata* I-38. It had a potential to be used as a biofertilizer for plant growth promotion in different types of soils.

Keywords: Sugarcane, plant growth promotion, *Burkholderia lata* I-38

Introduction

Sugarcane is used for the production of sugar because it contains highest percentage of sucrose of all plants. In terms of nutrition, one serving of sugarcane juice (28.35 g) contains 111.13 kJ of energy, 27.51 g of carbohydrates, 0.27 g of protein, 11.23 mg of Ca, 0.37 mg of Fe, 41.96 mg of K and 17.01 mg of Na (Mahata 2020, Singh *et al.* 2015) ^[19, 33]. The world demand for sugar is the primary driver of sugarcane agriculture. Sugarcane accounts for 80% of sugar produced; the rest is made from sugar beets (Dotaniya *et al.* 2016) ^[5]. Sugarcane is one of the most productive plant systems in the tropical and subtropical areas of different nations (FAOSTAT 2023) ^[9]. Brazil, India, Thailand, China and United states are the 5 major sugarcane producing countries in the world. India is the second largest producer after Brazil. In India, ~ 80% of the sugarcane produced is processed into white sugar, ~12% into jaggery and kandsari, and ~8% is used as seed cane (Kumar *et al.* 2022) ^[18]. In India, Gujarat is the 7th major producer of sugarcane.

The sugarcane is a monocot and perennial plant of the *Poaceae* family. It is sown once a year between January and March. Sugarcane, a C₄ plant, has a great capacity to store solar energy effectively and convert it to sugar. The four stages of sugarcane growth are germination, tillering, grand growth, and ripening. Commercially ripening of stems can be identified by the ration of refractometry indices of the juice taken from the seventh-eighth internodes and lower (0.95-0.98)). There are four main components of plants: a stalk, leaves, root system, and inflorescence. A plant that can grow up to 5 or 6 m tall, sugarcane produces several stems with jointed, sturdy, fibrous stalks that are rich in sugar content. In tropics at the harvesting time stems accumulate around 14-16% sugar, in subtropics 8-12% sugar. Apart from sugar, several derivatives generated from sugarcane are bagasse, molasses, press mud, spent wash, ethanol and trash. The fibrous portion called bagasse can be utilized for generation of electricity and paper production which is obtained as an end product after crushing sugarcane to squeeze out juice. Molasses (Thick, dark brown juice obtained from raw sugar during the refining process) is used for the production of ethyl alcohol, flavoring

agent and as a feed supplement for farm animals. Farmers use press mud (Residue of the filtration of sugarcane juice) in the form of manure (Gupta *et al.* 2011) [13].

Maintenance of soil fertility and warm atmosphere with adequate light are the necessary requirements of sugarcane plant to obtain desired growth. On an average the sugarcane plantation takes up great quantity of nutrients from soil such as 208 kg N, 53 kg P and 280 kg K from 1 ha area (10000 m²) besides that it takes 30 kg of S, 3.4 kg of Fe, 1.2 kg of Mn and 0.6 kg of Cu from the soil (Shukla *et al.* 2009) [32]. Application of N, P and K has significant effect on yield improvement of sugarcane crop. But, over use of these fertilizers is costly and hazardous. Land pollution, air pollution, global warming and climate change are the adverse effects of the excess use of chemical fertilizers (Sanghera *et al.* 2019) [27]. Hence, better approaches require are to determine the need of N, P and K for the individual crop with respect to production gain. Nitrogen (N) is very important for sugarcane yield improvement, but the excessive application of N fertilizer brings about N pollution and increase in cost. Globally, we are applying excessive nitrogen (N) fertilizers to agricultural crops, which ultimately cause nitrogen pollution to the ecosystem. The atmosphere is polluted by N₂O and NO_x gases that directly and indirectly increase atmospheric warming and climate change. N is also leached from agricultural lands as the water-soluble form NO₃⁻, which increases nutrient overload in rivers, lakes, and oceans, causing "dead zones", reducing property values and the diversity of aquatic life, and damaging our drinking water and aquatic-associated industries such as fishing and tourism.

Plant Growth Promoting Bacteria (PGPB) used as a biofertilizer leads to improved plant growth by both direct and indirect mechanism (Kloepper and Schroth 1978, Suthar *et al.* 2017) [17, 34]. In direct mechanism bacteria stimulate plant growth by N fixation, P solubilization, Fe sequestration and production of essential plant growth promoting hormones. Whereas production of ACC deaminase, antibiotics, cell wall degrading enzymes, competition, hydrogen cyanide, induced systemic resistance, quorum quenching and siderophores are the indirect mechanisms used for the control of phytopathogens. Gradually decreasing the dosage of chemical fertilizer and increasing the use of PGPB as biofertilizer leads to reduction in soil degradation and help in forming sustainable agriculture by being eco-friendly and cost-effective (Mahmud *et al.* 2021) [20]. Many environmental parameters such as soil type, pH and moisture content have significant impact on performance of biofertilizers. The kind of soil has a major impact on the diverse population of bacteria found in plant roots, which affects the efficiency of bacterial inoculants for successfully inoculating roots and promoting plant growth (Egamberdiyeva 2007) [7]. In the present study PGPB were isolated from different sugarcane growing areas of South Gujarat, followed by screening, selection, and identification of the most promising isolates having multiple plant growth promoting activities. Further, the potential of selected isolates was tested for its ability to promote sugarcane growth in various soil types by single eye bud treatment.

Materials and Methods

Soil samples were collected from different sugarcane growing areas of the South Gujarat, India (Table 1). Soil samples were taken to the laboratory in the sterile polythene

bags and analyzed on the same day. Isolation of PGPB collected from different types of soils were performed by serial dilution method using 1 g soil in 9 ml water to make homogeneous suspension further it was diluted upto 10⁻⁶. Isolation of bacteria from the diluted samples was carried out by spread plate method. The bacterial colonies were isolated on Luria Bertani (LB) agar plates providing incubation temperature of 37±2 °C for 48 h and microbial load in the collected soil samples was determined.

Table 1: Details of the sample collection sites selected for the isolation of PGPB

Sr. No.	Sample collection site	Longitude	Latitude
1	Bardoli	Sample-1 (Nandida)	21°06'40.5" N 73°05'17.1" E
2		Sample-2 (Barejadi)	21°07'44.0"N 73°04'27.0" E
3	Vyara	Sample 3 (Tichakpura)	21°06'02.8" N 73°20'28.0" E
4		Sample 4 (Songada)	21°06'11.6"N 73°20'53.4"E
5	Navsari	Sample 5 (Aat)	20°53'45.9" N 72°52'00.2" E
6		Sample 6 (Bhutsad)	20°54'08.6"N 72°53'18.1"E

Screening of bacterial isolates obtained from individual soil samples was performed based on their plant growth promoting characteristics. N fixation ability of each isolate was analyzed by observing growth of the isolate on N deficient Ashby's Mannitol Agar (AMA) medium (Ashby 1907) [2]. The P solubilizing capacity of the isolates was determined as Phosphate solubilization Index (PSI) using Pikovskaya's agar (Pikovskaya 1948) [23]. The K solubilization capacity of the isolates was assessed by inoculating the isolates on Aleksandrow agar medium and incubating at 37 °C for 48 h (Sheng 2005) [31]. Zn solubilizing efficiency of the selected isolates were determined using mineral salt agar containing 0.1% insoluble Zn compound (ZnO) as described by Saravanan *et al.* (2004) [28]. Production of siderophore by individual isolates was analyzed on CAS agar plate as described by Schwyn and Neilands (1987) [29]. IAA production capacity of individual isolates was determined using the method developed by Glickmann and Dessaux (1995) [11]. Further, compatibility with the insecticide and fungicide was determined using poison food technique (Jayasudha *et al.* 2018) [15]. Using the technique compatibility of the most promising PGPB was studied with Dimethoate 30% EC (2 ml l⁻¹) at different concentrations (450, 600 and 750 ppm) and with Carbendazim 50% WP (2 g l⁻¹) at different concentrations (750, 1000 and 1250 ppm). Out of all the isolates, the most promising PGPB isolate was identified based on its morphological, biochemical and molecular properties using standard procedure (Modi and Patel 2017) [22]. For morphological characterization of potential isolate Gram staining, colony characteristics, cell arrangement, cell shape and spore staining were analysed. For biochemical characterization of selected isolate certain tests namely Methyl red test, Voges-Proskauer test, Indole test, Citrate utilization test, sugar fermentation test were performed. Further confirmation was carried out by molecular characterization by 16S rRNA gene sequencing. The Genomic DNA was extracted from pure culture of the potential isolate. 16S rRNA gene based PCR amplification of purified DNA was done using 27F (5'AGA AAG GAG GTG ATC CAC3') and 1525R (5'GAG TTT GAT CCT GGC TCA3') primers. PCR amplified product was used for 16S rRNA gene sequencing and the BLAST tool was used to compare the obtained sequences and identify the potential isolate (Suthar *et al.* 2008) [35].

Results and Discussion

Total 38 bacteria were isolated from six soil samples collected from different regions of the South Gujarat. The

bacterial load in the samples ranged from 1.86×10^6 to 5.97×10^6 cfu g⁻¹ (Table 2).

Table 2: Bacterial load in different soil samples

Sr. No.	Sample collection site		Bacterial Count (cfu g ⁻¹)
1	Navsari	Sample 1	2.15×10^6
		Sample 2	1.86×10^6
2	Bardoli	Sample 3	5.97×10^6
		Sample 4	4.20×10^6
3	Vyara	Sample 5	4.35×10^6
		Sample 6	3.45×10^6

Further, screening of the isolates was carried out based on the multiple plant growth promoting activities. Out of 38 isolates, 34 (89.47%) of them demonstrated N fixation activity, 4 (10.52%) showed P solubilization, 6 (15.78%) K

solubilization, 3 (7.89%) Zn solubilization, 8 (21.05%) siderophore formation and 4 (10.52%) isolates were reported to have IAA production ability, respectively (Table 3).

Table 3: Plant growth promoting activity of the isolates

Isolates	N Fixation	P solubilisation	K solubilisation	Zn solubilisation	Siderophore production	IAA production
I-1	+	-	-	-	-	-
I-2	+	-	-	-	-	-
I-3	+++	-	-	-	+	-
I-4	+	-	-	-	-	-
I-5	+++	-	-	-	-	-
I-6	++	-	-	-	-	-
I-7	+++	+	+	+	+	+
I-8	+	-	-	-	-	-
I-9	+++	-	+	-	+	-
I-10	+	-	-	-	-	-
I-11	++	-	-	-	-	-
I-12	+	-	-	-	-	-
I-13	++	-	-	-	-	-
I-14	+++	-	-	-	-	-
I-15	+	-	-	-	-	-
I-16	++	-	-	-	-	-
I-17	-	-	-	-	-	-
I-18	+	-	-	-	-	-
I-19	+	-	-	-	-	-
I-20	+++	-	-	-	-	-
I-21	+++	-	-	-	-	-
I-22	-	-	-	-	-	-
I-23	+	-	-	-	-	-
I-24	+	-	-	-	-	-
I-25	++	-	-	-	-	-
I-26	-	-	-	-	-	-
I-27	+++	-	-	-	-	-
I-28	+++	-	-	-	-	-
I-29	+++	-	-	-	-	-
I-30	+	-	+	+	+	-
I-31	++	-	-	-	-	-
I-32	++	-	-	-	-	-
I-33	+	-	+	-	+	-
I-34	-	+	-	-	+	+
I-35	++	-	-	-	-	-
I-36	+	-	-	-	-	-
I-37	++	+	+	-	+	+
I-38	++	+	+	+	+	+

N plays a vital role in many physiological processes. It promotes the growth and development of the leaves, stem, roots and other vegetative parts. In addition to promoting rapid early growth, N assists the uptake and use of other nutrients like K and P, and controls overall plant growth (Shah *et al.* 2016) [30]. Synthesis of ammonia is crucial for the deposition of N and aids in stimulating root and shoot

growth as well as biomass production, all of which enhances the plant growth (Dutta and Thakur 2017) [6]. The identical N-deficient AMA agar medium was used by Hu *et al.* (2012) [14] to screen the PGPB, isolated from sugarcane rhizospheric soil. According to their findings, N could be fixed by 36 out of 148 isolates. Five of the 36 isolates (GX0703, GX0798, GX07104, GX08125, and GX0797)

were shown to have significant N fixation capacity. According to Wang *et al.* (2020) [36], N fixation on AMA agar was positive in 13 out of 39 isolates, with isolate N9 had the highest N fixing capacity. In the present study, N fixing ability on the N-deficient-AMA agar plates was demonstrated by 34 of the 38 isolates. Further, 10 isolates, namely I-3, I-5, I-7, I-9, I-14, I-20, I-21, I-27, I-28 and I-29, demonstrated the maximum levels of N fixation capacity (+++).

For plants to grow and perform many physiological processes P is a crucial element. It makes plants stronger and more resistant to diseases. In soil, P is found in insoluble and soluble forms. Plants cannot utilize the insoluble form of P. Some of the PGPB has the ability to convert the insoluble form of P into soluble form so that it becomes available to the plants (Anbuselvi *et al.* 2015) [1]. In the present study, 4 of the 38 isolates demonstrated positive P solubilization on Pikovskayas agar medium (Table 3). Isolate I-38 had the highest PSI among the 4 isolates. Hence, its PSI was measured up to 144 h at an interval of 24 h. It showed highest PSI value of 10.50 at 144 h (Fig. 1). Similar findings were made by Pereira *et al.* (2019) [24], who discovered that 70 isolates (19.23%) out of 364 isolates solubilized P on Pikovskayas agar medium. Wang *et al.* (2022) [37] reported that out of the 40 strains isolated from soil samples, *P. moraviensis*, *B. safensis*, and *F. pallidus* demonstrated the highest capacity for P solubilization. It had a substantial impact on higher grain output by promotion of the plant roots growth as well as by the enhancement of above-ground plant growth.

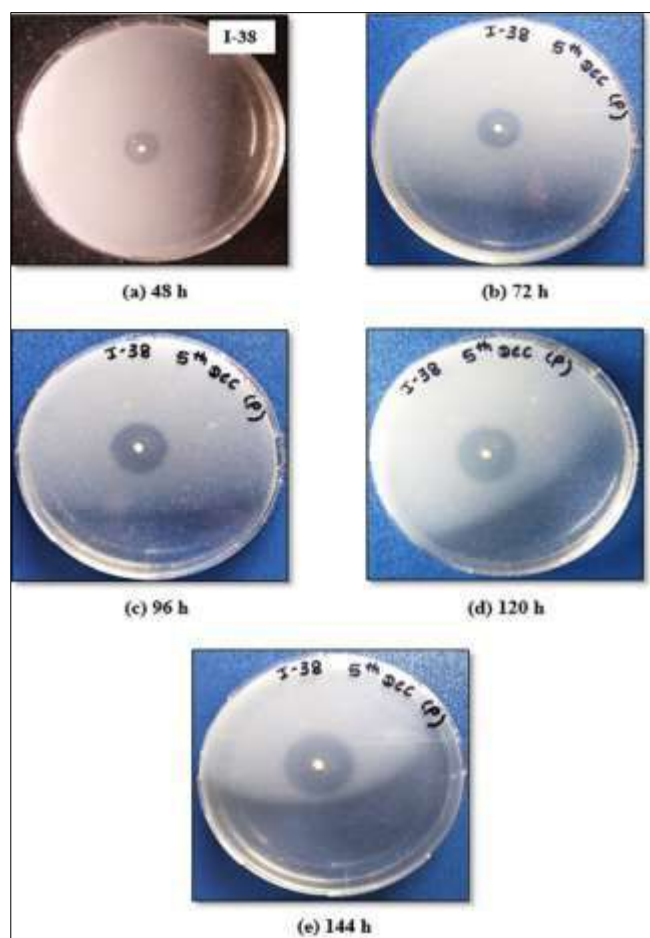


Fig 1: Determination of PSI by PGP isolate I-38 on Pikovskayas plates after different time intervals (a) 48 h, (b) 72 h, (c) 96 h, (d) 120 h and (e) 144 h

The third most important element after N and P, for plant growth is K. Many physiological activities such as activation many enzymes in the plant, translocation, and control over stomata closing and opening required K (Rawat *et al.* 2022) [26]. K works to promote plant growth by enhancing a number of key physiological processes including root growth, activation of numerous enzyme systems, maintenance of turgor, reduction of water loss and wilting, synthesis and food production, reduction of respiration, prevention of energy losses, enhancement of translocation of sugars and starch, growth of grain rich in starch, development of cellulose, reduction of lodging, and aid in the prevention of crop diseases (Meena *et al.* 2016) [21]. Out of 38 isolates, 6 (I-7, I-9, I-30, I-33, I-37, and I-38) produced clear halo surrounding the bacterial colony on Aleksandrow agar medium, indicated that the isolates had K solubilizing activity (Table 3). I-38 demonstrated the highest K solubilizing capacity among the 6 isolates tested. Therefore, its K solubilization activity was measured up to 120 h at a 24 h interval. I-38 showed highest zone of clearance of 29 mm at 120 h (Fig. 2). Similar findings were made by Ghevariya and Desai (2014) [10]. They used Aleksandrow agar medium to screen the K-solubilizing PGPB from sugarcane rhizospheric soil. Out of 50 isolates, 24 showed clear halos surrounding the colony by solubilizing K on media as a result of the formation of low molecular weight organic acid.

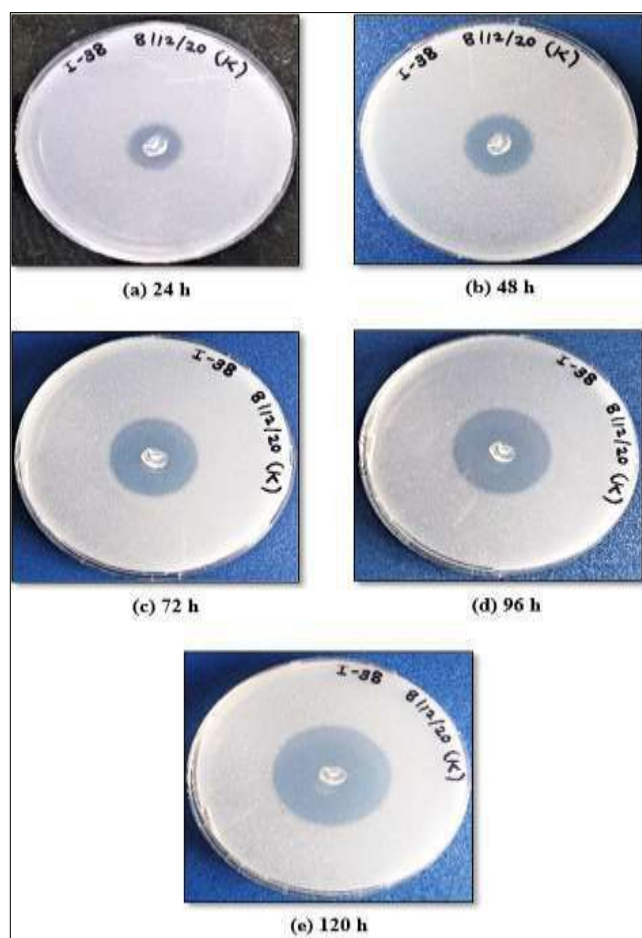


Fig 2: Determination of K solubilization by PGP isolate I-38 on Aleksandrow agar plates after different time intervals (a) 24 h, (b) 48 h, (c) 72 h, (d) 96 h and (e) 120 h

Zinc (Zn) is one of the essential minerals playing important role in the growth of plants. Out of 38 isolates, only 3 (I-7,

I-30 and I-38) produced the zone of clearance around the colonies (Table 3). I-38 demonstrated the strongest ability to solubilize Zn among the 3 isolates. Therefore, Zn solubilization activity of I-38 was measured up to 120 h at 24 h intervals. The highest zone of clearance was measured at 120 h (Fig. 3). Similar to this, 24 isolates were found in the rhizosphere of sugarcane and wheat by Kamran *et al.* (2017) [16]. Only 6 of them, *Pseudomonas fragi* (EPS1), *Pantoea dispersa* (EPS6), *Enterobacter cloacae* (PBS1 and PBS2), *Rhizobium* spp. (LHRW1) and *Pantoea* spp. (LS1-b) demonstrated Zn solubilization by generating a distinct halo around the colonies. The length and weight of the shoots and roots as well as the zinc content were increased in wheat plants after inoculation with these strains. Goteti *et al.* (2013) [12] discovered bacteria used variety of mechanisms, including the excretion of metabolites like organic acids, proton extrusion or the production of chelating agents, can be used to solubilize Zn. In their study, Out of 10 isolates, 2 (P17 and B40) displayed 31.0 mm zones in ZnO-incorporated medium.

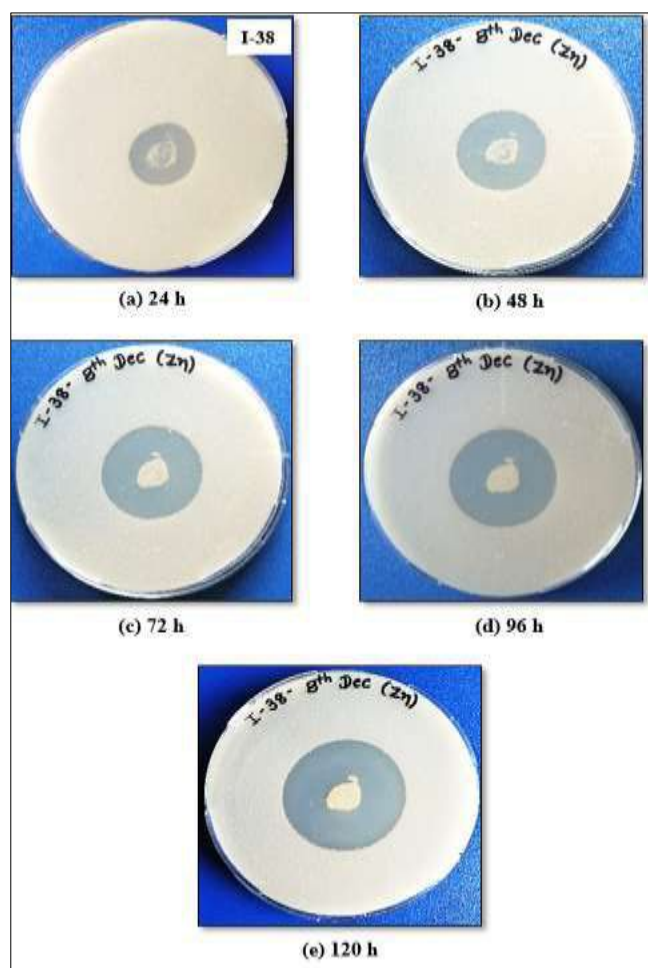


Fig 3: Determination of Zn solubilization by PGP isolate I-38 on Zn solubilization agar plates after different time intervals (a) 24 h, (b) 48 h, (c) 72 h, (d) 96 h and (e) 120 h

Phytohormones produced by bacteria include siderophores. In the presence of siderophores, insoluble Fe^{3+} might be utilized in the environment more readily, increasing its bioavailability to the plants. In the present study, 8 (I-3, I-7, I-9, I-30, I-33, I-34, I-37, and I-38) out of 38 isolates were found to produce orange halo around the bacterial colonies on CAS agar medium indicated positive siderophore production (Table 3). I-38 displayed the highest siderophore

production capacity among the 8 isolates tested. According to Rajasankar and Ramalingam (2012) [25], siderophore activity on CAS agar was detected in 5 out of 8 isolates. They stated that isolate VSC-14 (12.33 ± 0.57 mm) had the highest level of discolouration whereas isolate VSC-6 (8.33 ± 0.57 mm) had the lowest level. Recent research has demonstrated that siderophores during the germination and emergence phases, boost seedling activity and germination rates. Further, siderophores provide nutrients to plants in soils that have been contaminated with heavy metals hence lessen the stress and improve the plant growth (Zhang, 2023) [38].

Indole-3-acetic acid (IAA) is the most important plant hormone of the auxin class that play vital role in plant growth and development. IAA has no apparent function in bacterial cells, but it is speculated that it improve the fitness of the plant–bacterium interaction. The IAA produced by bacteria can loosen the plant cell wall and hence increase the release of root exudates rich in nutrients that support the growth of rhizospheric bacteria. Thus, IAA producing bacteria can provides more number of active sites and access to colonization for other PGPRs. Further, it increases the root surface area and length, and thereby provides the plant greater access to soil nutrients and water uptake. It is reported by Etesami *et al.* (2015) [8] that IAA should be the first PGP trait for screening bacteria isolated from plant rotated with rice for rice PGP agents. In the present study, the IAA production by all 38 isolates was analyzed and positive synthesis of IAA was displayed by 4 isolates (I-7, I-34, I-37, and I-38). Isolate I-34 ($139.76 \mu\text{g ml}^{-1}$) had the highest IAA production, which was followed by I-7 ($92.84 \mu\text{g ml}^{-1}$), I-38 ($73.61 \mu\text{g ml}^{-1}$) and I-37 ($56.69 \mu\text{g ml}^{-1}$). In a study of a similar nature, Beneduzi *et al.* (2013) [3] found that 368 (71%) out of 516 sugarcane bacterial isolates produced IAA in the range of 0.1 to $264 \mu\text{g ml}^{-1}$ in King's B medium supplemented with L-Trp and Salkowski reagent. In recent years, use of biocontrol agents along with chemical pesticides for effective management of pests, diseases and weeds has enhanced. Hence, it is important to test the compatibility of the PGPR with the chemical control agents used in the field. Hence, in the present study, the compatibility of the most promising plant growth promoting isolate I-38 was tested against fungicides (Carbendazim 50% WP) and insecticides (Dimethoate 30% EC) using poison food technique. Isolate I-38 was reported to grow well and showed compatibility with the different concentrations of the insecticide and fungicide tested. Similarly, Chari *et al.* (2015) [4] used agrochemicals like fungicides (Metalaxyl, Ziram, Propiconazole and Carbendazim) and insecticide (Chlorpyrifos, Dimethoate, Imidachloprid and Thiodicarb) to check the compatibility of 16 PGPR obtained from the different resource laboratories. They found that 5- isolates (PGP-1, PGP-9, PGP-13, PGP-14 and PGP-15) showed good compatibility with fungicide and 6 isolates (PGP-1, PGP-9, PGP-11, PGP-12, PGP-14 and PGP-15) with insecticides.

Based on the above mentioned studies, I-38 isolate having N fixation, P solubilization, K solubilization, Zn solubilization, siderophore production and IAA production ability was selected for further study. Its identification was done using morphological, biochemical and molecular tests. The results obtained are shown in the Table 4, 5 and 6, respectively. It was identified as *Burkholderia lata* I-38 (Gene bank Accession no. is OR048073). Effect of Multiple plant

growth promoting characteristics containing *B. lata* I-38 on sugarcane *Saccharum officinarum* CoN 13073 (GNS-10) growth in different types of soils was also tested by single eye bud treatment. It was reported to promote plant growth in terms of plant fresh weight, dry weight, number of leaves,

shoot length, root length, and germination percentage in three different types of soil (Data not shown). Thus, *B. lata* I-38 has the potential to be used as biofertilizer for the sugarcane growth promotion in different types of soils.

Table 4: Colony and morphological characteristics of potential isolate I-38

Colony Characteristics		Cell Characteristics	
Size	Small	Gram reaction	Gram negative
Shape	Round	Cell shape	Short rod shape
Margin	Entire	Cell arrangement	Single/pair
Elevation	Convex	Spore formation	Non-spore forming
Consistency	Smooth		
Pigmentation	Yellow		
Opacity	Opaque		

Table 5: Biochemical characteristics of potential isolate I-38

Sr. No.	Biochemical Test	Result
1	Methyl Red Test	+
2	Voges-Proskauer test	-
3	Indole test	-
4	Citrate utilization test	+
5	Glucose fermentation	+
6	Adonitol fermentation	-
7	Arabinose fermentation	-
8	Lactose fermentation	+
9	Sorbitol fermentation	-
10	Sucrose fermentation	+
11	Mannitol fermentation	-
12	Rhamnose fermentation	-

Table 6: 16S rRNA gene sequencing-based identification of potential isolate I-38

Sr. No.	Isolate Name	Max Score	Total Score	Query Cover	E value	Percent Similarity
1.	<i>Burkholderia lata</i> strain 38 16S rRNA gene partial sequence Gene bank Accession no. is OR048073	1589	1589	96%	0.0	99.88%

Conclusion

In conclusion, the study successfully isolated 38 bacterial strains from South Gujarat soils, with 34 isolates demonstrating nitrogen fixation and others exhibiting various plant growth-promoting activities, such as phosphorus, potassium, and zinc solubilization, siderophore production, and indole-3-acetic acid (IAA) production. Notably, *Burkholderia lata* I-38 showed superior growth-promoting traits, including high N fixation, P and K solubilization, and compatibility with agrochemicals. The isolate was further identified as *Burkholderia lata* I-38 (GeneBank Accession no. OR048073) and demonstrated significant growth enhancement in sugarcane under different soil conditions. These findings suggest that *B. lata* I-38 has great potential as a biofertilizer for promoting sustainable sugarcane cultivation.

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