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Isolation, screening and characterization of endophytic bacteria from root of finger millet (*Eleusine coracana* (L.) for different plant growth promotion (PGP) activities: An *in-vitro* study

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Abstract
Endophytic bacteria are ubiquitous in most plant species influencing the host fitness by disease suppression, contaminant degradation, and plant growth promotion. In the present study, an endophytic bacterium has been isolated from root of Finger millet (*Eleusine coracana* (L.)) and screened for different plant growth promoting activities *viz.* Phosphate solubilization, Nitrogen fixation, Indole acetic acid IAA production and 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity. The results showed that out of 30 endophytic isolates 7 isolate showed phosphate solubilization activity, 2 isolates showed nitrogen fixing activity, 4 isolates showed IAA production activity and 9 isolates showed ACC deaminase activity. On the basis of *In vitro* efficacy, isolate EP 6 showed phosphate solubilization, IAA production and ACC deaminase activity whereas, EP 17 showed nitrogen fixation, IAA production and ACC deaminase activity. Two most potent isolates were characterized and identified as *Bacillus subtilis* (EP 6) and *Achromobacter xylosoxidans* (EP 17) as per BIOLOG on the basis of biochemical tests.

Keywords: Finger millet, Endophytes, PGP activity, *Bacillus subtilis, Achromobacter xylosoxidans*

Introduction
Finger millet (*Eleusine coracana* (L.)) is an important cereal crop widely grown by subsistence farmers in Africa and South Asia (Goron and Raizada, 2015) [13]. Finger millet is known in many ancient languages, referred to as wimbi (in Swahili), bule (Bantu), dagussa/tokuso (Amharic), mugimbi (Kikuyu), tailabon (Arabic), and ragi (in South Asian languages) (Vietmeyer, 1996) [41]. Finger millet was domesticated in Western Uganda and Ethiopia around 5000 BC then reached India by 3000 BC. Finger millet is well known by local farmers as a crop that tolerates stress conditions and that resists diverse pathogens (Goron and Raizada, 2015) [13]. In Gujarat, finger millet is the staple food of the tribal's in Agro climatic Zone – I, II and III. It is grown as *kharif* rainfed crop in the least fertile hilly soils. Finger millet grains are rich source of protein, dietary fiber, minerals and amino acids.

Endophytic bacteria are bacteria that live in plant tissues without doing substantive harm or gaining benefit other than residency (Kobayashi and Palumbo, 2000) [22]. Bacterial endophytes can be isolated from surface disinfected plant tissue or extracted from internal plant tissue (Hallmann *et al.* 1997) [16]. Both gram-positive and gram-negative bacterial endophytes have been isolated from several tissue types in numerous plant species. Furthermore, several different bacterial species have been isolated from a single plant (Kobayashi & Palumbo, 2000) [22]. Endophytes enter plant tissue primarily through the root zone; however, aerial portions of plants, such as flowers, stems, and cotyledons, may also be used for entry (Kobayashi & Palumbo, 2000) [22]. Specifically, the bacteria enter tissues via germinating radicles (Gagne *et al.* 1987) [10, 11], secondary roots (Agarwal and Shende, 1987) [1], stomates (Roos and Hattingh, 1983) [34], or as a result of foliar damage (Leben *et al*. 1968) [25].

Plants are constantly involved in interactions with a wide range of bacteria. These plant associated bacteria colonize the rhizosphere (rhizobacteria), the phyllosphere (epiphytes) and the inside of plant tissues (endophytes). Endophytes are sheltered from environmental stresses and microbial competition by the host plants. Endophytic bacteria have been isolated and characterized from diverse type of plant hosts. These include agronomic crops, prairie plants, plants growing in extreme environments, and wild and perennial plants (Yuan *et al.* 2014; Zinniel *et al.* 2002) [43, 44]. Endophytic bacteria have been isolated from different plant parts that are above and below ground (Senthilkumar *et al.* 2011) [35]. These include roots, stems, leaves, seeds, fruits, tubers, ovules and nodules, where roots have the greatest number of bacterial endophytes as compared to above ground tissues. Abundant and diverse populations

of bacterial endophytes were identified in potato (Garbeva *et al.* 2001) [12], maize (Fisher *et al.* 1992) [9], rice (Stoltzfus *et al.* 1997) [38, 39], cotton (McInroy and Kloepper, 1995) [28] and cucumber (Mahafee and Kloepper, 1997) [27].

Some endophytic bacteria exert several beneficial effects on host plants, through one or more mechanisms, including biological nitrogen fixation (Verma *et al.* 2013) [40], phosphate solubilization (Krey *et al.* 2013) [24], production of hormones such as auxins, gibberellins and zeatin (Cassan *et al.* 2009) [6, 36], or act indirectly by means of biological control of pathogens (Wang *et al.* 2009) [42]. Thus, the present investigation was taken up to isolate and characterize endophytic bacteria from finger millet (*Eleusine coracana* (L.).

Material and Methods

Isolation of endophytic bacteria from finger millet root

The finger millet plant was collected from Rajendrapur farm of College of Agriculture, Navsari Agricultural University, Waghai (Dangs). The plant root was separated from the collected samples such that it contained approximately 2-3 cm in length portion of root. Soils around root were washed under tap water. Any visible damaged material was excluded. The tissue was put in beaker, soaked in distilled water and drained. It was rinsed in 70% ethanol for 30 seconds and then sterilized with 0.1% $HgCl_2$ for 3 minutes. The tissue was then washed ten times with sterile water (Gagne *et al.* 1987) [10, 11]. Surface disinfected tissue was aseptically macerated with homogenizers. Macerated tissue was diluted into 10^{-1} dilution by adding 9 volumes of sterile distilled water. Serial dilution was made up to 10^{-6} dilution by taking 1 ml of well shaken suspension and adding into 9 ml water blank tubes. 100 µl from appropriate dilutions were spread plated on nutrient agar (NA) and incubate at 28^0C for 48 hours. Endophytic bacterial strains growing on NA plates were isolated, purified and preserved on agar slant for further studies.

In-vitro screening of isolates for different plant growth promoting activities.

Phosphate solubilization

The isolates were screened for phosphate solubilization as per methodology described by Gupta *et al.* (1994) [15]. On modified Pikovskaya agar with insoluble tricalcium phosphate (TCP), a loop full of each culture was placed on the centre of agar plates and incubated at 30±0.1 °C for 5 days. Then the ability of PSM to solubilize the insoluble phosphate was studied by the determination of solubilization index: the ratio of the total diameter (colony + halozone) and the colony diameter (Edi-Premono *et al.* 1996) [7].

$$\text{Solubilization Index} = \frac{\text{Colony diameter} + \text{Halozone diameter}}{\text{Halozone diameter}}$$

Nitrogen Fixation

Each isolated strains were inoculated in Norris glucose nitrogen free medium plates and incubated at 28^0C for 7 days, bacterial growth was observed as qualitative evidence of the atmospheric nitrogen fixation Newton *et al.* (1976) [31].

Production of indole acetic acid (IAA)

IAA production was detected as described by Brick *et al.* (2004) [5]. Bacterial cultures were grown on Luria Bertani (LB) broth amended with 100mg/l tryptophan as the precursor of IAA and incubated in a shaker at 250 rpm at 30^0C for 3 to 5 days. Fully grown cultures were centrifuged at 10,000 rpm for 10 min. 2 ml of the supernatant was mixed with 4 ml of the salkowski reagent. Appearances of pink color in test tubes were indicating a positive result for IAA production.

1-aminocyclopropane-1-carboxylate (ACC) deaminase activity

The isolates were screened for ACC-deaminase activity on (Dworkin & Foster) DF medium containing 3mM ACC as sole source of nitrogen and incubated 28 ^{0}C. Presence of the colonies on agar was taken as a positive indicator of ACC deaminase production by the isolated bacteria (Kadioglu *et al.* 2018) [19].

Characterization and Identification of isolated Endophytic bacteria

Two most potent endophytic isolates were characterized in terms of different biochemical tests and identified by 96 well based biochemical identification method (BIOLOG).

Result and Discussion

Isolation of endophytic bacteria from finger millet root

Nutrient agar plates inoculated with root extract of finger millet showed morphologically different bacterial colonies. Morphological characterization of the 30 isolates based on Gram test revealed that 30 of the endophytic bacterial 17 were Gram positive and 13 were Gram negative (Table 1). The diversity of a collection of 30 endophytic bacteria isolated from root of Finger millet was assessed using phenotypic characterization methods. Colony morphology gave an indication of the variation among the endophytes. Earlier workers have reported a predominance of Gram negative bacteria in the tissues of various plants (Stoltzfus *et al.* 1997; Elbeltagy *et al.* 2000) [38, 39]. However, Zinniel *et al.* (2002) [44] reported an equal presence of Gram negative and Gram positive bacteria. A total of 65 bacterial endophytes were isolated from three tissues: stem, root and nodule of soybean *viz*. *Glycine max* and *G. soja*. and showed that approximately equal percentages of gram positive (49%) and gram negative (51%) bacteria were present (Hung and Annapurna 2004) [17]. Another study by Aravind *et al.* (2009) [2] revealed the presence of *Bacillus* spp., *Pseudomonads*, *Arthrobacter* spp., *Micrococcus* spp., *Curtobacterium* spp., and *Serratia* as endophytes in the different varieties of black pepper (*Piper nigrum* L.)

Table 1: Morphological characters of isolate

Sr. No.	Isolates	Shape	Arrangement	Gram Nature
1	EP 1	Round	Diplococci	+Ve
2	EP 2	Rod	Chain	+Ve
3	EP 3	Rod	Single	-Ve
4	EP 4	Rod	Diplobacilli	+Ve
5	EP 5	Round	Diplococci	+Ve
6	EP 6	Rod	Short chain	+Ve
7	EP 7	Round	Single	+Ve

8	EP 8	Rod	Diplobacilli	+Ve
9	EP 9	Rod	Chain	+Ve
10	EP 10	Round	Tetrad	+Ve
11	EP 11	Rod	Chain	+Ve
12	EP 12	Rod	Single	-Ve
13	EP 13	Rod	Chain	-Ve
14	EP 14	Round	Diplococci	+Ve
15	EP 15	Round	Staphylococci	+Ve
16	EP 16	Rod	Diplobacilli	-Ve
17	EP 17	Rod	Single	-Ve
18	EP 18	Round	Single	+Ve
19	EP 19	Rod	Single	-Ve
20	EP 20	Rod	Chain	+Ve
21	EP 21	Rod	Diplobacilli	-Ve
22	EP 22	Rod	Chain	+Ve
23	EP 23	Round	Diplococci	+Ve
24	EP 24	Rod	Pair	-Ve
25	EP 25	Rod	Single	-Ve
26	EP 26	Rod	Chain	+Ve
27	EP 27	Rod	Diplobacilli	-Ve
28	EP 28	Rod	Single	-Ve
29	EP 29	Rod	Chain	-Ve
30	EP 30	Rod	Single	-Ve

In-vitro screening of isolates for different plant growth promoting activities

In present study total 30 endophytic bacterial isolates were obtained from root of finger millet. All the isolates were screened for phosphate solubilization on Pikovyaskya's medium, nitrogen fixation capacity on Norris glucose nitrogen free medium, IAA production on LB medium amended with 100 mg/l tryptophan, and ACC deaminase activity on minimal DF (Dworkin and Foster) media containing 0.5% ACC (1-aminocyclopropane-1-carboxylic acid) as sole nitrogen source. Results are summarized in Table 2 and Fig. 1.

Phosphate solubilization activity of the endophytic isolates was successfully detected. It was observed that isolated endophytes solubilizing phosphate by forming hyaline halo around in periphery of colony. It was observed that highest phosphate solubilization zone ratio was found in EP 6 (5.8) followed by EP 10 (4.2), EP 20 (3.7), EP 2 & EP 12 (3.2) and EP 3 (2.5). However lowest phosphate solubilization zone ratio was found in EP 15 (0.7). Plant growth and yield are essentially dependent on the availability of minerals which they directly or indirectly acquire from soil in the soluble ionic forms. Soil contains 0.5% phosphorus, mostly in the form of insoluble mineral complexes which plants cannot absorb (Rengel and Marschner, 2005) [33], only 0.1% of the total phosphorous exists in a soluble form available for plant uptake (Zou *et al.* 1992) [45]. Phosphate solubilization is a common trait among plant endophytic bacteria. Phosphate-solubilising bacteria are able to solubilize bound phosphorous from organic or inorganic molecules, by secretion of organic acids and phosphatases thereby making it readily available for the plant (Kim *et al.* 1998) [21]. Borah *et al.* (2017) [4] reported that endophytic bacteria isolated from the *Oryza sativa* showed good phosphate solubilising activity and based on 16s rRNA gene sequence analysis the isolates were identified as *Pantoeaa ananatis*, *Pseudomonas putida*, *Brevibacillus agri*, *Bacillus subtilis* and *Bacillus megaterium*. Rani *et al.* (2018) [32] reported that Out of the 15 diazotropic endophytic isolates 11 isolates were shown to solubilize phosphate from different indigenous rice *i.e* Sada Gora (SG), Bala Gora (BG), Kala Gora (KG) and Kharani (KH).

Nitrogen (N) is an essential component of all proteins and enzymes, nucleic acids that make up DNA, and chlorophyll that enables the process of photosynthesis in plants (Leghari *et al.* 2016) [26]. It is a very common element in nature that is present in abundant amounts in atmosphere, lithosphere, and hydrosphere of the earth. However, much of this N is in the form of dinitrogen (N2), which is inert and cannot be used by plants. In order for plants to use this dinitrogen, it has to be reduced/fixed into forms like nitrate (NO_3^-) and ammonium (NH_4^+). N fixation, the process by which dinitrogen is reduced to plant available forms, is, therefore, a vital process for the sustenance of life on earth. In present investigation, it was determined that the two endophytic isolates EP 17 and EP 24 from finger millet showed nitrogen fixing activity as appearance of growth on norris glucose nitrogen free medium plates. Koomnok *et al*. (2007) [23] reported that the highest endophytic bacterial population (5.25×10^6 per gram fresh material) was found in the roots of *Oryza rufipogon*, and this population showed the highest nitrogenase activity. They also characterized diazotrophic bacteria as *Azospirillum*, *Herbaspirillum*, *Beijerinckia* and *Pseudomonas*. Majority of the endophytic isolates (15 out of 20 isolates) from *Ophioglossum reticulatum* L. were able to grow in N2-free medium indicating their ability to fix atmospheric nitrogen (Mukherjee *et al.* 2017) [29].

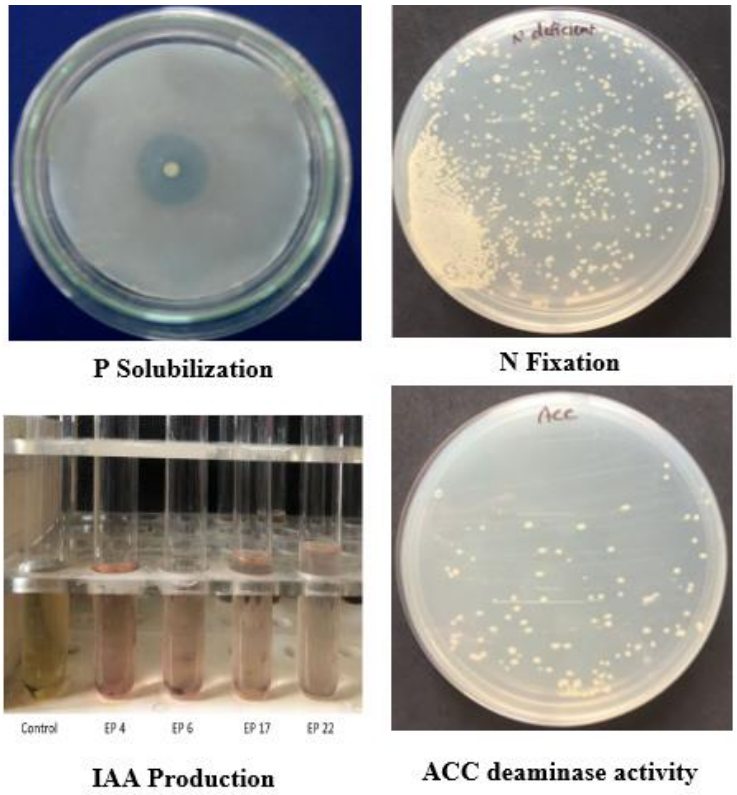
In the present study, it was determined that the endophytic isolates from finger millet have potential to produce indole acetic acid (IAA) as a stimulating phytohormone. IAA production was determined by sensitive salkowski's reagent to indol-3-acetic acid producing pink colour. Out of 30 isolates four isolates EP 4, EP 6, EP 17 and EP 22 showed IAA production activity. Bhutani *et al*. (2018) [3] reported that Endophytic bacteria *Bacillus aryabhattai*, *B. megaterium* and *B. cereus* isolated from nodules of *Vigna radiata* were producing significantly high amount of IAA. Shirsh and Jha 2020 [37] also reported that endophytic bacteria inhabiting the roots, stems and leaves of finger millet found positive for IAA, cellulase and protease and to solubilise phosphorous and zinc qualitatively. Endophytic bacteria can produce indole acetic acid (IAA) in the tissues of the root and stem of wheat Jha and Kumar (2009) [18].

The potential ACC deaminase activity was evaluated *in-vitro* in chemically defined medium. Endophytic isolates EP 2, EP 3. EP 6, EP 7, EP 10, EP 12, EP 16, EP 17 and EP 21 showed

positive growth on 1-aminocyclopropane-1-carboxylate medium through ACC deaminase activity. Khan *et al*. (2016) [20] reported that endophytic bacterial strain *Bacillus subtilis* LK14 have shown significant prospects in phosphate solubilization, ACC deaminase and acid phosphatase activity. Inoculation of plants with ACC producing bacteria has a positive effect on their growth under stress conditions (Grobelak *et al.* 2018) [14]

Table 2: Plant growth promoting activities of isolates

Sr. No.	P Solubilization Zone ratio	N Fixation	IAA Production	ACC Deaminase activity
EP 1	-	-	-	-
EP 2	3.2	-	-	+
EP 3	2.5	-	-	+
EP 4	-	-	+	-
EP 5	-	-	-	-
EP 6	5.8	-	+	+
EP 7	-	-	-	+
EP 8	-	-	-	-
EP 9	-	-	-	-
EP 10	4.2	-	-	+
EP 11	-	-	-	-
EP 12	3.2	-	-	+
EP 13	-	-	-	-
EP 14	-	-	-	-
EP 15	0.7	-	-	-
EP 16	-	-	-	+
EP 17	-	+	+	+
EP 18	-	-	-	-
EP 19	-	-	-	-
EP 20	3.7	-	-	-
EP 21	-	-	-	+
EP 22	-	-	+	-
EP 23	-	-	-	-
EP 24	-	+	-	-
EP 25	-	-	-	-
EP 26	-	-	-	-
EP 27	-	-	-	-
EP 28	-	-	-	-
EP 29	-	-	-	-
EP 30	-	-	-	-



P Solubilization N Fixation

IAA Production ACC deaminase activity

Fig 1: PGP activity of endophytic isolates

Characterization and Identification of isolated Endophytic bacteria: In present study two most potent endophytic isolates EP 6 and EP 17 were characterized in terms of different biochemical tests *viz.* catalase, citrate utilization, gelatin hydrolysi, methyl red, voges-proskauer, starch hydrolysis, urease, phenylalanine deamination, motility and utilization of different sugars. Results are summarized in Table 3. Identification was done by 96 well based biochemical identification method (BIOLOG). The result of the biochemical test and BIOLOG data shows that EP 6 was *Bacillus subtilis* (Table 4) and EP 17 was *Achromobacter xylosoxidans* (EP 17) (Table 5). Muzzamal *et al*. (2012) [30] reported that 76 endophytic bacterial isolates obtained from different plant tissues including root, stem and fresh and wilted leaves of various plants in Punjab, Pakistan were belongs to the genera *Bacillus, Pseudomonas, Serratia, Stenotrophomonas and Micromonospora*. Endophytic plant growth promoting bacteria (PGPB) associated to the halophyte *Prosopis strombulifera* were identified as *Bacillus, Lysinibacillus, Pseudomonas, Achromobacter* and *Brevibacterium* (Sgroy *et al*. 2009) [36]

Table 3: Biochemical characterization of isolates

Sr. No.	Test	EP 6	EP 17
1.	Catalase	Positive	Positive
2.	Citrate utilization test	Positive	Positive
3.	Gelatin hydrolysis	Positive	Negative
4.	Methyl Red test	Negative	Negative
5.	Voges-Proskauer test	Positive	Negative
6.	Starch hydrolysis	Positive	Negative
7.	Urease test	Negative	Negative
8.	Phenylalanine Deamination test	Negative	Negative
9.	Motility	Positive	Positive
10.	Sugar utilization test		
	Glucose	Positive	Positive
	Lactose	Negative	Negative
	Sucrose	Positive	Negative
	Mannitol	Positive	Negative
	Maltose	Positive	Negative
	Fructose	Positive	Negative
	Galactose	Negative	Negative

Table 4: Biochemical identification of *Bacillus subtilis* by BIOLOG

A1 Negative Control	A2 Dextrin	A3 D-Maltose	A4 D-Trehalose	A5 D-Celloblose	A6 Gentloblose	A7 Sucrose	A8 D-Turanose	A9 Stachyose	A10 Positive Control	A11 PH6	A12 PH5
B1 D-Raffinose	B2 α-D-Lactose	B3 D-Mellobiose	B4 β-Methyl-D-Glucoside	B5 D-Sallcin	B6 N-Acetyl-D-Glucosamin	B7 N-Acetyi-β-D-Mannosamine	B8 N-Acetyl-D-Galactosamlne	B9 N-Acetyl Neuraminic acid	B10 1% Nacl	B11 4% Nacl	B12 8% Nacl
C1 α-D-Glucose	C2 D-Mannose	C3 D-Fructose	C4 D-Galactose	C5 3-Methyl Glucose	C6 D-Fucose	C7 L-Fucose	C8 L-Rhamnose	C9 Inosine	C10 1% Sodium Lactate	C11 Fusidic acid	C12 D-Serine
D1 D-Sorbitol	D2 D-Mannitol	D3 D-Arabitol	D4 MYO-Inositol	D5 Glycerol	D6 D-Glucose-6-po4	D7 D-Fructose-6-PO4	D8 D-Aspartic acid	D9 D-Serine	D10 Troleadomycin	D11 Rifamycin SV	D12 Minocyclin
E1 Gelatin	E2 Glycyl-Proline	E3 L-Alanine	E4 L-Arginine	E5 L-Aspartic acid	E6 L-Glutamic acid	E7 L-Histidine	E8 L-Pyroglutamic acld	E9 L-Serine	E10 Lincomycin	E11 Guanldine HCI	E12 Nlaproof 4
F1 Pectin	F2 D-Galacturonic acid	F3 L-Galactonic acid Lactone	F4 D-Gluconic	F5 D-Glucuronlc acid	F6 Glucuronamide	F7 Mucle acid	F8 Qulnlc acid	F9 D-Saccharic acid	F10 Vancomycln	F11 Tefrazollum Vlolet	F12 Tefrazollum blue
G1 p-Hydroxy-Phenylacetic acld	G2 Methyl Pyruvate	G3 D-Lactic acid Methyl ester	G4 L-Lactic acid	G5 Cltric acid	G6 α-Keto-Glutaric acld	G7 D-Mallc acld	G8 L-Mallc acld	G9 Bromo-Succinic acid	G10 Nalldixic acid	G11 Lithium Chloride	G12 Potassium Tellurite
H1 Tween40	H2 y-Amino-Butyric acid	H3 α-Hydroxy-Butyric acid	H4 β—Hydroxy D-,L-Butyric acid	H5 α-Keto-Butyric acid	H6 Acetoacetic acid	H7 Proplonic acid	H8 Acetic acid	H9 Formic acid	H10 Azteonam	H11 Sodium Butyrate	H12 Sodium Bromate

Positive Borderline Negative

Table 5: Biochemical identification of *Achromobacter xylosoxidans* by Biolog

A1 Negative Control	A2 Dextrin	A3 D-Maltose	A4 D-Trehalose	A5 D-Celloblose	A6 Gentloblose	A7 Sucrose	A8 D-Turanose	A9 Stachyose	A10 Positive Control	A11 PH6	A12 PH5
B1 D-Raffinose	B2 α-D-Lactose	B3 D-	B4 β-Methyl-	B5 D-Sallcin	B6 N-Acetyl-D-	B7 N-Acetyi-β-D-	B8 N-Acetyl-D-	B9 N-Acetyl	B10 1% Nacl	B11 4% Nacl	B12 8% Nacl

		Mellobiose	D-Glucoside		Glucosamin	Mannosamine	Galactosamlne	Neuraminic acid			
C1 α-D-Glucose	C2 D-Mannose	C3 D-Fructose	C4 D-Galactose	C5 3-Methyl Glucose	C6 D-Fucose	C7 L-Fucose	C8 L-Rhamnose	C9 Inosine	C10 1% Sodium Lactate	C11 Fusidic acid	C12 D-Serine
D1 D-Sorbitol	D2 D-Mannitol	D3 D-Arabitol	D4 MYO-Inositol	D5 Glycerol	D6 D-Glucose-6-po4	D7 D-Fructose-6-PO4	D8 D-Aspartic acid	D9 D-Serine	D10 Troleadomycin	D11 Rifam ycin SV	D12 Minocyclin
E1 Gelatin	E2 Glycyl-Proline	E3 L-Alanine	E4 L-Arginine	E5 L-Aspartic acid	E6 L-Glutamic acid	E7 L-Histidine	E8 L-Pyroglutamic acld	E9 L-Serine	E10 Lincomycin	E11 Guanldine HCI	E12 Nlaproof 4
F1 Pectin	F2 D-Galacturonic acid	F3 L-Galactonic acid Lactone	F4 D-Gluconic	F5 D-Glucuronlc acid	F6 Glucuronamide	F7 Mucle acid	F8 Qulnlc acid	F9 D-Saccharic acid	F10 Vancomycln	F11 Tefrazollum Vlolet	F12 Tefrazollum blue
G1 p-Hydroxy-Phenylacetic acld	G2 Methyl Pyruvate	G3 D-Lactic acid Methyl ester	G4 L-Lactic acid	G5 Cltric acid	G6 α-Keto-Glutaric acld	G7 D-Mallc acld	G8 L-Mallc acld	G9 Bromo-Succinic acid	G10 Nalldixic acid	G11 Lithium Chloride	G12 Potassium Tellurite
H1 Tween40	H2 y-Amino-Butryric acid	H3 α-Hydroxy-Butyric acid	H4 β—Hydroxy D-,L-Butyric acid	H5 α-Keto-Butyric acid	H6 Acetoacetic acid	H7 Proplonic acid	H8 Acetic acid	H9 Formic acid	H10 Azteonam	H11 Sodium Butyrate	H12 Sodium Bromate

Positive Borderline Negative

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