

Rhizospheric bacteria of sugar cane from punjab

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Abstract

In an ecological survey twenty-nine strains of bacteria were isolated from the Rhizosphere of sugarcane. Different varieties of sugar cane were collected from Bahawalnagar, Okara, Hujra shah Muqem, and Kasur, (Punjab). These were grown on Nitrogen free malate (NFM), Luria Beratani Agar (LBA), and Mannitol Agar (MA). They include eight isolates of genus *Pasteurella*, four isolates of *Klebsiella*, three isolates of *Burkholderia*, two isolates of each *Salmonella*, *Yersinia*, *Proteus*, *Serratia* and *Stenotrophomonas*, one isolate of each *Pantoea*, *Providencia*, *Escherichia*, *Ralstonia*, *Enterobacter* and *Shigella*. All these cultures are available in preserved form in repository of FCBP.

Keywords: Bacteria, Rhizospheric, Sugarcane, Punjab.

Introduction

For the first time N₂-fixing bacteria were isolated from the sugar cane roots, soil and leaves in various agroecological zones of Brazil (Cavaleantae & Dobereiner, 1988). This finding lead to the opening of new horizons in the field of isolation of nitrogen fixing bacteria from roots stem and rhizosphere of sugarcane. A strain of nitrogen-fixing bacteria isolated from the root of sugar cane has been identified as *Pantoea agglomerans* using a biochemical test data. (Xinghong *et al.*, 1998). Mirza *et al.*, (2001) isolated strains of *Enterobacter* and *Klebsiella* from sugar cane plants. Reis *et al.*, (2004), isolated nitrogen fixing bacteria from the rhizosphere of sugarcane, maize and teosinte plants in Brazil, Mexico and South Africa, these worker identified a new phylogenetically homogeneous group of N₂-fixing bacteria within the genus *Burkholderia*. The occurrence of N₂-fixing *Burkholderia* species associated with different varieties of sugarcane and maize growing in regions of Mexico and Brazil was analyzed. Only 111 out of more than 900 isolates recovered had N₂-fixing ability (Perin *et al.*, 2006). An effort has been made to isolate some bacterial strains which may further be utilized for growth promotion and nitrogen fixation activities of non-leguminous plants.

Materials and Methods

Different varieties of sugar cane from Bahawalnagar, Okara, Hujra shah Muqem, and Kasur, (Punjab) were collected. The isolations were made from roots and stem of sugar cane plants. Fresh roots and stem samples were cut into 2- 3 cm pieces; surface sterilized, crushed and inoculated on media plates by optimized

serial dilution method. For inoculation a specific Nitrogen-Free Malate (NFM) medium (Döbereiner *et al.*, 1976) and LBA (Luria Bretani Agar) was used. These were incubated for 5-6 days at 30°C. The cultures were purified and single cell colonies were then projected to some tests for there confirmation at genus level i.e. morphological characterization including; single cell & colony morphology and biochemical tests by using Microbact and QTS-24 identification kit.

Results

The following bacteria were isolated during December 2006 and January 2007. Almost all the species and strains had plant growth promoting and nitrogen-fixing ability. The gram-negative rods were identified using diagnostic and bio-chemical tests (Table 2 & 3). They were isolated in pure culture (Table1).The pure culture was preserved in glycerol at -20°C.

1. *Burkholderia* (*Yabuuchi et al.* 1993 emend. *Gillis et al.* 1995.)

Burkholderia (previously part of *Pseudomonas*) genus name refers to a group of virtually ubiquitous gram-negative, motile, obligately aerobic rod-shaped bacteria including plant pathogens as well as some environmentally-important species have either a single polar flagellum or a tuft of polar flagella.

a) *Burkholderia pseudomallei*. (*Whitmore 1913 and Yabuuchi et al.* 1993)

Isolation Information

Isolate 1: Sugarcane stem, Hujra Shah Muqem, 29.10.06 (BCCP; 036).

Isolate II: Sugarcane stem, Kasur, 10.11.06, (BCCP; 055).

b) *Burkholderia mallei* (Zopf 1885) Yabuuchi *et al.* 1993, comb nov.).

Isolation Information

Sugarcane stem, Bahawalnagar, 6.11.06, (BCCP; 052).

2. *Pasteurella* (Trevisan 1887)

Pasteurella is a genus of Gram-negative, facultatively anaerobic bacteria. *Pasteurella* species are non-motile and pleomorphic. Spherical ovoid – rod shaped, 0.3-1.0µm in diameter occur singly or in short chains. Oxidase catalase positive.

a) *Pasteurella multocida* (Lehmann and Neumann 1899) Rosenbusch and Merchant 1939.

Isolation Information

Isolate I: Sugarcane stem, Kasur, 10.11.06(BCCP; 039).

Isolate II: Sugarcane root, Kasur, 10.11.06, (BCCP; 044a).

b) *Pasteurella hemolytical type T* (Newsom and Cross 1932).

Isolation Information

Sugarcane stem, Kasur, 10.11.06, (BCCP; 044b).

c) *Pasteurella hemolytical type A* (Newsom and Cross 1932)

Isolation Information

Isolate I: Sugarcane stem, Okara, 26.10.06, (BCCP; 053).

Isolate II: Sugarcane root, Okara, 26.10.06(BCCP; 057).

d) *Pasteurella ureae* (Jones 1962)

Isolation Information

Isolate I: Sugarcane root, Okara, 26.10.06, (BCCP; 054)

Isolate II: Sugarcane stem, Okara, 26.10.06(BCCP; 058)

Isolate III: Sugarcane stem, Bahawalnagar, 6.11.06(BCCP; 062)

3. *Klebsiella* (Trevisan 1885)

Klebsiella is a genus of non-motile, gram-negative bacteria with a prominent polysaccharide-based capsule. These are straight rods 0.3-1.0x 0.6-6.0µm, arranged singly, in

pairs, or in short chains, facultatively anaerobes, oxidase negative and catalase positive.

a) *Klebsiella pneumoniae* (Schroeter 1886, Trevisan 1887)

Isolation Information

Isolate I: Sugarcane stem, Bahawalnagar, 6.11.06(BCCP; 047)

Isolate II: Sugarcane root, Okara, 26.10.06 (BCCP; 050).

Isolate III: Sugarcane stem, Hujra Shah Muqem 22.04.07 (BCCP; 076).

b) *Klebsiella pneumoniae s sp.rhinoscleromatis* (Trevisan 1887 Ørskov 1984,)

Isolation Information

Sugarcane root, Okara, 26.10.06(BCCP; 059)

4. *Salmonella* (Lignieres 1900)

Salmonella is a genus Gram-negative, facultatively anaerobic, straight rod-shaped 0.7-1.5x 2-5 µm, usually motile, by peritrichous flagella, bacteria of the Enterobacteriaceae oxidase negative and catalase positive VP negative, H₂S is produced, urea is not hydrolysed, growth on KCN and utilization of malonate is variable.

a) *Salmonella galinarum* (Lignieres 1900)

Isolation Information

Sugarcane stem, Hujra Shah Muqem 29.10.06. (BCCP; 038).

b) *Salmonella typhi* (Schroeter 1886, Warren and Scott 1930)

Isolation Information

Sugarcane stem, Bahawalnagar, 6.11.06 (BCCP; 042).

5. *Yersinia* (Loghem, 1944)

Yersinia is a genus of bacteria in the family Enterobacteriaceae. *Yersinia* is gram negative rod shaped bacteria, facultative anaerobes, 0.5-0.8 x 1-3 µm non-motile at 37 °C, but motile with peritrichous flagella when grown below 30°C. Optimal temperature 28-30°C. urea is generally hydrolysed and few strains grew on KCN.

a) *Yersinia sp.(typical group-1)* (Loghem, 1944)

Isolation Information

Sugarcane stem, Hujra Shah Muqem
29.10.06(BCCP; 040).

b) *Yersinia enterocolitica* (Schleifstein and Coleman 1939) Frederiksen 1964)

Isolation Information

Sugarcane stem, Kasur, 10.11.06(BCCP; 056)

6. *Proteus* (Hauser 1885)

Straight rods 0.4-0.8 μ m in diameter., 1-3 μ m in length gram negative, motile by peritrichous flagella, forming concentric zones over moist surface of nutrient agar, facultatively anaerobic, glucose and few other carbohydrates are catabolyzed with production of acid and gas.

a) *Proteus vulgaris* (Hauser 1885)

Isolation Information

Sugarcane stem, Hujra Shah Muqem
22.04.07(BCCP; 064).

b) *Proteus merabillis* (Hauser 1885)

Isolation Information

Sugar cane stem, Okara, 26.10.06(BCCP; 043).

7. *Serratia* (Bizio 1823)

Serratia is a genus of straight rods, 0.5-0.8 x 0.9-2 μ m, gram negative facultatively anaerobe, glucose and other carbohydrates are catabolyzed with production of acid and often gas, indole negative, VP is positive, most strain produced the DN ase and hydrolysed corn oil and gelatin is usually hydrolyzed.

a) *Serretia rubidaea* (Stapp 1940, Ewing et al. 1973).

Isolation Information

Isolate I: Sugarcane stem, Bahawalnagar, 6.11.06, (BCCP; 045).

Isolate II: Sugarcane root, Okara, 26.10.06(BCCP; 060).

8. *Stenotrophomons* (Palleroni & Bradbury 1993)

Stenotrophomonas is a genus of Gram-negative bacteria, slightly helical rods 0.5-1x2-7 μ m, non-sporing motile by 2-8 flagella, inserted linearly on concave side, obtain energy by beta oxidation of fatty acids, in co culture with organisms utilize H₂S.

a) *Stenotrophomonas maltophilia* (Hugh 1981, Palleroni and Bradbury 1993)

Isolate I: Sugarcane stem, Kasur, 10.11.06(BCCP; 048).

Isolate II: Sugarcane stem, Okara, 26.10.06 (BCCP; 061).

9. *Pantoea* (Gavini et al. 1989)

Pantoea is a genus of gram-negative, straight rods, 0.5-1 x 1-3 μ m, family Enterobacteriaceae. Most strain produce yellow pigment, facultatively anaerobes, oxidase negative catalase positive indole negative no production of H₂S, urea is not hydrolyzed. Most strains grew on KCN.

a) *Pantoea-1* (Gavini et al. 1989)

Isolation Information

Sugarcane stem, Kasur 10.11.06 (BCCP; 037).

10. *Providencia* (Ewing 1962)

Providencia is a gram negative, motile bacteria of the family Enterobacteriaceae., straight rods 0.6-0.8 x 1.5-2.5 μ m, swarming doesnot occur, facultatively anaerobes oxidase negative catalase positive, indole positive grown on KCN glucose and other carbohydrates are catabolyzed with production of acid.

a) *Providencia rettgeri gp a* (Hadley 1918, Brenner *et al.* 1978)

Isolation Information

Sugarcane stem, Okara 26.10.06 (BCCP; 041).

11. *Escherichia* (Castellani & Chalmers 1919)

Escherichia is a genus of Gram-negative, non-spore forming, facultatively anaerobic, stiaight rod-shaped 1.1 -1.5 x 2-6 μ m occur singly or in pairs capsules are microcapsule occur in many stains, motile by peritrichous flagella oxidase negative catalase positive methyl red positive, VP negative citrate negative, negative for H₂S, urea hydrolysis and lipase.

a) *Escherichia coli* (Migula 1895, Castellani and Chalmers 1919)

Isolation Information

Sugarcane stem, Bahawalnagar 19.12.06 (BCCP; 046).

12. *Ralstonia* (Yabuuchi et al. 1996)

Ralstonia is a genus of proteobacteria, previously included in the genus *Pseudomonas*

Table 1: List of bacterial isolates from rhizosphere, stem and roots of sugar cane from Punjab.

Organism	Accession # of bccp at fcgp	Specimen examined	Place of collection	Date of collection
<i>Burkholderiapseudomallei</i>	036	Sugarcane stem	Hujra Shah Muqem	29.10.06
<i>Pantoea-1</i>	037	Sugarcane stem	Kasur	10.11.06
<i>Salmonella gallinarum</i>	038	Sugarcane stem	Hujra Shah Muqem	29.10.06
<i>Pasteurella multocida</i>	039	Sugarcane stem	Kasur	10.11.06
<i>Yersinia sp. (typical group-1)</i>	040	Sugarcane stem	Hujra Shah Muqem	29.10.06
<i>Providencia rettgeri gp a</i>	041	Sugarcane stem	Okara	26.10.06
<i>Salmonella typhi</i>	042	Sugarcane stem	Bahawalnagar	6.11.06
<i>Pasteurella multocida</i>	044a	Sugarcane root	Kasur	10.11.06
<i>Proteus merabillis</i>	043	Sugarcane stem	Okara	26.10.06
<i>Pasteurella sp (hemolytical type T)</i>	044b	Sugarcane stem	Kasur	10.11.06
<i>Serratia rubidaea</i>	045	Sugarcane stem	Bahawalnagar	6.11.06
<i>Escherichia coli</i>	046	Sugarcane stem	Bahawalnagar	19.12.06
<i>Klebsiella pneumoniae</i>	047	Sugarcane stem	Bahawalnagar	6.11.06
<i>Stenotrophomonas maltophilia</i>	048	Sugarcane stem	Kasur	10.11.06
<i>Ralstonia pickettii</i>	049	Sugarcane stem	Bahawalnagar	6.11.06
<i>Klebsiella pneumoniae</i>	050	Sugarcane root	Okara	26.10.06
<i>Enterobacter cloacae</i>	051	Sugarcane stem	Bahawalnagar	6.11.06
<i>Burkholderia mallei</i>	052	Sugarcane stem	Bahawalnagar	6.11.06
<i>Pasteurella sp. (hemolytical type A)</i>	053	Sugarcane stem	Okara	26.10.06
<i>Pasteurella ureae</i>	054	Sugarcane root	Okara	26.10.06
<i>Burkholderia pseudomallei</i>	055	Sugarcane stem	Kasur	10.11.06
<i>Yersinia enterocolitica</i>	056	Sugarcane stem	Kasur	10.11.06
<i>Pasteurella sp(hemolytical type A)</i>	057	Sugarcane root	Okara	26.10.06
<i>Pasteurella ureae</i>	058	Sugarcane stem	Okara	26.10.06
<i>Klebsiella pneumoniae s. sp rhinoscleromatis</i>	059	Sugarcane root	Okara	26.10.06
<i>Serratia rubidaea</i>	060	Sugarcane root	Okara	26.10.06
<i>Stenotrophomonas maltophilia</i>	061	Sugarcane stem	Okara	26.10.06
<i>Pasteurella ureae</i>	062	Sugarcane stem	Bahawalnagar	6.11.06
<i>Proteus vulgaris</i>	064	Sugarcane stem	Hujra Shah Muqem	22.04.07
<i>Shigella dysenteriae</i>	072	Sugarcane root	Hujra Shah Muqem	29.10.06
<i>Klebsiella pneumoniae</i>	076	Sugarcane stem	Hujra Shah Muqem	22.04.07

Culture Media used

NA; Nutrient Agar

LBA; Luria Bertani Agar

NFM; Nitrogen-free malate medium

.is a gram-negative, non sporing bacteria and rod-shaped.

a) *Ralstonia pickettii* (Ralston et al.1973, Yabuuchi et al. 1996)

Isolation Information

Sugarcane stem, Bahawalnagar 6.11.06 (BCCP; 049).

13. *Enterobacter* (Hormaeche & Edwards 1960)

Enterobacter is a genus of common gram-negative, facultatively-anaerobic, straight rod-shaped, 0.6-1 x 1.2-3 µm, optimal temperature 30-37°C, indole negative and VP positive, malonate is usually utilized and gelatin is slowly liquefied, H₂S and lipase are not produced, bacteria belong to the family *Enterobacteriaceae*.

a) *Enterobacter cloacae* (Jordan 1890, Hormaeche and Edwards 1960)

Isolation Information

Sugarcane stem, Bahawalnagar 6.11.06 (BCCP; 051).

14. *Shigella* (Castellani & Chalmers 1919)

Shigella is a genus of gram-negative, non-motile, non-spore forming rod-shaped bacteria facultatively anaerobes oxidase negative catalase positive production of indole varies, H₂S is not produced and no growth on KCN, closely related to *Escherichia coli* and *Salmonella*.

a) *Shigella dysenteriae* (Shiga 1897, Castellani & Chalmers 1919)

Shigella dysenteriae is a species of the rod-shaped bacterial genus *Shigella*. *Shigellae* are Gram-negative, non-spore-forming, facultatively anaerobic, non-motile bacteria.

Isolation Information

Sugar cane roots, Hujra shah Muqem 29.10.06 (BCCP; 072).

Table 2: Biochemical test for bacterial characterization

STRAINS	ONPG	CIT	MALO	LDC	ADH	ODC	H ₂ S	UREA	TDA	IND	VP	GEL	GLU	MALT	SUC	MANN	ARAB	SORB	ADO
<i>Burkholderiapseudomallei</i>	+	+	+	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-
<i>Pantoea-1</i>	+	-	+	-	-	-	-	-	+	-	+	+	+	-	+	+	-	+	-
<i>Salmonella gallinarum</i>	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+
<i>Pasteurella multocida</i>	+	+	+	-	-	-	-	+	+	-	-	+	+	+	+	+	+	+	-
<i>Yersinia p.(typical group-1)</i>	-	+	+	-	+	-	-	+	+	-	+	+	-	-	-	+	+	+	+
<i>Providencia rettgeri gp a</i>	+	+	+	-	-	-	-	+	+	-	-	+	+	-	-	+	+	-	+
<i>Salmonella typhi</i>	+	-	+	+	-	-	-	-	+	-	-	+	-	-	+	+	-	+	-
<i>Pasteurella multocida</i>	-	+	+	-	-	-	-	+	+	-	+	-	+	+	+	+	+	+	+
<i>Proteus merabillis</i>	+	+	+	-	+	-	+	+	+	-	+	+	+	-	-	+	-	-	+
<i>Pasteurella sp. (hemolytical type T)</i>	-	-	-	-	+	-	+	-	+	-	+	-	+	+	+	+	+	+	+
<i>Serratia rubidaea</i>	+	+	+	-	-	-	-	+	+	-	+	+	+	-	-	+	+	-	+
<i>Escherichia coli</i>	+	+	+	+	-	-	-	-	-	-	-	+	+	+	+	+	-	+	-
<i>Klebsiella pneumoniae</i>	+	+	+	+	-	-	-	+	-	-	-	+	+	+	+	-	-	-	-
<i>Stenotrophomonas maltophilia</i>	+	+	+	-	-	-	-	-	+	-	+	+	-	+	-	-	+	+	-
<i>Ralastonia pickettii</i>	-	+	+	+	-	+	+	+	-	-	-	+	+	+	+	-	-	-	-

Description of Biochemical Tests.

ONPG: Ortho-Nitrophenol test for beta-Galactosidase production

CIT: Citrate Utilization Test

LDC: Lysine Decarboxylase Test

ODC: Ornithine Decarboxylase Test

UREA: Urea Hydrolysis Test

IND: Indole Production Test

GEL: Gelatin Hydrolysis Test

MALT: Maltose Oxidation/Fermentation Test

SUC: Sucrose Oxidation/Fermentation Test

MANN: Mannitol Oxidation/Fermentation Test

ARAB: Arabinose Oxidation/Fermentation Test

SORB: Sorbitol Oxidation/Fermentation Test

ADO: Adonitol Oxidation/Fermentation Test

MALO: Malonate Test

ADH: Arginine Dihydrolase Test

H₂S: H₂S production Test

TDA: Tryptophan Deaminase Test

VP: Voges Proskauer

GLU: Glucose Oxidation/Fermentation Test

Table 3: Biochemical test for bacterial characterization

Strains	ONPG	CIT	MALO	LDC	ADH	ODC	H ₂ S	UREA	TDA	IND	VP	GEL	GLU	MALT	SUC	MANN	ARAB	SORB	ADO
<i>Enterobacter cloacae</i>	+	-	-	-	-	-	-	-	-	-	+	-	+	+	+	+	+	-	-
<i>Klebsiella pneumoniae</i>	+	-	+	+	+	-	-	+	-	-	+	+	-	+	+	+	-	+	-
<i>Burkholderia mallei</i>	+	-	-	-	+	-	+	-	-	-	-	+	-	+	+	+	+	+	+
<i>Pasteurella sp (hemolytical type A)</i>	+	-	+	-	-	-	-	-	+	-	+	+	+	+	+	+	-	-	-
<i>Pasteurella ureae</i>	-	-	+	-	-	+	+	+	-	-	-	+	+	+	+	+	-	-	-
<i>Burkholderia pseudomallei</i>	+	+	-	-	-	-	+	-	+	-	+	+	+	+	+	+	+	-	+
<i>Yersinia enterocolitica</i>	-	-	-	-	+	-	-	+	+	+	+	-	+	+	+	+	+	+	+
<i>Pasteurella sp.(hemolytical type A)</i>	+	+	+	-	-	-	-	-	+	-	-	-	-	+	+	+	+	-	-
<i>Pasteurella ureae</i>	+	+	+	-	-	+	+	+	+	-	-	+	+	+	+	+	+	-	-
<i>Klebsiella pneumoniae s. sp rhinoscleromatis</i>	-	-	-	-	-	-	-	-	+	-	-	+	+	+	+	+	+	+	-
<i>Serratia rubidaea</i>	+	+	+	-	-	-	-	+	+	-	+	+	+	-	-	+	+	-	+
<i>Stenotrophomonas maltophilia</i>	+	+	+	-	-	-	-	-	+	-	+	+	-	+	-	-	+	+	-
<i>Pasteurella ureae</i>	+	+	-	-	-	-	-	+	-	-	-	+	+	+	+	+	-	-	-
<i>Proteus vulgaris</i>	-	+	+	-	+	-	+	+	-	-	-	-	+	+	+	+	+	+	-
<i>Shigella dysenteriae</i>	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	+	-	-
<i>Klebsiella pneumoniae</i>	+	+	+	+	-	-	-	+	+	-	-	-	+	+	+	-	-	-	-

Description of Biochemical Tests.**ONPG:** Ortho-Nitrophenol test for beta-Galactosidase production**CIT:** Citrate Utilization Test**LDC:** Lysine Decarboxylase Test**ODC:** Ornithine Decarboxylase Test**UREA:** Urea Hydrolysis Test**IND:** Indole Production Test**GEL :**Gelatin Hydrolysis Test**MALT:** Maltose Oxidation/Fermentation Test**SUC:** Sucrose Oxidation/Fermentation Test**MANN :**Mannitol Oxidation/Fermentation Test**ARAB :**Arabinose Oxidation/Fermentation Test**SORB :**Sorbitol Oxidation/Fermentation Test**ADO :**Adonitol Oxidation/Fermentation Test**MALO:** Malonate Test**ADH:** Arginine Dihydrolase Test**H₂S :**H₂S production Test**TDA :**Tryptophan Deaminase Test**VP:** Voges Proskauer**GLU:** Glucose Oxidation/Fermentation Test**References**

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