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Rhizosphere microflora of *Saccaharum officinarum*: Characterization for sugar fermenting potential

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Abstract
Microorganisms play an important role in maintaining soil and plant health. They act as biofertilizers and increase the resistance to biotic and abiotic stress. Sugarcane, like wheat, rice, corn and other grains, is of Gramineae family characterized by segmented stems, blade-like leaves, and reproduction by seed. Analysis of 21 sugarcane samples from fields of chunni kalan village (FATEHGARH SAHIB, PB) was done to study the density of microorganisms by standard plate count (SPC) method.Total viable counts of rhizospheric sample on Potato Dextrose Agar media were calculated to be $45x10^7$ cell\g and samples on the nutrient agar were calcutaed to be $181x10^7$ cells\g of soil. Of the various microflora identified from the surface of these samples some are bacterium such as, *Pseudomonas, Azospillum, Bacillus, Proteous* some are fugus such as *Penicillum, fusarium Aspergillus* were found to be the pre-dominant species in most of the samples. On the basis of saccrholytic activity *pseudomonas* showed non saccrholytic, *bacillus,* showing weak saccrholytic *Azotobacter,* showing strong saccrholytic bacteria. Soil microbial population is immersed in framework of interaction known to affect the plant roots. Fungal and bacterial population helps the plant to maintain the nutrient level through their various interactions. Soil bioata multiplies rapidly when organic material, roots and their food sources are available, and also when the soil is moist and warm.

Keywords: Sugarcane, bacteria, rhizosphere, fungus

Introduction
Sugarcane is a tall growing monocotyledonous crop plant that is cultivated in the tropical regions of the world primarily for its ability to store high concentration of sucrose, a sugar in the internodes of the stem. It is the name given to sucrose producing species of the genus *Saccharum.* Sugarcane, like wheat, rice, corn and other grains, is of the grass family, Gramineae, characterized by segmented stems, blade-like leaves, and reproduction is by seed.
Plants growing under natural conditions interact continuously with a broad array of prokaryotic and eukaryotic microorganisms. Many of these are injurious, but various organisms are beneficial to plants. Numerous investigators have indicated that a number of microorganisms are associated with sugarcane phyllosphere and rhizosphere. The phyllosphere is a term used in microbiology to refer to leaf surfaces or total above-ground surfaces of a plant as a habitat for microorganisms. The below-ground bacterial habitat (i.e. the root surfaces) is referred to as the rhizosphere. Rhizosphere is the soil immediately surrounding the plant roots. (Oyeyiola and Hussein, 1991) [13]. Microorganisms are important for soil quality and fertility. They play a major role in the decomposition of organic matter, degradation of chemical pollutants and mineralization in soil (Brussard, 1994) [5]. Microbes also influence the soil structure and aggregate stability (Gupta and Germida 1988) [8]. The size of microbial mass is considered important, as a large biomass can store nutrients through the system (Stenberg *et al.,* 1998) [17]. The region of soil surrounding and including the plant root (the rhizosphere) is of crucial importance for plant health and nutrition (Marschner, 1995) [12]. Rhizosphere is a partnership between the plants, soil and soil microorganisms. Plants provide the carbon source for soil organisms, that bind the soil particles into aggregates and recycle soil nutrients, and provide the habitat, water, and mineral nutrients for both soil organisms and plants (Clapperton, 1997) [6].
Sugarcane micro flora includes bacteria, fungi, yeast, molds, coliforms etc. The rhizosphere-associated beneficial bacteria consist of the following genera: (1) *Pseudomonas* and *Bacillus*, which antagonize pathogenic or deleterious microorganisms (biological control) and (2) bacteria that enhance plant growth directly such as *Azospirillum, Herbaspirillum*, *Enterobacter*, *Acetobacter*, *Azotobater*, and *Pseudomonas*, as well as many unidentified

rhizosphere isolates. It is widely accepted that rhizosphere and rhizoplane microorganisms can influence plant growth and development. The term plant growth-promoting rhizobacteria (PGPR) was coined for the bacterial biocontrol agents of the rhizosphere (Kloepper *et al.*, 1980) [10]. The term plant growth promoting bacteria (PGPB) was proposed to designate rhizobacteria that enhance plant growth by other ways (Bashan & Holguin, 1998) [2]. Plant growth-promoting (PGP) activities have been reported for a series of bacterial species including *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Klebsiella*, *Enterobacter*, *Alcaligens*, *Arthobacter*, *Burkholderia*, *Bacillus* and *Serratia*. A saccharolytic bacterium is capable of hydrolyzing or otherwise breaking down a sugar molecule. Many important bacteria of ecosystems are obtaining energy by fermenting carbohydrates. Their fermentation products, which accumulate, include the volatile fatty acids (VFA) and essential nutrients. Saccharolytic organisms produce acid and gas from fermentation of carbohydrates This fermentation activity is known as saccharolytic activity. Soil biota (bacteria and fungi) can influence the growth of some organisms including larger life forms such as certain insects, crop plants and weeds. . Keeping the above facts in minds the present study on "Rhizosphere and rhizoplane microflora of *Saccaharum Officinarum*: characterization for sugar fermenting potential"" was designed with the following objectives:-

- To isolate and identify the micro flora of Sugarcane rhizosphere
- To study the biochemical activities of all isolated microorganisms.
- To study the saccharolytic activity of bacteria isolated from sugarcane

Materials and Method

All the chemicals used for preparing reagents and solutions were procured from Hi-media, Sd-fine chemicals and were of AR grade. For biochemical characterization of isolates, dehydrated media used were procured from Hi- media and were used as per the manufacturer's direction. All the glassware like test tubes; beakers and Erlenmeyer's flasks etc. used were of Borosil.

Collection of Sample

Rhizospheric Samples were collected from the sugarcane fields of village Chunni kalan, Dist. Fathegarh Sahib (Punjab).

Isolation of microorganisms

All the soil samples were air dried at room temperature and sieved. These samples were then stored in polythene bags.isolation was done by Agar Plate Methed and Pour Plate Methed.

Total viable count

After incubation the plates were observed for number and distribution of colonies of bacteria and fungi from each dilution by applying the formula:

$$\text{Viable cells/ g dry soil} = \frac{\text{Mean plate count x dilution factor}}{\text{Dry weight of soil}}$$

Isolation of Pure Colonies

This is a most practical method of obtaining discrete colonies and pure cultures. The inoculum was placed on the agar surface with the help of loop at the edge and was streaked from side to side in parallel lines across the surface of area. Plates were incubated at 37 °C for 24 to 48 hrs.

Morphological and Biochemical characterization of isolates

Morphological characterization viz. colony morphology (colour, chromo genesis, shape, margin, elevation and surface). Cell morphology (Shape, Gram reaction and arrangement) of recovered isolates was studied by Gram's staining. Identification of the isolates was done by the standard biochemical tests as per the criteria laid down in the Bergey's manual of systemic bacteriology like IMViC, catalase, and Sugar fermentation (K.R. Aneja, 2006) [1].

Study of Saccharolytic Activity

Saccharolytic activity is the capability to ferment sugar or carbohydrates. Microorganisms produce acid and gas from fermentation of carbohydrates or sugar. Acid production in carbohydrates was detected by sugar fermentation method. The fermentation medium was prepared. Broth was taken into tubes and sterilized by autoclaving. Then inoculate tubes with culture and kept one as control(uninoculated).Then incubate all tubes at 35 ºC for 24-48 hrs. and then carbohydrates were used at I % (w/v) in peptone water; the indicator was acid fuchsin. Inoculation was from an 18 hrs. Peptone water culture.

Results and Discussion

The present study was carried out to study the micro flora associated with sugarcane and their saccrolytic activity. Thirty one samples were collected from collected from sugarcane field of village Chunni Kalan and were analyzed for various micro flora associated with Rhizosphere. The bacterial strains were characterized on the basis of various morphological and biochemical tests. The results of present study are summarized as under:

Total viable count

Total viable count of Rhizospheric sample on Nutrient agar was calculated to be $181x10^7$ cells/g of soil.TVC of Rhizospheric sample on PDA was calculated to be $45x10^7$ cells/g. (Table I & Figure 1) bacterial count is more than fungus in total viable count were in confirmty with the results obtained by Bond, (1994) [3] who said that bacteria are the most numerous culturable organisms in soils.

Isolation and characterization of microflora associated with rhizosphere of sugarcane

In the present study, microorganisms have been isolated from the rhizosphere of the sugarcane fields. These microorganisms were further characterized in terms of cultural, morphological and biochemical properties and were then identified on the basis of specific characteristics with the use of Bergey's manual of determinative Bacteriology. Cultural characteristics were observed on Nutrient Agar.

Identification of rhizosoheric isolates (Table II)

On the basis of cultural, morphological and biochemical characterization, isolates RC_1, RC3 and RC_6 were observed with oval, low convex, rough, flat, circular, opaque, green pigments on nutrient agar found to be of *Pseudomonas sp.* These were in conformity with the results of Paul *et al.* (1996)

[14] and Schroth and Hancock (1982) [16]. *On* the basis of morphological and biochemical characterization isolate RC_2 and RC_5 wereobserved with oval thin growth on Nutrient Agar and assigned to genus *Azospirillum*. As Similar results were reported by Brady, (1994) [4]. Isolate RC_4 and RC_9 were having thick opaque moist greyish white colonies on Nutrient Agar and observed to be *Azotobacter* strain on the basis of results obtained with biochemical test and growth on media. While isolate RC_7 shows thick opaque greyish white colonies on Nutrient Agar. Isolate RC_8 and RC_{11} have been observed with greyish white circular colonies on Nutrient agar these isolates were assigned to genus *Bacillus*. On the basis of swarming movements of isolate RC_{10} on agar plates isolate was assigned as *Proteus* having s thin, colourless and transparent with fishy smell on Nutrient Agar *RC_{12}* having thick, opaque moist greyish white colonies on was identified as a member of *Enterobacteriaceae* family. similar results were reported by Fravel, (1988) [7], Raaijmakers *et al.* (2002) [15, 19], Weller *et al.* (2002) [19], Weller (1988) [20] Whipps (2001) [21]. Bond (1994) [3] also reported the emergence of rhizobacteria *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Enterobacter*, *Bacillus Enterobacter*,, contain root associated bacteria that enter bivalent interactions with plant and human host. Isolates RC_8 and RC_{11} were found to by Gram positive while rest of the isolates were found to by Gram negative. (Figure 1 and figure 2).

Morphological characterization and identification of fungal microflora of rhizosphere

In the present study different fungal cultures were isolated and were subjected to various morphological and cultural characteristics for their identification. Cultures were identified on the basis of mycelia growth and sporulation on PDA. All the cultures were stained with lacto phenol to observe their morphology under the microscope. (Table III). *Aspergillus* and *Fusarium* reported from these samples.

Determination of sugar fermenting ability of Rhizospheric bacterial isolates

From results obtained through experimental work isolates RC_1, RC_3 & RC_6 were found to be unable to ferment Dextrose, Sucrose and lactose. Strains RC8, RC_{10} and RC11 were able to ferment only Dextrose and Sucrose and unable to ferment Lactose. So they were found to be weak saccharolytic. RC_2, RC_5 RC_4, RC_9 and RC_7 were found to be able to ferment Dextrose, Sucrose and Lactose. So they were found to be saccharolytic strains. (Table IV) In the present work the sugar fermenting capacity of *Azospirillum* was established which made it the organism of our interest. This bacteria can be tested further for its sugar tolerance and saccharolytic capacities. *Azotobacter* and *Bacillus* species were also identified during the course of studies. The oxidation of glucose, maltose, lactose, and xylose was tested with 430 strains of *Pseudomonas*, *Acinetobacter*, *Achromobacter*, *Alcaligenes*, *Moraxella*, *Flavobacterium*, and *Bordetella* species by Hussain Qadri *et al.* (1982) [9]. More than 95% of the isolates tested gave correct oxidation reactions within 4 h in the rapid carbohydrate oxidation microtubes, whereas oxidation-fermentation media required 24 h to achieve the same sensitivity

Table I: Rhizosphere count on NA and PDA

CFU count on NA	Log_{10} CFU	CFU count on PDA	Log_{10} CFU
20×10^7	1.32	3×10^7	0.47
40×10^7	1.60	12×10^7	1.07
120×10^7	2.07	30×10^7	1.47

Table II: Microscopic and biochemical characterization of bacterial isolates of sugarcane rhizosphere

Strain	Gram Reaction	Shape	Motlity	MR	VP	Lactose Fermentation	Dextrose Fermentation	Sucrose Fermentation	Indole	Catalase	Citrate	H_2S production
RC_1	-ve	Rods	Motile	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	-ve
RC_2	-ve	Rods	Motile	-ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve
RC_3	-ve	Rods	Motile	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	-ve
RC_4	-ve	Rods & cocci	Motile	-ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve
RC_5	-ve	Rods	Motile	-ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve
RC_6	-ve	Rods	Motile	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve
RC_7	-ve	Rods & cocci	Motile	-ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve
RC_8	+ve	Rods	otile	-ve	+ve	-ve	A	A	-ve	-ve	-ve	-ve
Strain	Gram Reaction	Shape	Motlity	MR	VP	Lactose Fermentation	Dextrose Fermentation	Sucrose Fermentation	Indole	Catalase	Citrate	H_2S production
RC_9	-ve	Rods & cocci	Motile	-ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve
RC_{10}	-ve	Rods	Motile	+ve	-ve	-ve	AG	AG	+ve	+ve	-ve	+ve
RC_{11}	+ve	Rods	Motile	-ve	+ve	-ve	A	A	-ve	-ve	-ve	-ve
RC_{12}	-ve	Rods	Motile	+ve	-ve	AG	AG	A	+ve	-ve	-ve	-ve

AG- Acid Gas Production A- Acid Production

Table III: Morphological and Microscopic Characterization of fungal micro flora of rhizosphere

Strain	Morphological Observation	Microscopic Observation
RF-1	Black coloured sporulating growth	Sporongiospores arising from a foot cell, conidia are present in chains arises from the vesicles.
RF-2	White to green coloured colonies, fast growing	Conidia in balls
RF-3	White to pink coloured cottony growth	Sickle shaped transversely septate conidia arises from conidiophores
RF-4	Greenish or blue green colonies	Conidia in long chains on repeatedly branched conidiophores resembling a brush like head
RF-5	Greenish black and powdery colonies	Branched conidiophores conidia variable
RF-6	Greyish green or black coloured colonies	Transversly and longitudinally septate,beaked conidia

Table V: Saccharolytic fermentation by rhizospheric bacterial isolates

Strain	Dextrose Fermentation	Sucrose Fermentation	Lactose Fermentation	Result
RC_1,RC_3&RC_6	-ve	-ve	-ve	Non-saccharolytic
RC_2 & RC_5	+ve	+ve	+ve	Saccharolytic
RC_4 & RC_9	+ve	+ve	+ve	Saccharolytic
RC_7	+ve	+ve	+ve	Saccharolytic
RC8 & RC11	+ve	+ve	-ve	Weak-Saccharolytic
RC_{10}	+ve	+ve	-ve	Weak-Saccharolytic
RC_{12}	+ve	+ve	+ve	Saccharolytic

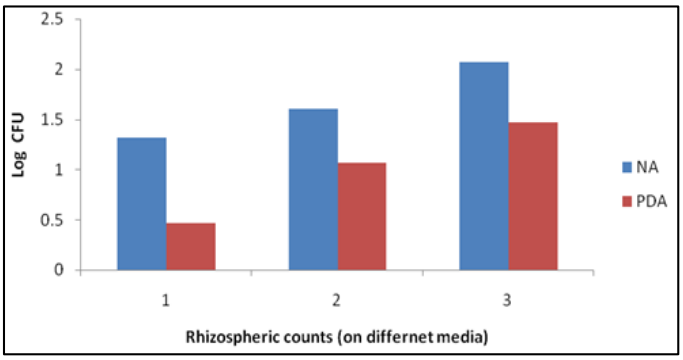
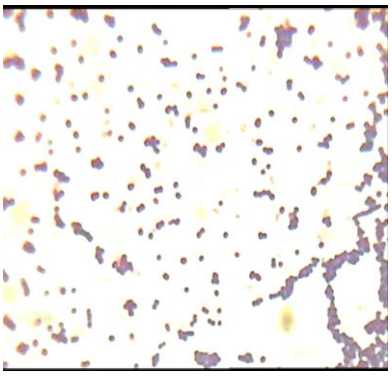


Fig 1: Growth of rhizospheric microflora on different media



Microscopic observation of of isolates RC_1, RC_2, RC_4 & RC_{12}

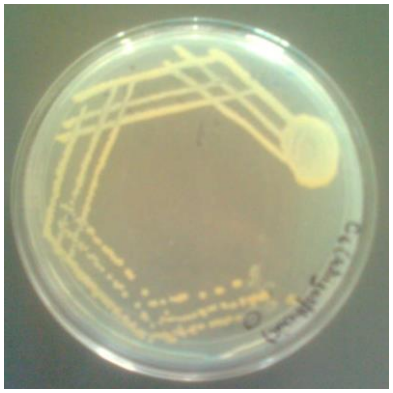
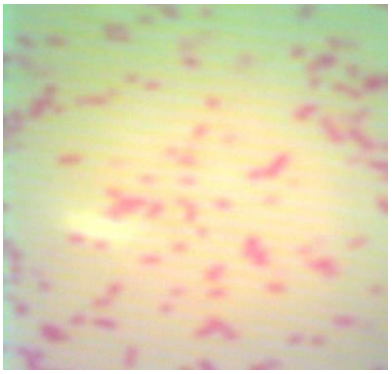


Fig 2: Growth of RC_1 and RC_6 on Nutrient agar



Microscopic observation of isolates C_8 and C_{11}

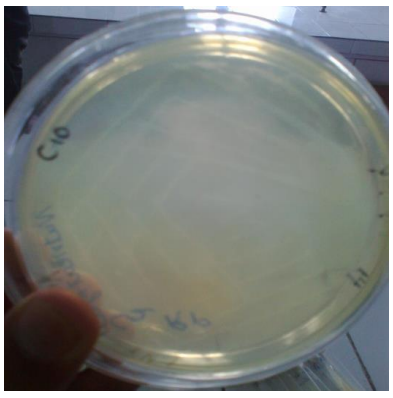
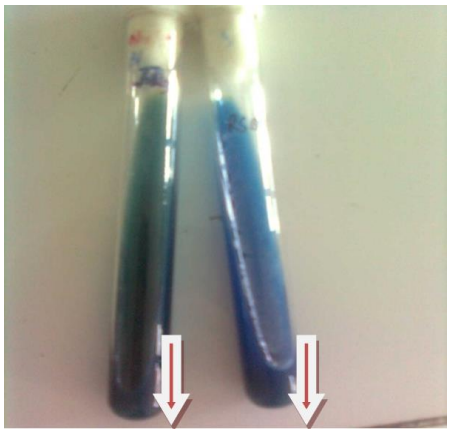


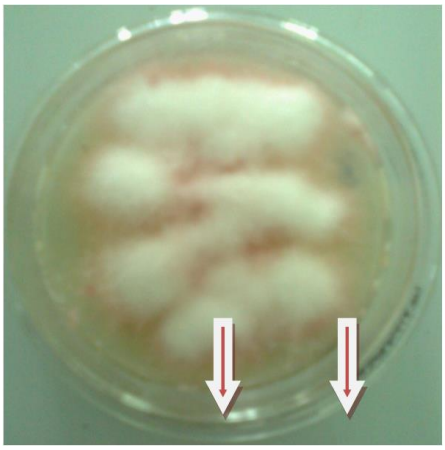
Fig: Growth of RC10 on NA

Positive Catalase test



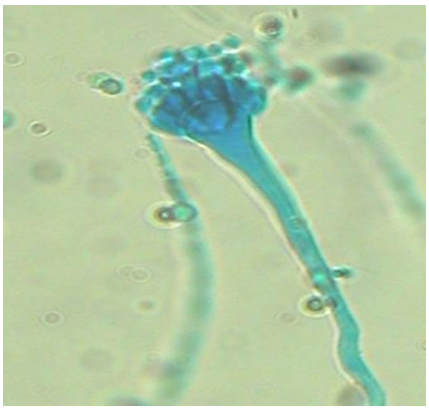
Control Positive Citrate utilization test

XIII Growth of RF2 on PDA



Growth of RF3 on PDA

Growth of RF1on PDA



Microscopic observation of RF1 (Aspergillus

Conclusions

The results obtained in the study were comparable to other studies carried out in this direction. It is quite conspicuous that approximately in all the studies undertaken always the similar genera of micro-species were obtained which shows their close association with the rhizospheric and rhizoplane habitat of the plant *Saccahrum officinarum*. Their close proximity also thereby implicates them with some essential functions such as nitrogen fixation, release of plant growth promoters and antimicrobial activities for preventing the host from plant pathogens. Some of the isolates which were found to be able to grow on sugar substrates can be studied further to work out their sacchrolytic potentials. The work assumes importance as industries utilizing microbes for sugar fermentation process constantly seek to find newer organisms with potentials that can be exploited and experimented with to better their natural sugar fermenting abilities that they are endowed with.

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