The Andhra Agric. J 57(4):418-421, 2010

Carbohydrate Utilization and Biochemical Properties of Some Phylloplane Bacteria

Key words : Phylloplane, biochemical properties, Bacteria, Carbohydrates

The surface of aerial plant parts provides a habitat for epiphytic micro-organisms, many of which are capable of influencing the growth of pathogens. The activity of both saprophytes and pathogens on leaves is dependent on the chemical environment at the plant surfacethat include presence of diverse carbohydrates, amino acids, organic acids, sugar alcohols, mineral trace elements, vitamins and hormones, as well as antimicrobial compounds such as phenols and terpenoids (Blakeman, 1981; Fiala *et al.,* 1990). Population of certain bacteria can often be elevated, atleast temporarily, by the natural deposition of complex substrates or by the presence of carbohydrates, aminoacids or mixes of nutrients (Fokkema *et al.,* 1979). The carbohydrate decomposition spectrum determined by production of acid from substrates is now regarded as a secondary tool in classification, but for the characterization of lower taxa and for epidemiological purposes it is still a valuable feature (Cowan, 1974).

In the present study, phylloplane bacteria were isolated from different plant species and the biochemical properties of the predominant bacteria were studied.

The present study was conducted during May to June 2009 at Agricultural College, Aswaraopet, Khammam district of Andhra Pradesh. Phylloplane bacteria from different plant species, *viz.,* maize, bittergourd, chilli, citrus, beans, bhendi and brinjal were isolated by leaf washing technique (Voznyakovaskaya and Khudyakov, 1960). The samples were collected from farmer's fields at Naravarigudem village of Khammam district on 09-05-09. The leaf washings were streaked on to nutrient agar plates and incubated at 25 ± 2^0C. Isolated colonies of bacteria with different colony morphology were purified by streaking thrice onto nutrient agar plates. Pure cultures of phylloplane bacteria were tested for utilization of carbohydrates and biochemical parameters by using biochemical test kits obtained from Hi Media Pvt. Limited, Mumbai. Each well of the biochemical test kits was stab inoculated with a loopful of bacterial culture and incubated at 25 ± 2^0C for 24 hours. Two morphologically distinct bacterial cultures were selected from phylloplane of chillies, citrus, bhendi, while only one colony was selected from the rest of the crops as all the colonies isolated from these crops are identical. The reaction of the bacteria to these carbohydrates and biochemical parameters were taken at 16 hrs after incubation. The results were interpreted as positive and negative on the basis of colour change of the medium as detailed in Table 1.

The results obtained in the present study indicated variation in utilization of various organic sources by the phylloplane bacteria isolated from seven crops, *viz.,* bean, bhendi, bittergourd, brinjal, chilli, citrus and maize (Table 2). Citrate was found to be utilized by the phylloplane bacteria isolated from six crops, *viz.,* maize, bittergourd, chillies, citrus, beans and bhendi; malonate from six crops, *viz.,* maize, chillies, citrus, beans, bhendi and brinjal. It is not known why two bacteria isolated from the phylloplane of the same crop (Bhendi and citrus) differ in utilization of citrate and malonate. In contrast, none of the bacteria isolated have not utilized α-methyl-D-mannoside, sorbitol and sorbose.

The bacteria on bittergourd is capable of utilizing seven out of fourteen sugars followed by five by those on citrus. Only the bacteria present on phylloplane of bitter gourd has utilized D-arabinose, lactose and cellobiose, while the bacteria isolated from all other crops could not utilize the above carbohydrates. Adonitol and xylitol were utilized by bacteria present on citrus and bacteria on no other crop could utilize them. One isolate of citrus bacteria has not utilized any of the carbohydrates tested, while other isolate from the same crop could utilize glucose, adonitol, xylitol, citrate and malonate. This could be because of differential activity of enzymes catalyzed by the metabolic processes of a particular bacteria.

Melezitose is not usually produced by plants. Melezitose is a trisaccharide found only in the honey dew of homopterous insects (Dik *et al.,* 1991). Melezitose has been found to be utilized by the bacteria isolated from the phylloplane of chilli, bhendi and brinjal. The presence of melezitose on the leaf surfaces is probably due to the susceptibility of

Table 1. Interpretation chart for carbohydrate utilization and biochemical tests.

S. No	Biochemical parameters	Principle	Color of the medium	Positive reaction	Negative reaction
1	Carbohydrate utilization	Detects utilization of carbohydrates	Red	Yellow	Red / Pink
2	ONPG decarboxy-lase	Detects β-galactosi-dase activity	Colourless	Yellow	Colourless
3	Esculin hydrolysis	Detects esculin hydrolysis	Cream	Black	Cream
4	Citrate utilization	Utlization of citrate as a sole carbon source	Yellowish - green	Blue	Yellowish-green
5	Malonate utilization	Utilization of sodium molybdate as a sole carbon source	Light-green	Blue	Light-green
6	Lysine decarboxy-lation	Detects lysine decar-boxylation	Olive green	Purple	Yellow
7	Ornithine decar-boxylase	Detects ornithine decarboxylation	Olive green	Purple	Yellow
8	Urease	Detects urease activity	Orange-yellow	Pink	Orangish-yellow
9	Phenylalanine deamination	Detects phenylalanine deamination activity	Colourless	Green	Colourless
10	H_2S production	Detects H_2S produc-tion	Orange-yellow	Black	Orangish-yellow

these crops to aphids. The ability of the bacteria to attack different substrates containing carbon, hydrogen and oxygen varies with the battery of enzymes present in the bacteria, (Cowan, 1974). It may be probable that the same kind of carbohydrates are produced on the leaf surfaces in the form of leaf exudates and may be because of habituation / adaptation of utilizing the carbohydrate on the leaf surface it is showing positive reaction.

All the test phylloplane bacteria isolates were found to be Gram negative, except on of the two isolates obtained from chilli. Blakeman and Brodie (1977) reported that most of the bacteria isolated from phylloplane of different platns are Gram negative. All the Gram negative phylloplane bacteria isolated from six of the seven test crops were found to be negative for esculine hydrolysis except bhendi which has shown positive reaction, while Gram positive phylloplane bacteria on chilli have shown positive reaction for esculin hydrolysis. The phylloplane bacteria on maize, bitter gourd, chilli and brinjal were found to be negative for urease activity. The phylloplane bacteria from bean and one bacteria each from citrus and bhendi were found to be positive for urease activity. All the phylloplane bacteria isolated were found to be positive for the decarboxylation of lysine and ornithine, while they were negative for H_2S production.

A Gram positive bacteria isolated from chilli phylloplane was found to be positive for esculin hydrolysis, decarboxylation of lysine and ornithine, reduction of nitrate and for O-Nitrophenyl-β-D-galactopyranoside (ONPG). However, the bacterium was found to be negative for urease activity, deamination of phenylalanine and H_2S production. Blazevic and Ederer (1975) advocated that many organisms produce H_2S and can be detected only if a very sensitive detection method is used.

Table 2. Carbohydrate utilization of Biochemical properties of phylloplane bacteria isolated from different crops crops

	Phylloplane bacteria from									
	Maize	Bitter groud	Chilli		Citrus		Bean	Bhendi		Brinjal
			Isolate 1	Isolate 2	Isolate 1	Isolate 2		Isolate 1	Isolate 2	
L-Arabinose	+	+	-	-	-	-	-	+	-	-
D-Arabinose	-	+	-	-	-	-	-	-	-	-
Glucose	-	+	-	-	-	+	+	-	+	+
Sorbose	-	-	-	-	-	-	-	-	-	-
Rhamnose	-	+	-	-	-	-	-	+	-	-
Lactose	-	+	-	-	-	-	-	-	-	-
Cellobiose	-	+	-	-	-	-	-	-	-	-
Melezitose	-	-	+	-	-	-	-	+	-	+
Adonitol	-	-	-	-	-	+	-	-	-	-
Sorbitol	-	-	-	-	-	-	-	-	-	-
Xylitol	-	-	-	-	-	+	-	-	-	-
α-methyl-D-Mannoside	-	-	-	-	-	-	-	-	-	-
Citrate	+	+	+	+	-	+	+	-	+	-
Malonate	+	-	+	+	-	+	+	-	+	+
Gram reaction	-	-	-	+	-	-	-	-	-	-
Esculin hydrolysis	-	-	-	+	-	-	-	+	+	-
Lysine decarboxylase	+	+	+	+	+	+	+	+	+	+
Ornithine decarboxylase	+	+	+	+	+	+	+	+	+	+
Urease	-	-	-	-	-	+	+	-	+	-
Phenylalanine deamination	-	-	+	-	-	+	-	+	+	+
Nitrate reduction	-	+	-	+	-	+	+	-	+	-
H2S production	-	-	-	-	-	-	-	-	-	-
ONPG	-	+	+	+	-	-	+	+	+	+

+ positive; - negative; ONPG = O-Nitrophenyl-β-D-galactopyranoside

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(Received on 11.01.2010 and revised on 28.06.2010)