INTERNATIONAL JOURNAL OF ADVANCED BIOLOGICAL RESEARCH

www.scienceandnature.org

CHARACTERIZATION AND IDENTIFICATION OF PHYLLOSPHERIC BACTERIAL ISOLATES OF RICE (*Oryza sativa*)

*Ojha, R.K., Jha, A.K. & Jha, S.K.
Department of Soil Science & Agricultural Chemistry, Birsa Agricultural University, Ranchi-834006 (Jharkhand)
*Corresponding author email: rajanojha@gmail.com

ABSTRACT
Present investigation was carried out to isolate, characterize & identify bacterial isolates from the Rice phyllosphere at different locations of Ranchi districts. Reduction in Rice phyllospheric bacterial population was found at polluted site as compared to unpolluted site. Twenty six isolates were observed & examined on the basis of their morphological, physiological & biochemical characters for identification. Out of these, fourteen bacterial isolates were found in unpolluted while, twelve were at polluted site. Dominant genera observed in present investigation were *Azotobacter* sp. in unpolluted site & *Bacillus* sp. in polluted site. However, *Pseudomonas* sp. was found only in unpolluted site and *Aeromonas, Erwinia* & *Flavobacterium* sp were found only in polluted site.

KEY WORDS: Phyllosphere, Rice, Bacterial isolates, Characterization, Azotobacter, Bacillus,

INTRODUCTION

Plant surfaces particularly the phyllosphere are an ideal habitat of microorganisms that support the growth of a variety of microorganisms and has an important role in biological control of disease, nitrogen fixation, degradation of waxes, acceleration of senescence, production of growth regulators, decomposers and performers of other biochemical process. Studies on the identify of organisms in the phyllos have focused on bacteria and to a lesser extent fungi (Vorhalt, 2012). The phyllosphere comprises the aerial parts of plants and is dominated by the leaves, most. It is frequently considered that the magnitude of the bacterial population is an important indication of the fertility of the soil. Eventually the nitrogen fixing bacteria will supply the necessary combined nitrogen for the development and maintenance of the other phyllospheric population and the physiological state of supporting foliage will be determining which of the micro-organisms will become dominant in the phyllosphere. The kinds of micro-organisms occurring in the phyllosphere population and the degree to which the leaf surface is covered vary for different plant species under different environmental condition. The occurrence of a microbial population rich in nitrogen-fixing organisms in phyllosphere of vegetation may be of decisive importance for the nutrition. Keeping these facts in mind, present investigation were done to isolates, characterize and identify the rice phyllospheric bacterial isolates on the basis of their morphological, physiological and bio-chemical properties.

MATERIALS & METHODS

Selection of location & sample collection

Leaf samples of rice were collected from polluted (near road, industrial sites) and unpolluted (one km away) sites from six different locations of Ranchi district in Jharkhand.

Location selected for leaf sample collection

Location	Area name	Geographical situations	Site name	
			Polluted site (PS)	Unpolluted site (UPS)
L1	Ranchi – Hazaribagh	23^0 22′ N - $85^0$21′ E	Road side (RSL1)	Remote side (RRL1)
L2	Ranchi – Jamshedpur	23^0 22′ N - $85^0$21′ E	Road side (RSL2)	Remote side (RRL2)
L3	Ranchi – Gumla	23^0 22′ N - $85^0$19′ E	Road side (RSL3)	Remote side (RRL3)
L4	HEC, Sector – II, Ranchi	23^0 19′ N - $85^0$17′ E	Industrial site (ISL4)	Remote side (IRL4)
L5	HEC, Sector – III, Ranchi	23^0 18′ N - $85^0$17′ E	Industrial site (ISL5)	Remote side (IRL5)
L6	Usha Martin, Ranchi	23^0 22′ N - $85^0$25′ E	Industrial site (ISL6)	Remote side (IRL6)

15 –20 leaves/plant from three plant replicates were taken randomly from the sites of all location. Matured whole leaf samples were removed with the help of sterilized scissors between 9.00 AM to 3.00 PM during bright sunshine and were kept separately in sterilized poly bags. The leaf samples were stored at low temperature (4^0 C) till completion of the experiment.

Isolation and Identification of phyllospheric bacteria

Isolation

Counts of bacteria was done by serial dilution plate technique (Aneja, 2007) using the nutrient agar medium. Discs of 1 cm diameter were excised from the leaves with the help of a sterilized cork borer. Fifty such discs per sample were placed in a 250 ml conical flask containing 100 ml of sterilized distilled water. The flask was shaken

vigorously for 15–20 minutes to detach the surface propagules. For the counting of bacteria 10^{-7} dilution were taken. Pure cultures of phyllospheric bacterial isolates were obtained through repeated streaking of well differentiable colonies on solidified media for the microbial groups. Pure cultures thus obtained were maintained by frequent sub culturing on slants throughout the experiment.

Characterization

During total bacterial count, the plate showing maximum number of colonies was taken for further investigation in all cases. Assignment of appropriate genera to the phyllospheric bacterial isolates were done based on morphological (Shape and arrangement of cells, size, gram stain, and colony size, colour & shape on agar), physiological (O_2 relation & motility) and biochemical (Ammonia production, Urease test, Catalase test, Starch hydrolysis, Casein hydrolysis, H_2S production, NO_3 reduction, Growth on N free media & Growth on PSB media) characters as described in Bergey's Manual of Determinative Bacteriology (Holt *et al.*, 1994).

Morphological characterization

For examination of size and shape, smears on slides fixed by gentle heat were stained with carbol fuchsin. Gram stained bacterial cells on clean micro slides were used for measurement of bacterial size (length and width) using ocular micrometer. 24 hours old cultures of bacteria were stained and examined under microscope for shape and arrangement of cells. Isolates were examined for Gram staining characters. Shape, size and colour of isolates colony under study were streaked and plate were incubated as needed, and growth were checked after 18-24 hours (Chhonkar *et al.*, 2007) in which above observation were taken.

Physiological characterization

To determine whether a culture is aerobic or unaerobic, nutrient broth (containing 0.005% bromocresol purple) columns were prepared in culture tubes. Observations were recorded at 48 hr. interval for 7 days. Hanging drop technique was performed for demonstrating motility of bacteria using 12 hr. old nutrient broth culture (Aneja, 2007)

Biochemical characters

(i) Ammonia production- Culture was incubated in peptone broth for 5 days at small piece of filter paper with Nessler reagent was placed in upper part of culture tube. Test tube was warmed on a water bath at 50 - 60^{o} C. Filter paper shown brown to black, was indicator of ammonia production.

(ii) Urease test - Urease test was performed on urea agar medium**.** Basal medium was poured in 90.0ml quantities in flasks, autoclaved and cooled to 45^{0}C. 10ml filter sterilized urea solution (20%) was added to each flask, after mixing it was poured in 5ml culture tubes. After solidification of medium in slanting position, tubes were inoculated with test bacterium, incubated (30 ±2^{0}C) and observations were recorded at regular intervals up to 15 days. Presence of urease gives red colour and for no urease yellow colour.

(iii) Catalase test - Catalase test was performed by adding H_2O_2 to trypticase soy agar slant. Culture medium was poured in culture tubes and sterilized by autoclaving. Test bacterium was inoculated in agar slant and inoculated at 35^{0}C for 24-48 hrs. observations were taken by adding 3 - 4 drops of H_2O_2 to flow over the growth of each slant culture and the reaction obtained was recorded. Presence of gas bubbles indicates catalase +ve test.

(iv) Hydrolysis of starch- It was performed by adding soluble starch (0.2 percent) in nutrient agar medium, pH was adjusted to 7.0 - 7.2 with the help of bromothymol blue indicator. The liquid was dispensed in test tube and sterilized. When cooled the liquid was incubated with culture and incubated at 30^{0}C. After incubation a small portion of liquid was treated with diluted iodine solution. No change in colour indicates complete hydrolysis, brown colour indicates partial hydrolysis and blue colour indicates that there was no hydrolysis of starch.

(v) Casein hydrolysis- Autoclaved and cooled basal medium was poured into sterilizes Petri plates, inoculated with culture. A single line streak inoculation across the surface of medium was produced and plates incubated for 2 -3 days at 37^{0}C in an inverted position. A clear zone around streak showed a positive reaction while absence of clear area around the growth of an organism is a negative reaction.

(vi) Hydrogen Sulphate production test - Production of H_2S by different isolates was tested with the help of impregnated strips of filter paper with 10% solution of lead acetate and dried. A lead acetate strip was placed at the top of a nutrient broth culture and incubated. Blackening of paper indicated H_2S production.

(vii) Nitrate reduction- Nitrate broth was used with following composition: Peptone - 10.0g, Beef -extract- 5.0 g, KNO_3-1.0g, Distilled water - 1000.0 ml. The ingredients were dissolved and poured in tubes (5 cm) and autoclaved. The broth was inoculated with the test bacterium and incubated at 25^{0}C. Reduction of nitate was checked upto 15 days at regular intervals by adding a few drops of sulfanilic acid (0.8% in 5M acetic acid) and dimethyl alpha-nephthylamine (0.5% in 5M acetic acid) to the nitrate broth culture subsequently. Presence of nitrite was detected by the red Colour given by addition of sulfanilic acid, α–naphthylamine reagent to a portion of the liquid.

Determination of specific properties

Nitrogen fixing bacteria - For the assessment of Nitrogen -fixing bacteria, Burk's Nitrogen free medium was used and incubated in 250 ml conical flasks inoculated with culture of test organisms. The flasks were then incubated for 14 days at 30^{0}C. After the incubation, the contents of the flasks along with uninoculated contents as control were digested for the determination of nitrogen by the Kjeldahl's method (Jackson, 1973).

Phosphate solubilizing bacteria - For the assessment of P –solubilizing bacteria, Bunt and Rovira (1955) medium was used. 100 ml of the medium replacing Tricalcium Phosphate by Rock Phosphates equivalent to 50mg % P_2O_5 in 250ml conical flasks were inoculated with different isolates. Inoculated flasks were incubated for 15 days at 30 ±0.2 ^{0}C under static condition and shaken once in 12 hr. along with control un-inoculated flasks. 5 ml of growth medium from each flask was withdrawn aseptically after 15 days and passed through Whatman No. 42 filter paper. The filtrates were assayed for P_2O_5 by Amino-Molybdate Ascorbic acid blue method (Jackson, 1973).

By comparing the results of the above tests as described in Bergey's Manual of Determinative Bacteriology (Holt *et*

al., 1994), isolates was tentatively identified up to generic level.

RESULTS & DISCUSSION

Bacterial population

Bacterial population of rice phyllosphere at different locations is presented in (Table 1). It is evident from the data that population of bacteria decreased significantly in polluted site as compared to unpolluted sites of rice phyllosphere. Bacterial population of rice phyllosphere was reduced to the extent of 30.70 per cent. The reduction in bacterial colonization located in the vicinity of industries and roadsides may be attributed to increased absorption of heavy metals, dust, traffic fumes. Location L1 recorded significantly higher bacterial count which was at par with Locations L4 and L6. Our results are in agreement with the findings of (Joshi, 2008).

TABLE 1. Bacteria population cm^{-2} (x 10^7) on *Oryza sativa* phyllosphere at different location

Locations/ Crops	*Oryza sativa*		
	UPS	PS	Mean
L1	12.2	9.1	10.7
L2	8.9	5.2	7.1
L3	11.6	8.2	9.9
L4	13.0	7.9	10.5
L5	11.1	8.4	9.8
L6	11.5	8.7	10.1
Mean	11.4	7.9	9.7
	L	P	L x P
S.Em. ±	0.37	0.12	1.26
C.D. 5%	0.75	0.25	NS
C.V. %		5.35	

UPS – Un-polluted site, PS – Polluted site.

Morphological, Physiological and biochemical characteristics

Rice phyllospheric isolates

The bacterial isolates found on rice phyllosphere from different locations have been placed in eight different groups on the basis of morphological, physiological and biochemical characteristics (Table, 2a and 2b).

Group (A) cells morphology of isolates was observed as straight rods, short rods, occurring singly, in pairs or in chains. Individual cells ranged from 1.0 µ to 2.5 µ in length and 3.0 µ to 4.5 µ in width. Size of colony varied from 10 to 12 mm in diameter. Colony growth on nutrient agar showed creamy white to yellow, convex, wrinkled with entire margin. Physiologically they showed Gram positive, positive growth in presence of oxygen and cells were motile. Biochemically, isolates hydrolyzed urea and starch, liquefied casein and was catalase positive. However, isolates failed to produce H_2S and reduce NO_3^-. Cells solubilized inorganic phosphate on appropriate medium. The isolates $RRL1_1$, $RSL2_2$, $ISL4_1$ and $ISL5_2$ resembling in above characters were placed in this group and were tentatively identified as members of genus *Bacillus* sp.

Group (B) Cells were short rods, rods, occurring in pairs and chains. Individual cells ranged from 0.5 µ to 3.0 µ in length and 2.0 µ to 5.0 µ in width. Size of colony on agar varied from 7 to 20 mm in dia with yellow to yellowish and creamy white to white in colour, scattered, irregular, raised to highly raised and granular. Physiologically they were aerobic, Gram negative and motile. Biochemically these isolates produced ammonia, hydrolyzed urea, reduced NO_3^- and failed to produce H_2S. Cells showed positive growth in mineral nitrogen free medium. On the basis of above characteristics, the organisms ($RRL1_2$, $RSL1_2$, $RRL3_1$, $IRL4_1$, $IRL5_1$, $IRL6_1$ and $ISL6_2$) may tentatively be identified belonging to genus *Azotobacter* sp.

Group (C) Cells were straight, spherical occurring singly or in chains, clumps with diameter ranging between 3.5 to 4.5 µ, colony size on nutrient agar 10 to 15 mm, white in colour, shape undulate, flat, glistening, smooth and wrinkled. Aerobic to anaerobic nature with oxygen pressure, Gram positive and motile. Hydrolyzed urea, catalase test and H_2S production were positive. Starch hydrolysis and NO_3^- reduction were negative. Cells showed positive growth on inorganic nitrogen free medium. On the basis of above parameters, organisms ($RSL1_1$, $RRL2_3$, and $IRL4_3$) may tentatively be identified as the member of genus *Klebsiella* sp.

Group (D) Cells were ellipsoidal, diameter ranging between 0.5 µ to 1.2 µ in length and 1.5 to 2.5 in width. Size of colony 5 to 10 mm on nutrient agar, white to dull white in colour with circular, smooth, slimy and glistening colonies. Aerobic nature of growth & Gram negative and were motile. Produced ammonia and H_2S, but failed to reduce NO_3^- and hydrolyze starch. Positive growth of cells on specific N- free medium. With above characters organisms ($RRL2_1$, $RSL2_1$, $RRL3_2$, $IRL4_2$ and $IRL6_2$) were tentatively identified as the genus *Beijerinckia* sp.

Group (E) Rod shaped cells with round ends, diameter 1.6 µ to 2.5 µ in length and 0.8 µ to 3.5 µ. Colony size on agar 10 mm, creamy white to shiny white in colour, raised, glistening & smooth. Cells showed positive growth with pressure of O_2, Gram negative and were motile. Urease and catalase test showed positive, starch hydrolyzed and did not reduce NO_3^-.

TABLE 2(a): Cultural characteristics and identification of rice phyllospheric bacterial isolates

Location	Isolate no.	Morphological						Physiological	
		Shape and arrangement of cells	Size, µm	Gram stain	Colony on agar			O_2 relation	Motility
					Size, mm	Colour	Shape		
RRL1	1^A	Rods, chain	1.2 x 3.0	+	12	Yellow	Entire, convex	Aerobic	+
	2^B	Short rods, pairs	2.5 x 3.5	–	7	White	Irregular, scattered	„	+
RSL1	1^C	Straight	3.5	+	10	White	Undulate, glistening, smooth	„	+
	2^D	Short rods, chain	2.0 x 4.5	–	10	Yellowish	Highly raised, granular	„	+
RRL2	1^D	Ellipsoidal	0.5 x 1.7	–	5	White	Circular, smooth, slimy	„	+
	2^E	Rod shaped, chain	2.5 x 3.5	+	15	Creamy white	Raised, glistening	„	+
	3^C	Spherical, singly in chains	4.5	+	12	White	Undulate, flat	„	+
RSL2	1^D	Ellipsoidal	1.2 x 2.0	–	7	Dull white	Circular, glistening, slimy	„	+
	2^A	Rod shaped, pairs	1.5 x 3.0	+	10	Creamy white	Entire growth, convex	„	+
RRL3	1^B	Short rods, pairs	2.0 x 5.0.	–	10	Whitish	Irregular, scattered	„	+
	2^D	Ellipsoidal	1.2 x 2.5	–	7	Dull white	Circular, glistening, slimy	„	+
RSL3	1^F	Rods, singly in pairs	0.6 x 5.0	–	5	Yellowish grey	Dull, round	Anaerobic	–
	2^G	Rod, packets	2.0 x 0.7	–	10	Brown white	Granular, raised	Aerobic	–
IRL4	1^B	Short rods, chain	3.0 x 4.0	–	10	Yellowish	Raised, granular	„	+
	2^D	Ellipsoidal,	0.8 x 2.0	–	5	Dull white	Circular, slimy	„	+
	3^C	Spherical, clumps	4.0	+	15	White	Undulate, flat, wrinkled	„	+
ISL4	1^A	Short rod, chain	2.5 x 4.0	+	10	Yellow	Convex, wrinkled	„	+
	2^H	Spherical,	4.0	–	10	White	Irregular	„	+
IRL5	1^B	Rods, single chain	0.5 x 4.0	–	7	White	Glistening, slimy, raised	„	+
	2^E	Rods, pairs	1.6 x 0.8	+	5	Shiny white	Raised, glistening, smooth	„	+
ISL5	1^G	Rods, cells in packets	2.5 x 4.0	–	7	White	Highly raised, granular	„	+
	2^A	Straight rods, single chain	1.0 x 4.5	+	12	Yellow	Convex, entire growth	„	–
IRL6	1^B	Rods, pairs	0.7 x 2.0	–	12	Yellow	Granular, circular raised	„	–
	2^D	Ellipsoidal	0.6 x 1.5	–	10	White	Circular, smooth, slimy	„	+
ISL6	1^H	Spherical, singly in chains	5.0	–	10	White	Irregular, scattered	„	+
	2^B	Rod	1.5 x 4.0	–	20	Creamy white	Highly raised, granular	„	+

+ Positive, – Negative.

ISSN 2250 – 3579

TABLE 2(b) Biochemical characteristics and identification of rice phyllospheric bacterial isolates

Location	Isolate no.	Ammonia production	Urease test	Catalase test	Starch hydrolysis	Casein hydrolysis	H_2S production	NO_3 reduction	Growth on N free media	Growth on PSB media	Identified Genus
RRL1	1^A	+	–	+	+	+	–	–	–	+	*Bacillus*
	2^B	+	+	–			–	+	+	–	*Azotobacter*
RSL1	1^C	–	+	+	–	–	+	–	+	–	*Klebsiella*
	2^D	+	+	–	–	–	–	+	+	–	*Azotobacter*
RRL2	1^D	+	+	–	+	–	+	–	+	–	*Beijerinckia*
	2^E	–	+	+	+	–	–	–	–	+	*Pseudomonas*
	3^C	–	+	+	–	–	+	–	+	–	*Klebsiella*
RSL2	1^D	+	+	–	+	–	+	–	+	–	*Beijerinckia*
	2^A	+	–	+	+	+	–	–	–	+	*Bacillus*
RRL3	1^B	+	+	–	–	–	–	+	+	–	*Azotobacter*
	2^D	+	+	–	+	–	+	–	+	–	*Beijerinckia*
RSL3	1^F	–	+	+	+	+	–	+	–	–	*Flavobacterium*
	2^G	–	–	+	–	–	+	+	–	–	*Aeromonas*
IRL4	1^B	+	+	–	–	–	–	+	+	–	*Azotobacter*
	2^D	+	+	–	+	–	+	–	+	–	*Beijerinckia*
	3^C	–	+	+	–	–	+	–	+	–	*Klebsiella*
ISL4	1^A	+	–	+	+	+	–	–	–	+	*Bacillus*
	2^H	–	–	+	–	–	+	–	–	+	*Erwinia*
IRL5	1^B	+	+	–	–	–	–	+	+	–	*Azotobacter*
	2^E	–	+	+	+	–	–	–	–	+	*Pseudomonas*
ISL5	1^G	–	–	+	–	–	+	+	–	–	*Aeromonas*
	2^A	+	–	+	+	+	–	–	–	+	*Bacillus*
IRL6	1^B	+	+	–	–	–	–	+	+	–	*Azotobacter*
	2^D	+	+	–	+	–	+	–	+	–	*Beijerinckia*
ISL6	1^H	–	–	+	–	–	+	–	–	+	*Erwinia*
	2^B	+	+	–	–	–	–	+	+	–	*Azotobacter*

+ Positive, – Negative.

Cells solubilized inorganic phosphate on medium. On the basis of above the ($RRL2_2$, $IRL5_2$) organisms may tentatively be identified as the genus *Pseudomonas* sp.

Group (F) Cells were rods occurring singly and in pairs with round ends, 0.6 µ in length 5.0 µ in width. Colony size 5 mm, yellowish grey, agar colonies were dull, round. Anaerobic, Gram negative and non motile. Hydrolyzed urea and starch, catalase test positive, lignified casein and reduced nitrate, but did not produce H_2S. On the basis of above characteristics, only one $RSL3_1$ organisms were tentatively identified as genus *Flavobacterium* sp.

Group (G) cells contained rod shaped, occurring in packets ranged between 2.0µ to 2.5µ in length and 0.7µ to 4.0µ in width. Colony size on agar varied 7 to 10 mm, colour brown white to white raised to highly raised and granular. They were aerobic, Gram negative and motile. Failed to hydrolyze urea, but catalase, H_2S production and NO_3^- reduction were positive. On the basis of above parameters, the organisms $RSL3_2$ & $ISL5_1$ were tentatively identified as genus *Aeromonas* sp. Group (H) Cells were spherical, occurring singly or in chains with diameter ranging between 4.0 µ to 5.0 µ. Colony size on nutrient agar 10 mm, white in colour, irregular and scattered. Aerobic, Gram negative and motile. Catalase test and H_2S production positive, Urease, starch, casein hydrolysis and NO_3 reduction were negative. Cells showed inorganic phosphate solubilization on medium. On the basis of above, organisms ($ISL4_2$ and $ISL6_1$) may tentatively be identified as to genus *Erwinia* sp.

Among the total bacterial population, twenty six isolates were observed & examined. Out of these, fourteen were found in unpolluted sites while twelve were found in polluted sites. Reduction in number of isolates at polluted site may be due to the sensitivity of the organisms to pollution (Joshi, 2008). The dominant genera observed in present investigation were *Azotobacter* sp. in unpolluted sites and *Bacillus* sp. in polluted site. However, *Pseudomonas* sp. was found only in unpolluted site and *Aeromonas*, *Erwinia* and *Flavobacterium* were found only in polluted site. Predominance of *Bacillus* sp. in polluted site may be attributed to their resistance to pollutants (Joshi, *et al.*, 2008). *Azotobacter* sp. was predominant in unpolluted sites but also found in some polluted sites. This is in conformity with Brighigna, *et al.* (1999), who reported that non spore forming nitrogen fixing organisms were more susceptible to pollution.

REFERENCES

Aneja, K.R. (2007) Experiments in Microbiology. 4th edition of cultivation techniques for Isolation and Enumeration of microorganisms. New age International Publishers. New Delhi, 157-188.

Brighigna, L., Gori, A., Gonnelli, S. and Favilli, F. (1999) The influence of air pollution on the phyllosphere microflora composition of *Tillandsia* leaves (Bromeliaceae). Am. J. Bot., 85 (7), 723-27.

Bunt, J.S. and Rovira, A.D. (1955) Microbiological studies of some physiological cases of *Fusarium oxysporum*. Trans.

Brit. Mycol. Soc. 40, 145-154.

Chhonkar, P.K., Bhadraray, S., Patra, A.K. and Purakayastha, T.J. (2007) Experiments in soil biology biochemistry, Westville publishing house, New Delhi.

Holt, J.G., Krieg, N.R., Sneath, P.H.A., Staley, J.T. and Williams, S.T. (1994) Bergey's Manual of Detereminative Bacteriology, 9th edn. Baltimore: Williams and Wilkins.

Jackson, M.L. (1973) Soil chemical analysis. Prentice hall of India, New Delhi.

Joshi, S.R. (2008) Influence of roadside pollution on the phyllosplane microbial community of *Alnus nepalensis* (Betulaceae). Int. J. Trop. Biol., 56 (3), 1521-1529.

Joshi, S.R., Bhagobaty, R.K. and Kumar, R. (2008) Microbial community on leaf surfaces of broad leaved Alder (*Alnus nepalensis* D. Don) and needle leaved Khasi pine (*Pinus kesiya* Royle Ex Gordon) as influenced by atmospheric dry deposition of roadside pollution in Eastern Himalayas. Res. J. of Environ. Sci. 2(4), 234-42.

Vorhalt, J.A. (2012) Microbial life in the phyllosphere. Natural Res. Microbial. 10: 828 – 840.