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BACTERIAL DIVERSITY IN SOME SELECTED AGRICULTURAL FOOD PRODUCTS

AMNA ALI*, MUHAMMAD SALEEM HAIDER, SANA HANIF AND MUHAMMAD ASHFAQ

Institute of Agricultural Sciences, University of the Punjab, Quaid-e-Azam Campus, Lahore, 54590, Pakistan

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Abstract

A total of 27 bacterial strains were isolated from apple, potato, turnip, onion, cucumber, zinger, lemon, spinach, radish and mint marketted in Labore, Pakistan. Highest frequency of occurrence (4%) was recorded for each of *Enterobacter aggglomerans, Ensifer adhaerens* and *Bordetella pertussis*, *Brassica rapa* and *Kurthia gibsonii* showed 30% frequency of occurrence. Frequency of all other bacterial strain ranged from 10 - 20%. Highest number (7) of bacterial speices were recorded from lemon and potato while the minimum number (4) was represented by each of apple, onion, ginger, spinach and radish.

Bacteria 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. Specifically, the bacteria enter tissues via germinating radicles, secondary root, stomata, or as a result of foliar damage. Bacteria inside a plant may either become localized at the point of entry or spread throughout the plant. These microorganisms can reside within cells, in the intercellular spaces, or in the vascular system (Zinniel *et al.* 2002). The aim of the present study was to isolate and identify the bacterial species found in different agronomic crops for their diversity.

Ten infected agricultural products *viz:* apple (*Malus domestica* L. Borkh.), potato (*Solanum tuberosum* L.), turnip (*Brassica rapa* L.), onion (*Allium cepa* L.), cucumber (*Cucumis sativus* L., ginger (*Zingiber officinale* L.), lemon (*Citrus limon* L. Burm. f.), spinach (*Spinacia oleracea* L.), radish (*Raphanus sativus* L.) and mint (*Mentha sativa* L.) were collected from markets of Lahore, Pakistan, during September to December 2012. The collected materials were stored in sterilized polythylene bags for further investigation. Surface sterilization of samples was done by stepwise washing in 70% ethanol for 5 min, sodium hypochlorite solution for 5 min, and 70% ethanol for 30 sec, followed by three rinses in sterile distilled water (Ishaq and Khan 2011). Different types of media *viz:* Luria Bertani agar (LBA), nutrient agar (NA), trypton agar (TA) and yeast extract agar (YEA) were used for isolation and identification (Ali and Naseem 2011, 2012). The surface of infected samples was removed with a sterilized razor blade, and the inner infected tissue was cut into pieces 4 to 6 mm long and were placed on media plates. Incubation was carried out at 37°C for 24 hrs to allow growth of endophytic bacteria. Moreover, fragments of diseased samples were homogenized in 5 ml of sterile saline solution with a blender. The serial dilutions (1 ml of 10^5) were spread with sterilized spreader onto media (Ishaq and Khan 2011) incubated at 37°C for 24 hrs. After incubation, distinct individual colonies were selected and sub-cultured by streaking on an agar plate for purification and preservation. Identification of bacterial species was done by following morphological, microscopic characteristics and biochemical tests and consulting the pertinent literature (Holt *et al.* 2000, Koneman *et al.* 1997, Benson 1996). After the identification of bacterial species, following values were determined: (i) percentage of each bacterial isolate in

*Author for correspondence: <write2amna@gmail.com>.

the sample, (ii) number of occurrences of each bacterial taxa = number of samples colonized by a specific bacteria, (iii) frequency of occurrence of all taxa (%) = number of samples colonized by a specific bacteria divided by the number of sample examined × 100 (Bajwa *et al.* 2009).

Table 1. Frequency of occurrence (%) of bacterial species isolated from agricultural food products.

Bacteria	Food product	Number of occurrence	Occurrence frequency (%)
Klebsiella sp.	Apple	1	10
Burkholderia pseudomallei	Apple, lemon, mint	3	30
Yersinia ruckeri	Apple	1	10
Corynebacterium minutissium	Apple, radish	2	20
Bacillus farraginis	Potato, onion, spinach	3	30
Kurthia gibsonii	Potato, turnip, lemon	3	30
Enterobacter aggglomerans	Potato, cucumber, spinach	4	40
Ralstonia solanacearum	Potato	1	10
Azospirillium lipoferum	"	1	10
Acinetobacter lwoffii	Potato, onion	2	20
Peptococcus sp.	Turnip, mint	2	20
Acidovorax facilis	Turnip	1	10
Ensifer adhaerens	Turnip, cucumber, lemon, mint	4	40
Acinetobacter calcoaceticus	Turnip, lemon	2	20
Curtobacterium albidum	Onion, ginger	2	20
Acetobacter aceti	Cucumber	1	10
Acidovorax temperans	"	1	10
Bordetella pertussis	Cucumber, lemon, radish, mint	4	40
Lactococcus lactis	Ginger, radish	2	20
Pantoea sp.	Ginger	1	10
Spirillospora albida	"	1	10
Aerococcus sp.	Lemon	1	10
Syntrophospora sp.	"	1	10
Micrococcus luteus	Spinach	1	10
Proteus vulgaris	"	1	10
Microbacterium lacticum	Radish	1	10
Arthrobacter sp.	Mint	1	10
Total		48	480

A total of 27 bacterial strains representing 24 genera were isolated from ten different types of agricultural products. Two species belonged to genus *Acinetobacter* and *Acidovorax* whereas the rest of the genera *Klebsiella* sp., *Burkholderia pseudomallei*, *Yersinia ruckeri*, *Corynebacterium minutissium*, *Bacillus farraginis*, *Kurthia gibsonii*, *Enterobacter aggglomerans*, *Ralstonia solanacearum*, *Azospirillium lipoferum*, *Peptococcus* sp., *Ensifer adhaerens*, *Curtobacterium albidum*, *Acetobacter aceti*, *Bordetella pertussis*, *Lactococcus lactis*, *Pantoea* sp., *Spirillospora albida*, *Aerococcus* sp., *Syntrophospora* sp., *Micrococcus luteus*, *Proteus vulgaris*, *Microbacterium lacticum* and *Arthrobacter* sp. were represented by a single species (Table 1). The

Table 2. Percentage of composition of bacteria in individual agricultural food products.

Sample No.	Substrate/ host	Place of collection	Bacteria	No. of colonies	% of bacteria
01	Apple	Fruit market, Lahore, Cantt.	*Klebsiella* sp.	04	26
			Burkholderia pseudomallei	03	20
			Yersinia ruckeri	05	33
			Corynebacterium minutissium	03	20
02	Potato	Vegetable market, Lahore, Cantt.	*Bacillus farraginis*	08	25
			Kurthia gibsonii	07	22
			Enterobacter agglomerans	07	22
			Ralstonia solanacearum	03	9.6
			Azospirillum lipoferum	04	12
			Acinetobacter lwoffii	02	6.4
03	Turnip	Vegetable market, Lahore, Cantt.	*Peptococcus* sp.	03	18
			Kurthia gibsonii	04	25
			Acidovorax facilis	02	12.5
			Ensifer adhaerens	03	18
			Acinetobacter calcoaceticus	04	25
04	Onion	Vegetable market, Lahore, Cantt.	*Bacillus farraginis*	04	25
			Enterobacter agglomerens	03	18
			Curtobacterium albidum	06	37
			Acinetobacter lwoffii	03	18
05	Cucumber	Vegetable Market, Lahore, Cantt.	*Acetobacter aceti*	04	24
			Acidovorax temperans	05	29
			Bordetella pertussis	03	18
			Ensifer adhaerens	02	12
			Enterobacter agglomerans	03	18
06	Ginger	Vegetable market, Lahore, Cantt.	*Lactococcus lactis*	04	31
			Pantoea sp.	03	23
			Spirillospora albida	02	15
			Curtobacterium albidum	04	31
07	Lemon	Fruit market, Lahore, Cantt.	*Bordetella pertussis*	04	22
			Acinetobacter calcoaceticus	03	17
			Ensifer adharrens	04	22
			Aerococcus sp.	01	05
			Kurthia gibsonii	02	11
			Syntrophospora sp.	01	05
			Burkholderia pseudomallei	03	17
08	Spinach	Vegetable market, Lahore, Cantt.	*Micrococcus luteus*	03	25
			Enterobacter agglomerans	02	17
			Proteus vulgaris	04	33
			Bacillus farraginis	03	25
09	Radish	Vegetable market, Lahore, Cantt.	*Microbacterium lacticum*	02	20
			Lactococcus lactis	03	30
			Corynebacterium minutissium	03	30
			Bordetella pertussis	02	20
10	Mint	Vegetable market, Lahore, Cantt.	*Peptococcus* sp.	04	27
			Burkholderia pseudomallei	03	20
			Ensifer adhaerens	02	13
			Arthrobacter sp.	03	20
			Bordetella pertussis	03	20

dominance of genera in the present study accorded with the results of (Hung and Annapurna 2004). Bacterial isolates from different agronomic plants and especially *Enterobacter aggglomerans, Ensifer adhaerens* and *Bordetella pertussis* those one with highest occurrence frequency showed that these are physiologically more versatile, not only in coping with the harshness of the climate but also have strong resistance to pollutants like heavy metals, herbicides, pesticides and antibiotics produced by other microorganisms and/or plants (Bajwa *et al.* 2009). Conversely, some species were isolated from a single locality, perhaps because they prefer a particular type of chemicals present in that locality or they lack competitive saprophytic ability to fight against antibiotics/ toxic substances produced by other flora and/or plants. Data also showed that percentage composition of bacterial flora was considerably influenced by the type of the plant (Table 2). The performance and stability of terrestrial ecosystems are determined by biodiversity and species composition. Distribution patterns of microorganisms provide important clues about the underlying mechanisms that structure ecological communities and are central to setting conservation priorities (Bajwa *et al.* 2009).

Bacterial strains isolated in the present investigation are a novel addition to the microdiversity of agricultural crops in Pakistan.

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