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Isolation, cultivation, purification and identification of bacterial species from microfauna of soil

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Abstract

Soil is an excellent source of unknown microorganisms since bacteria, algae, protozoans, yeasts, moulds, and microscopic worms are routinely found in this environment. Therefore, soil is a medium in which life is sustained in a fragile biological balance. Bacteria play an important role in nutritional chains that are an important part of biological balance. In the present study, four different soil samples were collected from the rhizosphere of i) *Sapota zapotilla*, ii) Eucalyptus species, iii) *Ficus religiosa* from Lahore and iv) soil from Changa manga, Pakistan. A Total of 28 bacterial species were isolated and classified in the period between November 2008 and December 2009. All species were cultured on recommended media for verification of biochemical characteristics. The results showed that at least fifteen Gram-positive bacterial species were present in samples and these were considered as the major group constituting the bacterial population strains.

Key words: bacterial strains, soil, gram positive

Introduction

In a balanced soil type, plants grow in an active and vibrant environment. The mineralcontent of the soil and its physical structure are important for a plants' well-being, and it is this life on earth that powers its cycles and provides its fertility. Without the activities of soil organisms, organic materials would accumulate and litter the soil surface, and there would be no food for plants [1]. Bacteria play key roles in maintaining a healthy soil. They act as decomposers that break down organic materials to produce detritus and other breakdown products. Most soil bacteria live in close proximity to plant roots and are often referred to as *rhizobacteria*. Aerobic bacteria are most active in a soil that is moist (but not saturated, as this would deprive aerobic bacteria of the air that they require to function), and neutral in pH, and where there is plenty of food (carbohydrates and micronutrients from organic matter) available [2]. Bacteria are able to perform an extremely wide range of chemical transformations, including the degradation of organic matter, disease suppression and nutrient transformations inside roots (e.g. reducing bacteria in roots, bacteria cause nitrogen fixation) [3].

In general, bacteria in soil are the organisms that are mainly responsible for transforming inorganic constituents from one chemical form to another. Their system of external digestion means that some of the metabolites released by the use of extracellular enzymes may be used by other organisms, such as plants. The bacteria gain nutrients and energy from these processes and provide other organisms with suitable forms of chemicals that they require for their own processes as, for example, in the conversion of nitrate to nitrite, sulphate to sulphide and ammonium to nitrite [2].

The aim of the present work was to isolate, cultivate, purify and identify the bacterial species found in microfouna of soil for their diversity.

Materials and methods

Collection of samples

Four soil samples of 30grams were collected from different locations of Lahore and Changa manga and placed in separate sterile cellophane or paper bags. Each soil sample was collected at different depths (the lowest of which was 3metres), under 40°C, and with a 6.5 pH. All four samples were placed in a refrigerator at 4°C for further studies.

Preparation of medium

The Luria Bertani (LB) Agar media and Nutrient Agar (NA) media, (Panreac. Quimica. SA) were

sterilized at 121°C, temperature15 psi pressure for 15 minutes [4]. After autoclaving, the medium was poured into plates under aseptic conditions.

Isolation of bacterial species

Isolation from soil was achieved by the direct soil plating method [5] and standard dilution method [6] in LB and NA Agar plates. Such plates were then incubated at 37°C for 48 to 72 h for recovery of bacteria from different samples.

Purification of bacterial species

After 24 hours of incubation at 37°C, single colonies were removed from these plates and sub-cultured by streaking on the surface of LB and NA agar plate for purification and preservation.

Identification of bacterial species

Identification of bacterial species was done by recording macroscopic and microscopic characteristics. The purified colonies were subjected to gram staining and characterized using biochemical tests and consulting the pertinent literature. [7-9].

Results

A total of 28 bacterial species were identified from different soil samples (Table 1). They were isolated in pure culture on LB and NA agar media. The quantitative estimates of the microbial population are shown in Table 2. The morphological and physiological characteristics of the cultures are given in Table 3. We found

Table 1. List of Bacterial species Isolated from different microfauna.

Sample no	Substrate and Place of Collection	Date of Collection	Bacterial species
1	Soil, Changa Manga	12th December 2009	*1. Curtobacterium pusillum* *2. Bordetella parapertusiss* *3. Xanthobacter flavus* *4. Sarcina ventriculi* *5. Peptostreptococcus sp* *6. Pediococcus sp.* *7. Lactococcus citreum* *8. Kurthia gibsonii* *9. Staphylococcus caprae* *10. Curtobacterium sp* *11. Micrococcus agilis* *12. Aerococcus sp* *13. Curtobacterium albidum* *14. Enterobacter sp* *15. Ensifer adhaerens* *16. Moellerella visconsensis*
2	Rhizospheric soil of *Sapota zapotilla* (chiko) plant, Lahore	13th November 2008	*17. Bacillus sp.* *18. Escherichia coli* *19. Listeria sp.* *20. Xanthomonas axonopodis* *21. Brochothix sp* *22. Pseudomonas flourescene* *23. Bacillus subtilus* *24. Bacteriodes uniformis*
3	Rhizosopheric soil of Eucalyptus plant, Lahore	16th November 2009	*25. Bordetella parapertussis* *26. Brucella melitensis*
4	Rhizosopheric soil of *Ficus religiosa* (peepal) plant, Lahore	09th December 2009	*27. Trichococcus sp* *28. Acidovorax facitis*

that out of twenty eight isolates, fifteen of them were Gram-positive and nine were Gram-negative. Most of the strains produced yellow pigment on LB agar plates. Further examinations were carried out on the cultures (Table 4). The comparison of results with Bergey's Manual showed that seven Gram-positive bacilli isolates were identified as corresponding to the genus *Xanthomonas, Listeria, Kurthia, Bacillus*, *Xanthobacter, Curtobacterium and Brochothix.*

Table 2. Number of Bacterial colonies by direct soil plating and standard dilution method.

Sample No	Number of Bacterial colonies in 1gm of soil (cfu/g of soil)						
	Direct soil plating method	Standard Dilution method					
		10^{-0}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
1	>69	>78	>65	>54	42	31	24
2	52	>56	>47	>39	28	16	11
3	42	>46	>35	>27	18	09	05
4	48	>48	>31	>24	14	07	05

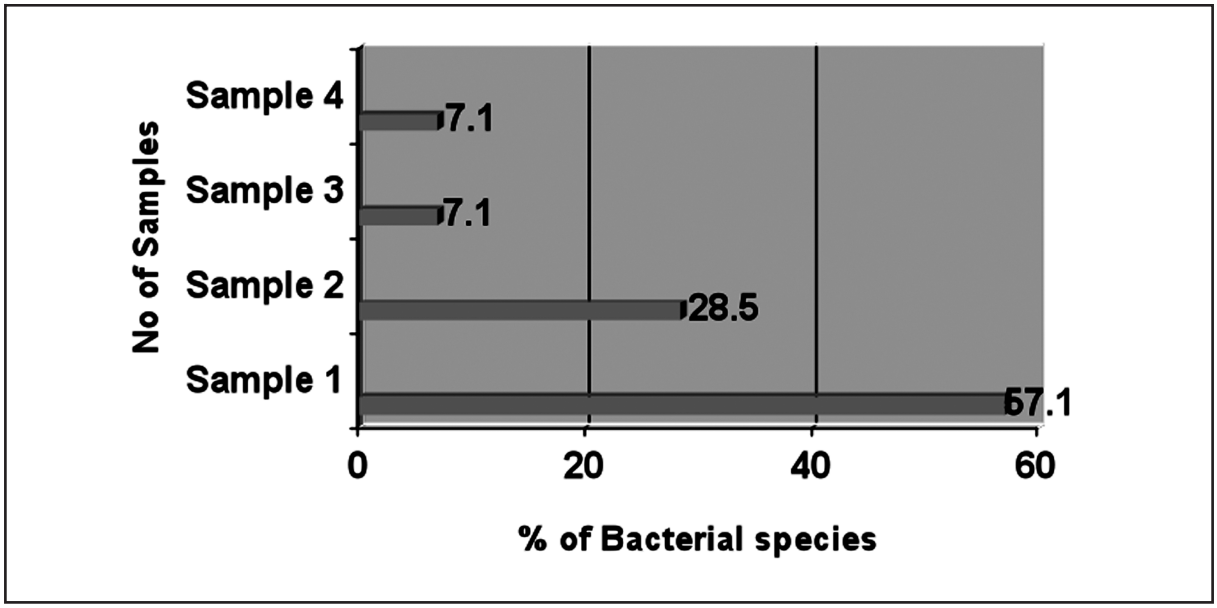
Table 3. An outline of the morphological and physiological characteristic of bacteria isolated from microfauna.

Bacterial genus	Morphology	Gram reaction	Spore	Motility	Pigment
Staphylococcus sp.	cocci	+	-	-	Yellow
Micrococcus sp.	cocci	+	-	+	Yellow
Trichococcus sp.	cocci	+	-	-	Yellow
Xanthomonas sp.	rod	+	-	+	Yellow
Listeria sp.	rod	+	-	+	-
Moellerella sp.	rod	-	-	-	Yellow
Ensifer sp.	rod	-	-	+	White
Aerococcus sp.	cocci	+	-	-	Yellow
Bordetella sp.	cocci	-	-	-	Yellow
Kurthia sp.	rod	+	-	+	-
Enterobacter sp.	rod	-	-	+	-
Lactococcus sp.	cocci	+	-	-	Yellow
Pseudomonas sp.	rod	-	-	+	-
Bacillus sp.	rod	+	+	+	Yellow
Pediococcus sp.	cocci	+	-	-	-
Peptostreptococcus sp.	cocci	+	-	-	Yellow
Sarcina sp.	cocci	+	-	-	Yellow
Xanthobacter sp.	rod	+	-	+	-
Curtobacterium sp.	rod	+	-	+	Yellow
Escherichia sp.	rod	-	-	+	Yellow
Brochothix sp.	rod	+	-	-	-
Bacteriodes sp.	rod	-	-	-	-
Acidovorax sp.	rod	-	-	+	Yellow
Brucella sp.	cocci	-	-	-	Yellow

Table 4. An outline of biochemical tests used in classifying the isolated bacterial species from microfauna

Bio-chemical tests	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Oxidase	+	+	+	+	-	-	-	-	-	-	-	+	+	+	+	+	+	+	-	+	-	-	+	-	-	-	-	-
Catalase	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-
Nitrate	+	-	+	-	-	-	-	-	-	-	+	+	+	+	+	-	+	-	-	-	-	-	+	+	+	+	-	-
Lysine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	+	+	-	-	-	-	-	-
Ornithine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	-	+	+	-	-	-	+	-	-
H2S	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	+	-	-	-	-	-
Glucose	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	+	-	-	-	-	-
Mannitol	+	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Xylose	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
ONPG	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
Indole	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-
Urease	-	-	-	-	+	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
V.P	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Citrate	+	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	+	-	-	-	+	-	-	+	-
TDA	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Gelatine	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-
Malonate	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	+	-	-	-	-	-
Inositol	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	+	-	-	+	-	-	-	-	-
Sorbitol	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-
Rhamnose	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-	-	-	+	-	-	-	-	-	-
Sucrose	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-
Lactose	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	-	+	-	-	-	+	+	-	-	-	-	+
Arabinose	-	-	-	-	-	-	-	-	+	-	+	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
Adonitol	-	-	+	-	-	-	-	-	-	-	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-
Raffinose	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
Salicin	+	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
Arginine	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+

Figure 1. Percentage of Bacterial species Isolated from microfauna of Pakistan



The other genus of *Staphylococcus, Micrococcus*, *Trichococcus*, *Aerococcus, Lactococcus, Pediococcus, Peptostreptococcus* and *Sarcina* were identified as Gram-positive cocci. Seven strains of Gram-negative bacilli were identified as corresponding to the genus *Moellerella, Ensifer, Enterobacter*, *Pseudomonas*, *Escherichia, Bacteriodes* and *Acidovorax.* The percentage of bacterial species isolated from the soil of Changa Manga (sample 1) was high as compared to other samples (Figure 1). In addition, different species of fungi were also isolated from each soil sample and submitted to the collection of the First Fungal Culture Bank of Pakistan (FCBP), Institute of Mycology and Plant Pathology, University of the Punjab, Lahore for identification and preservation.

Discussion

Soil is an excellent source of unknown microorganisms since bacteria, algae, protozoans, yeasts, moulds, and microscopic worms are routinely found in this environment. The results of the present work indicated that gram-positive bacteria constituted the majority of species in the soil microfauna of Pakistan. In this study, the isolated gram-positive bacteria belonged to the genus *Staphylococcus, Micrococcus, Trichococcus, Xanthomonas, Listeria, Aerococcus, Kurthia, Lactococcus, Bacillus, Pediococcus, Peptostreptococcus, Sarcina, Xanthobacter, Curtobacterium* and *Brochothix*. These results are in accordance with Qazi *et al.* [10] who isolated different bacterial species from Lahore, Pakistan and identified these species on the basis of physio-biochemical characterization. Gram-positive bacteria belonging to the genus *Moellerella, Ensifer, Bordetella, Enterobacter, Pseudomonas, Escherichia, Bacteriodes, Acidovorax* and *Brucella* were also isolated and identified. Kamil *et al.* [6] reported four hundred bacterial isolates from the rhizosphere of different plants collected from three different localities in Egypt. These isolates were subjected to a study of their chitinase activity using plate assay.

These bacterial strains are a novel addition to the micro-diversity of soil in Pakistan and further work on these strains may throw light on their therapeutic and anti-mycotic usefulness.

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