Readers Insight
Publisher of open access books and journals
PJMLS
Print ISSN: 2707-4471. Online ISSN: 2707-448X

Research Article	Pak-Euro Journal of Medical and Life Sciences
DOI: 10.31580/pjmls.v6i4.2980	Copyright © All rights are reserved by Corresponding Author
Vol. 6 No. 4, 2023: pp. 441-452	
www.readersinsight.net/pjmls	**Revised:** December 13, 2023 **Accepted:** December 22, 2023
Submission: August 31, 2023	**Published Online:** December 31, 2023

CULTURE-DEPENDENT ANALYSIS OF BACTERIAL DIVERSITY ASSOCIATED WITH *MALUS DOMESTICA* (B.)

Ayesha Rahat[1], Sana Tanveer[1], Basharat Ali[1]*

[1]**Institute of Microbiology and Molecular Genetics**, University of the Punjab, Quaid-e-Azam Campus, Lahore-54590, Pakistan

***Corresponding Author:** Basharat Ali. E. mail: basharat.ali.mmg@pu.edu.pk

Abstract

The surfaces of the fresh fruits harbor a wide variety of bacterial communities, but less is known about their type. The main objective of the current study was to analyze the bacterial diversity isolated from apple (Malus domestica (B.). Samples collected from different locations of Lahore; Pakistan were processed by direct plate count method using 0.1 % peptone water. Sampling was done periodically four times from moderate to cold temperatures (September, October, November and December). Almost 60 strains isolated were characterized based on morphological and biochemical characterization. A total of 24 screened bacterial strains were identified by using 16S rRNA gene sequencing. Results revealed that these bacteria belonged to Bacillus, Marichromatium, Staphylococcus and Enterobacteriaceae genera. The antibiotic susceptibility profile of bacterial strains was assessed against Gentamycin (10 µg), Streptomycin (10µg), Tetracycline (30 µg) and Chloramphenicol (30 µg). All bacterial strains were resistant to Tetracycline. Colorimetric estimation of bacterial auxin manifested significant levels of auxin biosynthesis. B. aerius, Enterobacter cloacea, Klebsiella pneumoniae and B. marisflavi exhibited production of 62.10 µg/ml, 37.67 µg/ml, 126.67 µg/ml, 66.38 µg/ml, 20.53 µg/ml conc. of auxin, respectively. The presence of E. cloaceae, B. cereus, K. pneumoniae on the surface of apple fruit manifests that it was contaminated with different types of potential pathogenic bacterial strains. Albeit bacterial auxin production suggests that they may be beneficial for agricultural productivity.

Keywords: *Antibiotic susceptibility, Auxin, Bacterial diversity, Malus domestica, Pathogenic bacteria*

INTRODUCTION

Fruit and vegetable products are recommended internationally for the daily diet due to their high number of vitamins, low caloric content, fibers, and minerals. These foods are also a source of numerous phytochemicals such as flavonoids, polyphenols, and sterols, exerting antioxidant activity. Despite all these beneficial traits of raw fruits and vegetables, the quality and safety of these products are still a source of concern since they can be quickly spoiled and have a very short shelf-life. Moreover, they are more prone to microbial communities and maybe a vehicle of pathogenic microorganisms (1).

Fruits and vegetables are prone to bacteria post-harvesting. Bacterial communities that usually inhabit fresh fruits and vegetables are *Klebsiella, Proteus sp., Enterobacter, Citrobacter, Escherichia coli, Staphylococcus* and *Salmonella*. The major family of pathogens observed is *Enterobacteriaceae*. These may be pathogenic or may include in the normal flora of fruits that neither cause any harm to fruits nor to human beings who eat them. It is found that the *E. coli* isolated from samples may be Enterohemorrhagic and may ultimately lead to food poisoning (2)

Apple is the most consumed fruit around the globe. It is an important source of vitamins, dietary fibers, and antioxidants. Limited research has been performed on its microbiome to date. Several studies have characterized microbial communities from the apple phyllosphere, flower microbiome and apple endosphere. Studies have reported the presence of *Methylobacterium, Cronobacter, Hymenobacter* and *Sphingomonas* as the most abundant genera on apple skin. However specific insights into the presence of pathogenic bacteria and their antibiotic profile are still missing (3).

The development of antibiotic resistance in the colonized microorganisms is one of the main food safety problems associated with fresh fruits. Particularly, gram-negative bacteria's resistance to antibiotics should

be given careful thought. The antimicrobial-resistant bacteria may be transported and stored on the surfaces of fruits. Drug resistance may be transmitted horizontally through bacterial populations associated with plants. The utilization of pesticides, antimicrobial agents, irrigation wastewater, and farming techniques that involve maneuvers as fertilizers are some of the primary factors contributing to the horizontal spread of antibiotic resistance in microorganisms (4).

There are certain plant beneficial bacteria that colonize fruits and vegetables, for instance; *Azospirillum, Proteus vulgaris, P. mirabilis, K. pneumoniae, E. coli.* These bacteria can stimulate plant growth by production of Indole-3-acetic acid (IAA), cytokinins, gibberellins, or abscisic acid. They can also help in plant growth indirectly by solubilization of phosphate in the soil, production of siderophores, or biological control (5).

The main objective of this study was the assessment of bacterial load present on the surface of an apple i.e. *Malus domestica*. All the samples were taken from different localities in the city of Lahore. Taxonomic classification was evaluated by 16S rRNA gene sequencing and bacteria strains were assessed for their auxin biosynthesis and antibiotic susceptibility profile.

MATERIALS AND METHODS

SAMPLE COLLECTION

Samples of apple fruit (*M. domestica* (B.)) were collected four times from three different locations in Lahore, Pakistan i.e., Karim Block Market, Township Market and Barkat Market. Samples were collected in duplicate i.e., two samples from each location. A total of twenty-four samples were used for the culture-dependent analysis of bacterial load on apple fruit.

ISOLATION OF BACTERIA

Peptone water was used for culture enhancement. Each collected sample without washing with tap water was added to a beaker having sterilized Peptone water. Samples were shaken properly and stood for about 5 minutes. L-agar, S-S agar, MacConkey agar, mannitol salt agar and EMB agar were used for isolation and purification of bacterial strains from peptone water samples. After spreading 25 µl peptone water, these spread plates were incubated at 37ºC for 24 hours. Next day bacterial colonies were observed.

Bacteria strains with distinct morphology were selected from each plate and streaked 2-3 times L-agar plates to get their pure culture.

MORPHOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF BACTERIAL STRAINS

The selected bacterial strains were characterized morphologically and biochemically according to the protocol of Cappuccino and Sherman's protocol (6). Initially, strains were subjected to gram staining. Afterward, a catalase test was performed to detect catalase production by bacterial strains. Cytochrome oxidase test was conducted to detect the presence of the activity of cytochrome oxidase in bacteria. Methyl red test was carried out to identify the bacteria that have the ability to produce stable acidic products after glucose fermentation. Voges Proskauer was performed to analyze the production of neutral products, e.g. acetoin from organic acids by bacteria. Motility test was conducted to determine the motility of bacteria. Bacteria can be observed as motile only when they are not restricted to a line of inoculation and the medium becomes turbid. Urease test was performed to analyze the ability of bacterial strains to produce urease enzyme.

IDENTIFICATION OF BACTERIAL CELLS

The genomic DNA of the isolated bacterial strains was extracted as per the manufacturer's protocol of FavorPrep™ Tissue Genomic DNA Extraction Mini Kit. After isolation, DNA was observed by agarose gel electrophoresis. Amplification of 16S rRNA gene was done by using Dream Taq™ Green PCR Master Mix (Fermentas). The 1.5 kb fragment of 16S rRNA was amplified with a pair of 27f forward (5'-AGAGTTTGATCCTGGCTCAG-3') and 1522r reverse (5'AAGGAGGTGATCCA(AG)CCGCA-3') primers (7). Amplification of 16S rRNA gene was done by using Dream Taq™ Green PCR Master Mix (Fermentas). Steps of PCR carried out in thermocycler along with different conditions are: Denaturation (95ºC for 5

minutes), annealing (55ºC for 1 min, 30 cycles), extension (72ºC for 2-5 min) and final elongation step (72ºC for 10 min). After the confirmation of PCR amplification, gel purification was done by using FavorPrep™ Gel Purification Mini Kit. Finally, the gel purification product of all bacterial strains was sent to First Base Sequence (Singapore).

PHYLOGENETIC ANALYSIS

All the sequences were aligned by a multiple sequence alignment program known as ClustalW by using MEGA 4 software (8) and by Neighbour-Joining method, phylogenetic tree was constructed.

COLORIMETRIC AUXIN ESTIMATION OF BACTERIAL STRAINS

For the estimation of auxin production by isolated bacterial strains, colorimetric method was used as described by Tanveer and Ali (9). About 25 ml of L-broth supplemented with 200 ug/ml of L-tryptophan was added to each flask and inoculated with the bacterial culture. Inoculated broth having no L-tryptophan was used as a control. All the flasks along with controls were incubated at 37ºC for 24 hours. Bacterial cells were removed by centrifugation of media at 5000 rpm for 10 minutes. After that, 2 ml of Salkowski reagent was added to the 1 ml bacterial supernatant. Following that, samples were incubated for 30 min in the dark to allow pink to red color formation. At 535 nm, optical density was measured. To measure bacterial auxin production, the standard curve was plotted utilizing various concentrations of standard IAA.

ANTIBIOTIC SUSCEPTIBILITY TEST

Mueller Hinton agar was used to check the antibiotic sensitivity profile of bacterial strains. One day old bacterial culture was swabbed on the medium by sterile cotton swabs and bacterial lawn of each strain or respective plates was made. Then, antibiotic discs of Streptomycin (10 µg), Gentamycin (10 µg), Chloramphenicol (30 µg) and Tetracycline (30 µg) were placed on the bacterial lawn of each strain with the help of sterile forceps. All plates were incubated at 37ºC for 24 hours. The next day, results were recorded by measuring the zone of inhibition around each disc.

RESULTS

ISOLATION OF BACTERIA

Sampling was done four times from moderate to cold temperatures i.e., September, October, November, and December labeled as SM1, SM2, SM3 and SM4 respectively. A total of 60 distinct colonies were selected and purified. The highest bacterial load was observed with samples collected at moderate temperatures after that it decreased and very few colonies were observed from samples collected at the cold temperature. Isolated colonies were purified and maintained on L-Agar plates. Gram staining of selected strains showed SM3-11 as gram-positive cocci, SM1-4 as gram-positive rods and SM3-8 as gram-negative cocci and SM3-5 as gram-negative rods (Fig. 1, Fig. 2, Fig. 3, Fig. 4, Fig. 5 and Fig. 6).

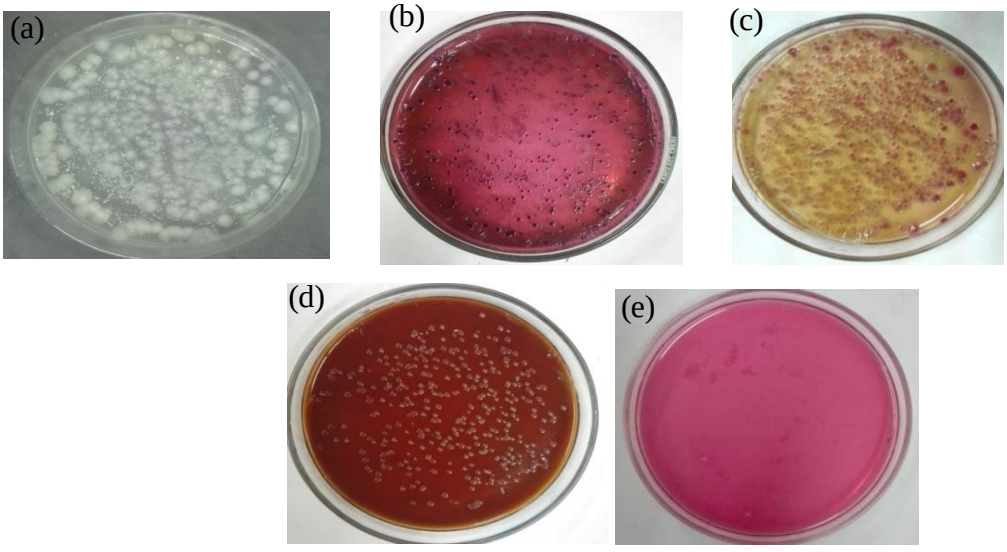


Fig. 1. Results of first sampling. (**a)** L- agar; **(b)** EMB agar; **(c)** Mannitol salt agar; **(d)** S-S agar; **(e)** MacConkey agar

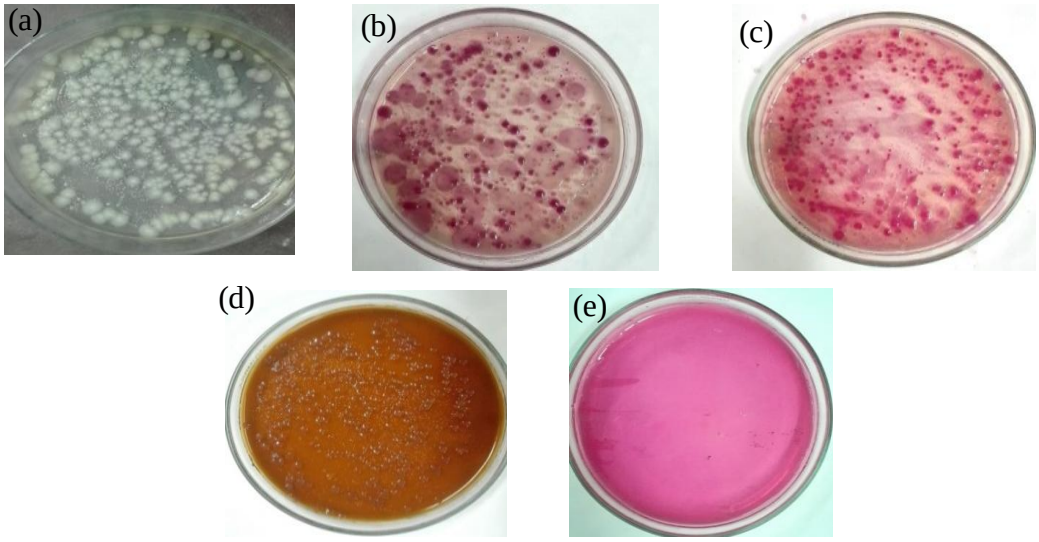


Fig. 2. Results of second sampling. **(a)** L- agar; **(b)** Manitol salt agar; **(c)** MacConkey agar; **(d)** S-S agar; **(d)** EMB agar

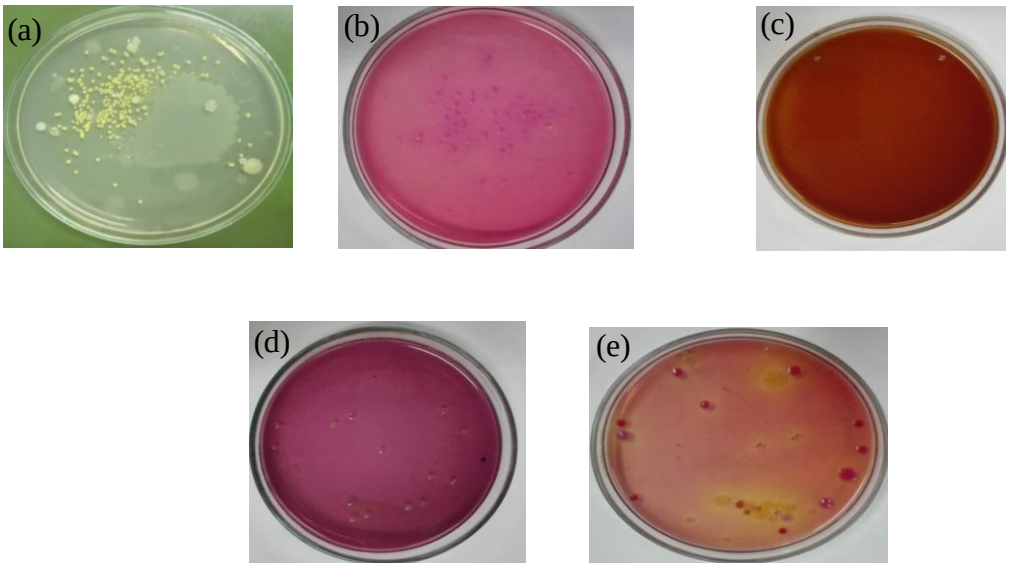


Fig. 3. Results of third sampling. **(a)** maximum growth on L- agar; **(b)** MacConkey agar; **(c)** S-S agar; **(d)** EMB agar; **(e)** Manitol salt agar

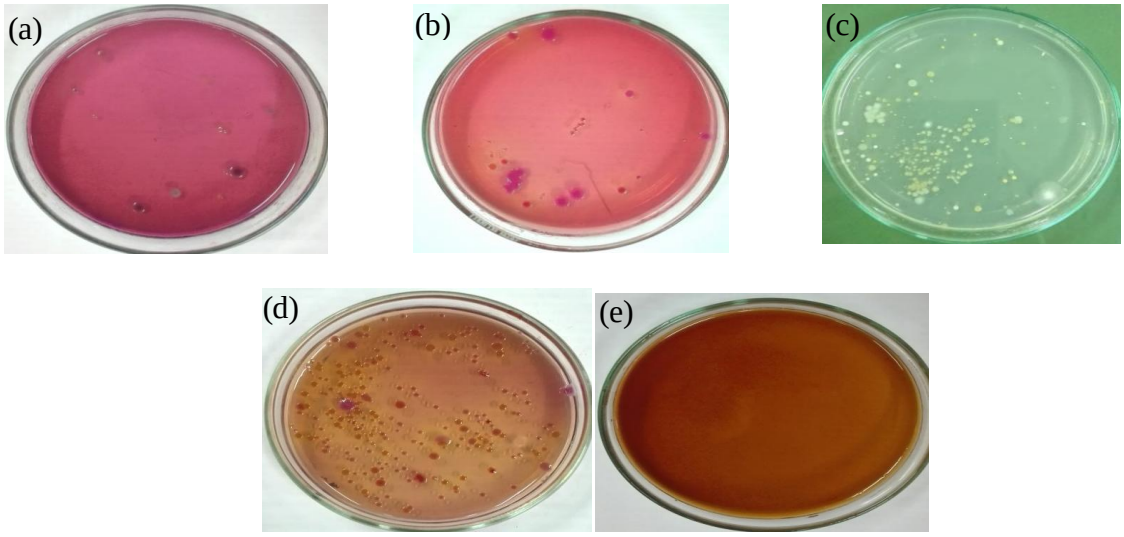


Fig.4. Spreading results of fourth sampling. **(a)** EMB agar; **(b)** MacConkey agar; **(c)** L- agar; **(d)** Manitol salt agar; **(e)** S-S agar

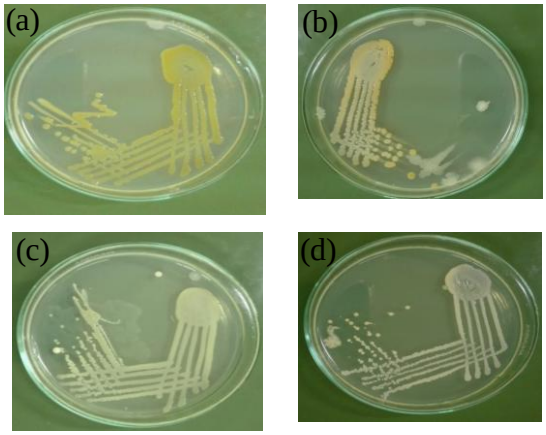


Fig. 5. Bacterial strains isolated from the infected apple. **(a)** *B. cereus* SM3-5; **(b)** *B. safensis* SM1-2; **(c)** *B. pseudomycoides* SM2-8; **(d)** *B. tequilensis* SM3-16

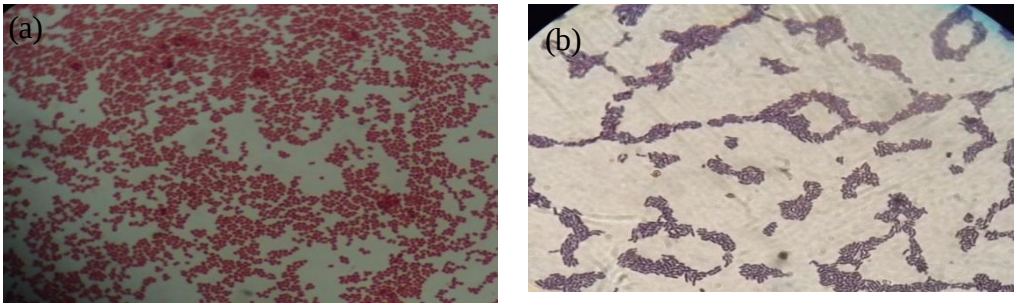


Fig. 6. Gram staining. **(a)** *K. pneumonia* SM3-8; **(b)** *B. aerius* SM1-4

MORPHOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF BACTERIAL STRAINS

Morphological characterization parameters of bacterial colonies were recorded which included margins, elevation, form, size, shape, and color. Most of the bacterial colonies such as SM3-5, SM3-8, SM3-18, SM1-2, SM2-9, SM2-6, and SM3-8 showed entire margins while on the other hand, SM3-12, SM4-8, SM1-4, SM2-3 showed undulate margins (Table I). Bacterial strains were also identified according to biochemical tests which included catalase test, urease test, oxidase test, motility test, MR and VP tests. SM1-4, SM2-9 and SM3-5 showed positive results for the motility test. VP results showed that only SM3-5, SM3-8, SM2-9 and SM1-4 are positive. While SM3-11 exhibited positive results for the methyl red test. For the oxidase test, SM3-5, SM3-11, and SM1-4 revealed positive results. For the urease test, SM3-8 and SM2-9 showed positive results. The majority of strains SM3-8, SM3-5, SM3-11, SM1-4, and SM2-9 showed positive results for catalase test (Table II).

Table I. Morphological characteristics of bacteria

		Colony Morphology Parameters					
S. No.	**Strains**	**Shape**	**Margin**	**Consistency**	**Elevation**	**Size**	**Color**
1	SM3-5	Rods	Ent	Mucoid	Raised	S	OF
2	SM3-18	Rods	Ent	Mucoid	Raised	S	OF
3	SM1-2	Rods	Ent	Dry	Raised	M	Y
5	SM2-6	Rods	Ent	Mucoid	Flat	S	W
6	SM3-8	Rods	Ent	Mucoid	Raised	M	W
7	SM3-12	Rods	Undo	Dry	Raised	M	OF
8	SM4-8	Rods	Undo	Dry	Raised	M	W
9	SM1-4	Rods	Undo	Mucoid	Raised	M	Y
10	SM2-3	Rods	Undo	Mucoid	Flat	M	Y
11	SM2-8	Rods	Ent	Mucoid	Flat	S	Y
12	SM3-4	Rods	Ent	Mucoid	Flat	S	Y

13	SM3-7	Rods	Ent	Mucoid	Flat	S	OF
14	SM3-11	Cocci	Undo	Dry	Flat	L	W
15	SM3-16	Rods	Undo	Mucoid	Flat	L	W
16	SM4-5	Rods	Undo	Dry	Flat	S	W
17	SM4-20	Rods	Ent	Dry	Convex	M	W
18	SM1-3	Rods	Undo	Mucoid	Flat	L	OF
19	SM2-2	Rods	Undo	Mucoid	Flat	M	OF
20	SM2-7	Rods	Ent	Dry	Raised	L	Y
21	SM3-3	Rods	Ent	Dry	Raised	L	Y
22	SM3-10	Rods	Ent	Mucoid	Raised	L	Y
23	SM3-8	Cocci	Ent	Mucoid	Raised	M	W
24	SM3-13	Rods	Undo	Dry	Raised	M	W

Abbreviations: Ent=Entire; Undo=Undonated; L= Large; S=Small; W=White; Y=Yellow; OF=Off white

Table II. Identification of bacteria by biochemical testing

Biochemical tests	Strains				
	SM3-8	SM3-5	SM3-11	SM1-4	SM2-9
Catalase	+	+	+	+	+
Oxidase	-	+	+	+	-
Urease	+	-	-	-	+
MR	-	-	+	-	-
VP	+	+	-	+	+
Motility	-	+	-	+	+
Gram staining	-	-	+	+	-

Abbreviations: MR= Methyl Red; VP= Voges Proskauer; += positive; –=negative

16S rRNA GENE SEQUENCING

Based on gene amplification by PCR and purification of PCR product, 24 bacterial strains were sent for 16S rRNA gene sequencing to First Base Sequence Laboratories in Singapore (Fig. 7 a and b). The homology of bacterial sequences was then compared with GenBank sequence database by BLAST. Most of the bacterial sequences showed homology with *Bacillus* genus. Some of the strains belonged to *Staphylococcus* genus. All sequences were then submitted to GenBank, and accession numbers were acquired (Table III).

Table III. Sequencing of 16S rRNA gene of bacteria associated with *Malus domestica*

Name of strains	Source of samples	Identified as	% Homology	Accession number
SM3-5		*B. cereus*	99	KX417249
SM3-18		*B. safensis*	100	KX417250
SM1-2		*B. safensis*	99	KX417251
SM2-9		*E. cloacae*	99	KX417252
SM2-6		*Serratia marcescens*	98	KX417253
SM3-8	Apple fruit	*K. pneumoniae*	99	KX417254
SM3-12	(*Malus domestica*)	*B. mojavensis*	100	KX417255
SM4-8		*B. thuringiensis*	99	KX417256
SM1-4		*B. aerius*	99	KX417257
SM2-3		*B. weihenstephanensis*	90	KX417258
SM2-8		*B. pseudomycoides*	79	KX417259
SM3-4		*M. purpuratum*	73	KX417260
SM3-7		*M. purpuratum*	88	KX417261
SM3-11		*S. sciuri*	100	KX417262
SM3-16		*B. tequilensis*	99	KX417263
SM4-5		*S. warneri*	99	KX417264

SM4-20	*Planococcus plakortidis*	99	KX417265
SM1-3	*B. aerius*	100	KX417266
SM2-2	*E. cloacae*	99	KX417267
SM2-7	*B. marisflavi*	99	KX417268
SM3-3	*B. toyonensis*	99	KX417269
SM3-10	*S. warneri*	100	KX417270
SM3-13	*B. aerius*	100	KX417271
SM3-13	*B. aerius*	100	KX417272

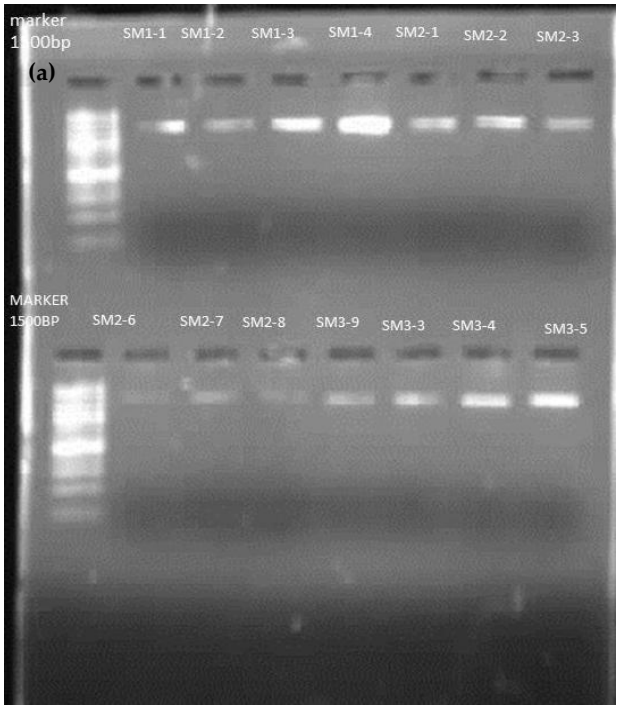
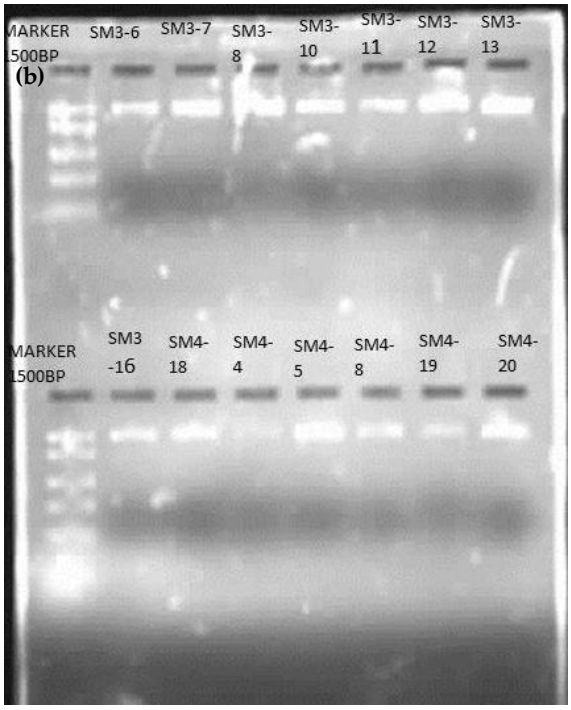


Fig. 7 (a). Agarose gel showing distinct bands of PCR product



(b). Agarose gel showing distinct bands of PCR product

PHYLOGENETIC ANALYSIS

Neighbor-joining algorithm technique was used for the phylogenetic analysis of bacteria by using MEGA 4 software. The constructed phylogenetic tree divided all bacteria into three major groups (Fig.8). *Bacillus* is clustered at the top, *Staphylococcus* in the middle while *Marichromatium* and *Enterobacteriaceae* are clustered at the bottom.

COLORIMETRIC ESTIMATION OF AUXIN PRODUCTION BY BACTERIA

Auxin production by bacteria was determined by using salkowski's reagent. Pink to red color was established after incubation (Fig. 10). Maximum auxin production was shown by SM2-9 *E. cloacae* (126 µg/ml), and SM3-8 *K. pneumoniae* (66 µg/ml) (Table IV, Fig. 9).

Table IV. Concentration of auxin produced by bacteria

S. No	Strains	Auxin production (µg/ml)	
		Without L-TRP	With L-TRP
1	SM1-4	17.10286	37.67429
2	SM2-9	15.96	126.6743
3	SM2-2	16.96	62.10286
4	SM2-7	45.67429	20.53143
5	SM3-18	24.24571	30.96
6	SM3-8	25.67429	66.38857

Abbreviation: L-TRP, L-Tryptophan

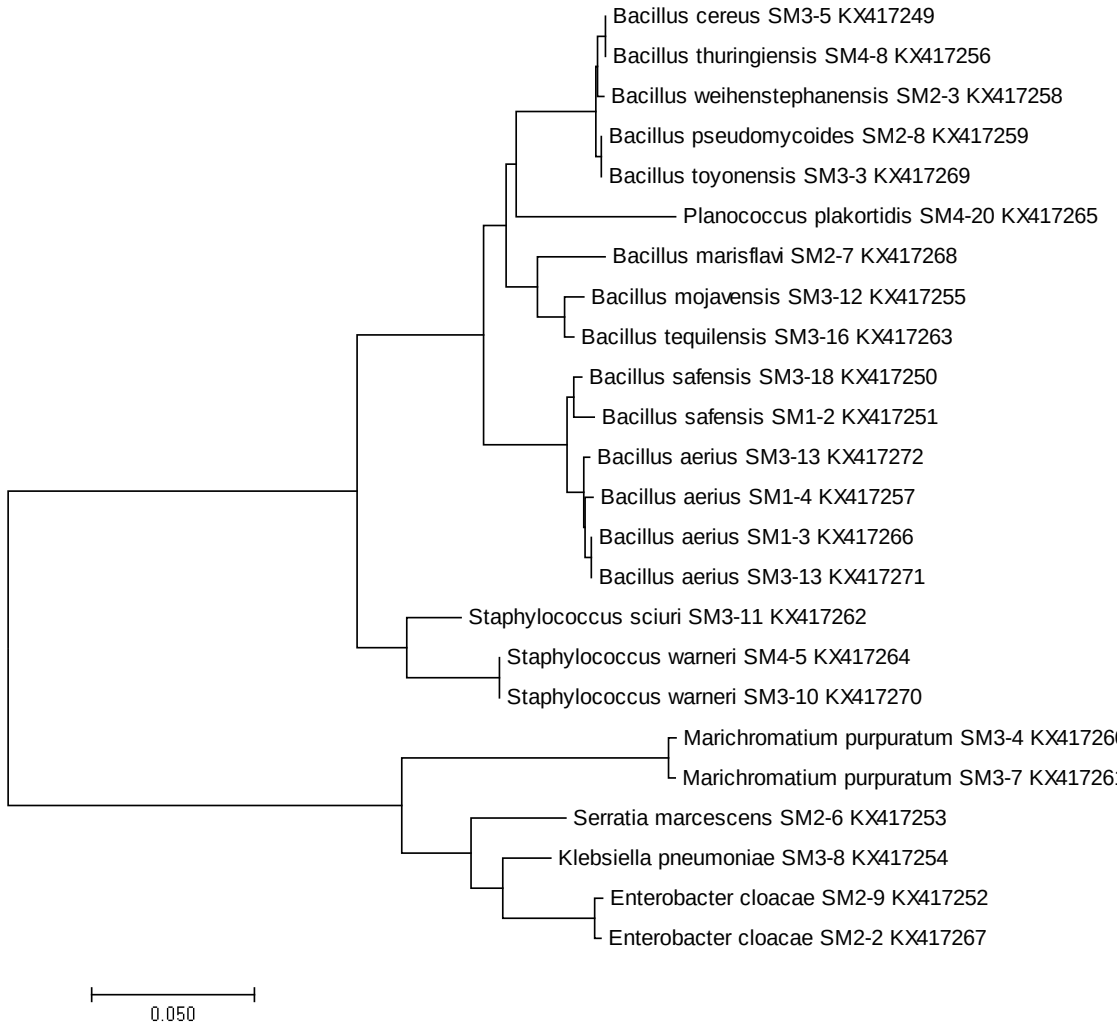


Fig. 8. Combined phylogenetic tree showing a comparison of different 24 strains isolated from the apple fruit. Gram-Positive *Bacillus* were clustered together at the top

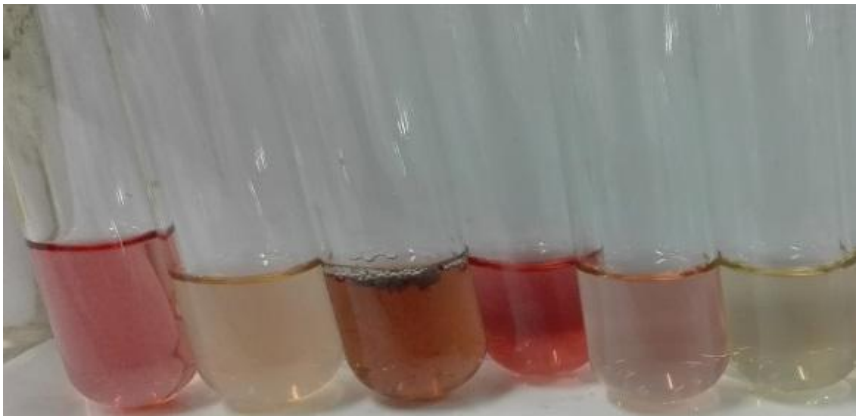


Fig. 9. Auxin production by bacteria. A) *E. cloacae* SM2-2, B) *B. aerius* SM1-4, C) *E. cloacea* SM2-9, D) *K. pneumoniae* SM3-8, E) *B. marisflavi* SM2-7, F) Control

ANTIBIOTIC SUSCEPTIBILITY PATTERN OF BACTERIA

Antibiotic susceptibility of bacteria isolated from *M. Domestica* was observed against Gentamycin (10 µl), Streptomycin (10 µl), Chloramphenicol (30 µl) and Tetracycline (30 µl). Finally, the results obtained were compared with antibiotic susceptibility standard charts of Bioanalyse®. Bacterial strains SM1-3 *B. aerius*, SM1-2 *B. safensis*, SM2-3 *Bacillus weihenstephanensis*, SM3-8 *K. pneumoniae*, SM3-11 *S. sciuri*, SM3-18 *B. safensis* are all sensitive to Gentamycin with zone of inhibition of 16, 22, 12, 12, 14 and 16 respectively. All of them were also sensitive to Streptomycin with zones of inhibition 16, 20, 16, 16, 16 and 18 respectively. On the other hand, only SM3-8 *K. pneumoniae* and SM3-11 *S. sciuri* were sensitive to Chloramphenicol. While all the above strains were resistant to Tetracycline (Fig. 10, Table V).

Table V. Antibiotic susceptibility pattern of bacteria

Name of Strains	Name of antibiotics			
	CN	S	C	TE
	Zone of inhibition-mm (Susceptibility)			
SM1-3	16 (S)	16 (S)	12 (R)	12 (R)
SM1-2	22 (S)	20 (S)	12 (R)	12 (R)
SM2-3	12 (S)	16 (S)	8 (R)	12 (R)
SM3-8	12 (S)	16 (S)	16 (S)	12 (R)
SM3-11	14 (S)	16 (S)	16 (S)	12 (R)
SM3-18	16 (S)	18 (S)	14 (I)	12 (R)

Abbreviations: CN, Gentamycin; S, Streptomycin; C, Chloramphenicol; TE, Tetracycline; S, sensitive; I, Intermediate; R, Resistant

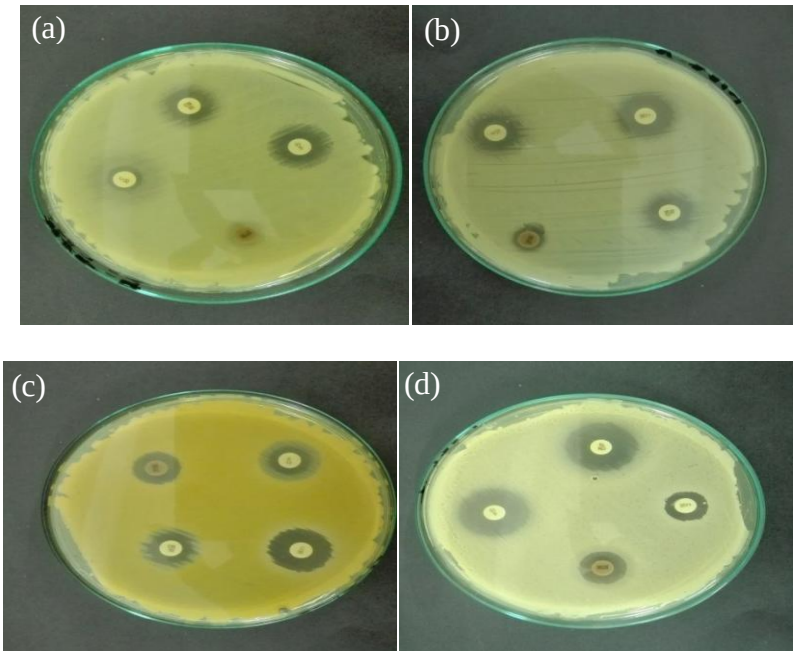


Fig. 10. Antibiotic susceptibility pattern of bacteria shown by clear zones around each disc. **(a)** SM1-3 *B. aerius*; **(b)** SM1-2 *Bacillus safensis*; **(c)** SM2-3 *B. weihenstephanensis*; **(d)** SM3-8 *K. pneumoniae*

DISCUSSION

In this study, the microbial content, especially bacterial load that inhabits the surface of fresh *Malus domestica* B. was analyzed. Apple is a member of the Rosaceae family of plants, which has over 300 species and over 100 genera worldwide. Temperate areas are home to most of these species and harbor numerous microbial communities (10). It was observed that bacterial load was maximum on the samples that were taken in the month of moderate temperature i.e. September. It is because the slightly hot climate proved to be favorable for bacterial growth, so sampling in this month showed the highest bacterial growth with a great diversity. While it gradually decreases with the decrease in temperature. Interestingly, very low growth was observed on S-S agar. It showed that *Salmonella* and *Shigella* species could not survive in lower temperatures. No growth was observed on the plate of EMB agar, S-S agar, mannitol salt agar and MacConkey agar. Very few bacterial colonies were observed on L- agar. This demonstrates that cold temperature is unfavorable for this bacterial growth.

For cultural dependent analysis 0.1% solution of peptone water was used to promote the growth of bacteria that live on the surface as peel is the least favorable spot for microbes to inhabit. 16S rRNA gene sequencing revealed that most of the identified bacteria belonged to *Bacillus* species. Among them, *B. cereus* SM3-5, *B. safensis* SM3-18, *B. mojavensis* SM3-12, *B. thuringiensis* SM4-8, *B. aerius* SM1-4, *B. weihenstephanensis* SM2-3, *B. pseudomycoides* SM2-8, *B. tequilensis* SM3-16, *B. marisflavi* SM2-7, *B. toyonensis* SM3-3 were included. Others belonged to *Staphylococcus* which included *S. warneri* SM4-5. *Enterobacteriaceae* were also found e.g. *S. marcescens* SM2-6, *K. pneumoniae* SM3-8. Some distinct species were also identified e.g. *M. purpuratum* SM3-4, *B. weihenstephanensis* SM2-3, *B. mojavensis* SM3-12. A recent study indicated that if you eat only peel and fruit

pulp then you will ingest 3.87×10^7 and 3.39×10^6 16S rRNA gene copies with one apple, respectively, they have reported *Bacillus*, *Sphingomonas*, *Pseudomonas*, and *Methylobacterium* as abundant bacterial genera (11).

After purification of all bacterial strains on L- agar, gram staining was done and all varieties were obtained e.g. Gram-positive cocci and rods, and Gram-negative cocci and rods were obtained. Some of the biochemical tests such as catalase test, urease test, Methyl Red (MR) test, Voges Proskauer (VP) test, motility test and oxidase test. Most of the strains were catalase positive e.g. SM3-8, SM3-5, SM3-11, SM1-4 and SM2-9. Very few strains showed positive results for MR test e.g. SM3-11. Positive results for VP test were obtained by SM3-5, SM3-8, SM1-4 and SM2-9. On the other hand, SM3-11 showed a negative result for VP test. Some of the strains gave positive results for the urease test including SM2-9 and SM3-8. When the oxidase test was performed, the strains showing positive results were SM1-4, SM3-11 and SM3-5 while SM3-8 and SM2-9 showed negative results. SM1-4, SM2-9 and SM3-5 showed positive results for motility test. For the urease test, SM3-8 and SM2-9 gave positive results while most strains were negative for the urease test e.g. SM1-4, SM3-5 and SM3-11. Mairami *et al.*, (12) have also reported *B. cereus* and *Klebsiella* isolated from tomato, guava, and banana to be catalase and VP positive for MR negative results.

Antibiotic susceptibility testing for all bacterial strains was done. The antibiotics used were Streptomycin (10 µg), Gentamycin (10 µg), Tetracycline (30 µg) and Chloramphenicol (30 µg). All the strains showed resistance towards tetracycline while only SM3-8 *K. pneumoniae* and SM3-11 *S. sciuri* were sensitive to Chloramphenicol. Auxin is a plant growth-promoting hormone synthesized from L-tryptophan. The concentration of auxin was estimated quantitatively by Salkowski's reagent, and the results manifested the highest levels of auxin production by *E. cloacea* SM2-9 at 200 µg/ml conc. of L-tryptophan i.e., 126.67 µg/ml. *Acinetobacter ursingii* isolated from *Pyrus communis* (pear) has been reported to produce 19.48 mg/L of auxin (5).

CONCLUSION

In conclusion, hygienic conditions of ready-to-eat and fresh raw fruits post-harvesting are not so good in Pakistan. The surface of apple fruit was contaminated with different types of potentially pathogenic bacterial strains; especially, *E. cloaceae, B. cereus, K. pneumoniae*. These pathogenic microorganisms can cause severe food-borne diseases, ultimately food poisoning. SM3-8 *K. pneumoniae* and SM3-11 *S. sciuri* were sensitive to Chloramphenicol while all are resistant to Tetracycline. Nevertheless, colonized bacteria exhibited auxin production that may be beneficial for plant growth and productivity.

Authors' Contribution:

A. Rahat conducted the experimental work and collected the data. S. Tanveer prepared the draft of the manuscript. B. Ali conceived this study and checked the final draft of this study.

Conflict of Interest:

Authors have no conflict of interest.

References:

1. Sequino G, Valentino V, Torrieri E, De Filippis F. Specific microbial communities are selected in minimally-processed fruit and vegetables according to the type of product. Foods. 2022;11(14):2164.
2. Saksena R, Malik M, Gaind R. Bacterial contamination and prevalence of antimicrobial resistance phenotypes in raw fruits and vegetables sold in Delhi, India. Journal of Food Safety. 2020;40(1):e12739.
3. Bösch Y, Britt E, Perren S, Naef A, Frey JE, Bühlmann A. Dynamics of the apple fruit microbiome after harvest and implications for fruit quality. Microorganisms. 2021;9(2):272.
4. Ahsan A, Tanveer S, Shahzadi K, Gull S, Ali B. Genetic and Functional Diversity of Bacterial Strains Associated with *Prunus persica* (L.). Journal of Advances in Microbiology. 2022;22(10):75-89.
5. Nadal MC, Ferreira GM, Andrade GV, Buttrós VH, Rodrigues FA, da Silva CM, Martins AD, Rufato L, Luz JM, Dória J, Pasqual M. Endophytic Bacteria Can Replace the Need for Synthetic Auxin during In Vitro Rooting of *Pyrus communis*. Agronomy. 2022;12(5):1226.

6. Cappuccino JG, Sherman N. In: Microbiology: A Laboratory Manual, sixth ed. Pearson Education, Signapore; 2002.
7. Bano I, Tanveer S, Ali B. Plant Growth Promoting Potential of Rhizobacteria Isolated from *Cannabis Sativa* L. Pak-Euro Journal of Medical and Life Sciences. 2022;5(2):291-300.
8. Tamura K, Nei M, Kumar S. Prospects for inferring very large phylogenies by using the neighbor-joining method. Proceedings of the National Academy of Sciences. 2004;101(30):11030-5.
9. Tanveer S, Ali B. Evaluation of Bacillus and Rhizobium Strains to Enhance the Growth of *Vigna radiata* (L.) under Drought Stress. Pak-Euro Journal of Medical and Life Sciences. 2022;5(1):101-12.
10. Padder SA, Mansoor S, Bhat SA, Baba TR, Rather RA, Wani SM, Popescu SM, Sofi S, Aziz MA, Hefft DI, Alzahrani OM. Bacterial endophyte community dynamics in apple (*Malus domestica* Borkh.) germplasm and their evaluation for scab management strategies. Journal of Fungi. 2021;7(11):923.
11. Wassermann B, Müller H, Berg G. An apple a day: which bacteria do we eat with organic and conventional apples?. Frontiers in microbiology. 2019:1629.
12. Mairami FM, Negbenebor HE, Ali M. Determination of bacterial isolates associated with fruits spoilage in Gwagwalada market, Abuja Nigeria. Clinical Biotechnology and Microbiology. 2018;2(4):401-7.