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Spices are indispensible in the Indian culinary. A variety of spices are used to enhance the taste, flavor, and aroma of several Indian delicacies. While on one hand, spices are known to possess several health benefits because of their antimicrobial and antioxidant properties, spices may also be responsible for certain health hazards. From the time that seeds are sown, the crops are harvested, transported and stored, these spices constantly comes in contact with microorganisms. Sources of microorganisms include the plant itself, soil, water, air, storage containers and handlers. Thus it is evident that a variety of microorganisms can be found residing in these spices. In this study, microflora (bacteria) from 16 different spices from 4 different regions in India, namely, Kolkata, Gujarat, Ranchi and Chennai were isolated, characterized and identified. A total of 40 different bacterial isolates could be obtained. Simple traditional methods like colony characteristic was initially used to screen dominant types. 16 bacterial isolates were selected based on dominance. Staining, study of cultural properties and enzyme (catalase, amylase, lipase, pectinase, laccase, lignin peroxidase) assays of the 16 isolates were done. Finally molecular techniques using 16s rRNA sequencing were studied for identification of some selected isolates. While on one hand some of the isolates could be identified as potentially harmful and pathogenic ones, like *Acinetobacter baumanii*, *Bacillus anthracis*, *Bacillus cereus* and some other isolates, like *B. tequilensis* and *B. subtilis* which have the potential to play beneficial roles.

Key words: Colony characteristics, Coriander, Cumin, microbial association, microflora

INTRODUCTION

Spices, in the Oxford dictionary, is defined as "any vegetable substance which is aromatic or pungent that is used to flavor food, e.g. cumin, cloves, or coriander". Elsewhere, a spice has been defined as "a fruit, seed, root, flower, bark, or other plant parts used mainly for the purpose of coloring or adding flavor to food."

Spices are often available and used in various forms, like, fresh, entire but dried, or powdered and dried. Spices are generally stored in dry forms. Fresh spices, like ginger and garlic have shorter shelf life than the whole dried and powdered dried ones. The whole dried spices are often more flavorful than the powdered dried ones. The flavors in a spice are partly contributed by the volatile oils which may evaporate or undergo chemical reactions (e.g. oxidation/reduction) on aerial exposure. When a particular spice is ground to a powder from its whole form, it increases the surface area. This result in increased rates of evaporation or chemical reaction like oxidation and hence flavor loss. Thus spices are better stored whole and ground whenever needed. The shelf life for whole dried spices can be for two years while that for powdered ones are six months. When it comes to the "flavor life", powdered spices have it even shorter than their shelf life.

Cumin spice is the dried seed of the plant *Cuminum cyminum*, belonging to the Apiaceae family. It is an annual herb and seeds are harvested manually by hand. The seeds of cumin are oblong in shape,

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yellowish brown in color with eight longitudinal ridges with oil canals (Sastry and Anandraj, 2013). India is the top producer of cumin in the world and is maximally produced in Gujarat and Rajasthan.

The typical flavor and aroma of cumin is contributed by a large number of compounds, mostly volatile. The principal component is cuminaldehyde (Bettaieb *et al*. 2011).

Coriander (*Coriandrum sativum*), like cumin, is also an annual herb belonging to the Apiaceae family. While all the parts of the plant are edible but it's the seeds that find all time use in cooking. The seeds possess a lemony citrus flavor due to the principal component, linalool and pinene.

Both cumin and coriander seeds are widely used in Indian curries and possibly each and every family makes regular use of these two spices in everyday cooking. Thus it becomes important to find out what are the common types of microorganisms usually associated with these spices. Earlier reports have shown that different spices can be heavily contaminated by various kinds of microorganisms. Reports show the presence of total aerobic mesophilic bacteria in 89% of the spice samples tested (Karam *et al*.2021). There had been some studies on the microflora of different spices in the 20th century, but the later research documented the beneficial aspects of these spices. Several studies have already been done on the antimicrobial, antifungal, antioxidant, anticancerous and antidiabetic properties of different spices. This trend is still continuing. Not much of work is documented on the microflora of the spices and their roles in spoiling/degrading the concerned spices. Hence, this research deals with studying the microflora of some Indian spices, namely, cumin and coriander. The microflora after isolation is purified, characterized and identified. This investigation was undertaken to understand the microbial plethora of the different spices samples and to identify and characterize the most prevalent ones.

MATERIALS AND METHODS

Samples used

Two different spice samples, Cumin and Coriander, both whole dried and powdered samples from different regions of India, namely, Kolkata, Gujarat, Chennai and Ranchi were collected. All the samples used were non branded. The spices after collection were stored at room temperature in air tight containers and away from light.

Isolation of bacteria

A stock solution of 100 mg/ml of each spices sample was prepared in sterile distilled water. Isolation of bacteria was done by serially diluting the stock samples upto 10^{-4} followed by plating 100µl of the dilutions onto Nutrient Agar [HiMedia] (Peptone 5g/l, beef extract3g/l, NaCl 5g/l, Agar 18g/l, pH 7±0.2) and Chrome Agar [Hi media; AlianRambach] (Peptic digest of animal tissue 15g/l, chromogenic mixture 2.5g/l, Agar 13.5 g/l, Casein enzymic hydrolysate 4g/l, pH 7.2±0.2). Nutrient Agar and Chrome Agar were prepared according to the composition specified, sterilized using an autoclave at 121°C, 15 lbs/sq. inch pressure for 15 minutes, cooled and followed by pour plating of the dilutions of the spice samples. Plates were then incubated in inverted position for 24 h at 37°C. Individual colonies from each plate were purified by further subculturing them into Nutrient Agar plates.

Characterization of the purified colonies by colony morphology and Gram staining

Colony characteristics, like colony count, color, shape, size, form, opacity and elevation were noted down. This was followed by Gram staining for bacterial colonies.

Bacterial colony identification by cultural methods

The bacterial colonies were streaked onto Bacillus HiChrome Agar (Hi Media) and depending on the colour of the colony obtained on Bacillus HiChrome Agar, a probable identification was done. The media was prepared according to instructions provided by Hi Media, sterilized using an autoclave maintained at 121°C for 15 min. followed by plating into sterilized Petri dishes. Individual selected isolates based on dominance were streaked and incubated at 37°C for 24 h.

Enzyme assays of the bacterial isolates

The bacterial isolates were assayed qualitatively for enzymes like catalase, amylase, pectinase, lipase, lignin peroxidase and laccase.

Catalase assay was performed taking a drop of 3% Hydrogen Peroxide on a slide and mixing the bacterial culture with the Hydrogen Peroxide and checking for effervescence by following standard microbiological protocol.

Amylase producing ability of the isolates was detected by streaking them on Starch Agar and adding of Iodine solution after incubation by following standard microbiological protocol. A clear zone around the colonies indicated presence of amylase producing ability of the isolate.

Pectinase assay was done on Pectinase Screening Agar Medium (PSAM) plates by streaking the isolates on it and incubating for 2 days to 2 weeks. PSAM was prepared measuring g/L; $NaNO_3$ 2g/L, KCl 0.5g/L, $MgSO_4$ 0.5g/l, K_2HPO_4 1g/L, trypton 0.5g/L, agar 20g/L and pectin 10g/L. After incubation, 50mM Potassium iodide-iodine solution was flooded onto the plates. A clear transparent halo zone is indicative of pectinase producing ability of the isolates (Beg *et al*. 2000).

Lipase assay was done using Tributyrin Agar (HiMedia) plates. The media contains peptone 5 g/l yeast extract 3 g/l, agar 15 g/l and pH 7 is maintained. 10 ml of Tributyrin (FD081) was added to make up the volume of the medium to 1lt which was sterilized using an autoclave maintained at 121^0C for 15 min. Plates were poured, solidified and then streaked with the isolates followed by incubation at 37^0C for 24-48 hours. Tributyrin is degraded by lipolytic microorganisms which are indicated by clear zones surrounding the colonies.

Lignin peroxidase assay was done by the primary plate screening method using Guaiacol indicator. A 3% nutrient agar plate with 0.01% of guaiacol was inoculated with the cultures to be tested and incubated at 30°C for 5 days. Plates were observed for dark brown halo due to oxidation of guaiacol (Shukla and Padhiar, 2019).

Laccase assay was also performed using guaiacol indicator. Nutrient agar medium containing 0.5mM of guaiacol was used to streak the isolates. Laccase producing cultures developed a reddish brown colour after incubation. (Sheikhi *et al*. 2012).

Isolates that gave positive tests for qualitative analysis were subjected to quantification of the respective enzymes that they could produce following the same reference used for qualitative analysis.

16s rRNA sequence analysis of bacteria

Based on the colony morphology, staining, selective media screening, and enzyme assays, five selected bacterial isolates were characterized by molecular sequencing of 16s rRNA by Sanger sequencing method. (Kumar, 2016).

RESULTS AND DISCUSSION

Isolation of microflora

Upon careful and repeated isolation procedures from all the sixteen different spice samples, a total of 40 different bacterial colonies could be isolated.

Colony characteristic study and gram staining

CFU count and colony morphology of the bacterial isolates have been illsutrated in Fig. 1 and Table1 respectively. CFU count for powdered spice samples is higher than that of whole spice samples. CFU count for whole spice samples ranges from $31x10^4$ to $73x10^4$ CFU/g; while for powdered samples, it ranges from $28x10^4$ to $77x10^4$ CFU/g. Two of the samples have shown higher than 300 x 10^4 CFU/g heavy load and possibly a contaminated sample. Eliminating the repeating isolates and considering the dominant types, 16 different bacterial colonies were used for further studies. Results of Gram staining of the 16 bacterial isolates are tabulated in Table. 2. Fifteen of the bacterial isolates were Gram Positive rods and only one was Gram negative rod.

Bacterial colony identification by cultural methods

When grown on Bacillus HiChrome Agar, species of *Bacillus*, ranging from *Bacillus cereus, Bacillus subtilis, Bacillus licheniformis, Bacillus megaterium, Bacillus mycoides, Paenibacillus* species and *Lysinibacillus* species were identified. The plates showing the growth of the isolates on Hi Chrome Agar are given in Fig. 2 and the probable identification on HiCrome agar of the 16 bacterial isolates are also presented in Table 2. The Gram negative rod did not grow on Bacillus HiChrome

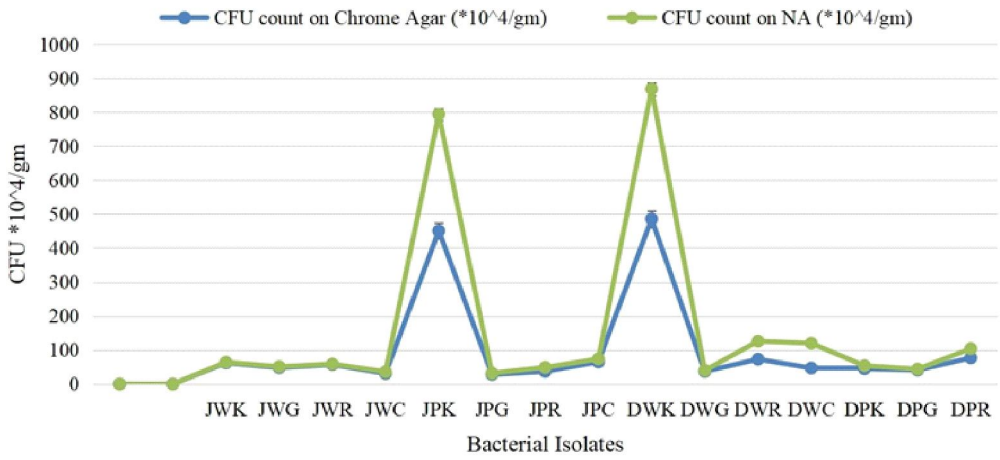


Fig. 1: CFU count of the 10^{-2} Chrome Agar and Nutrient Agar plates
(J- Cumin, W -Whole, P -Powder, D - Coriander, K- Kolkata, G - Gujarat, R - Ranchi, C - Chennai)

Agar. Banerjee and Sarkar (2003) have earlier reported the presence of bacteria like *Bacillus cereus, Staphylococcus aureus, Clostridium perfringenes, E. coli* and other coliforms from different spice samples collected from different regions in India. Noel *et al*. (2017) studied the presence of several contaminating microbes in spices and spice blends. They could detect pathogens like *E.coli* (3.14 $\log_{10}$cfu/ml-1.17±1.07 $\log_{10}$cfu/ml) and *Salmonella* spp. (0-0.9 $\log_{10}$cfu/ml). Prevalence of *B. cereus* and *B. thuringensis* and their subsequent risk effects in different spice samples have been discussed by Cufaoglu and Ayaz (2021). These studies are in accordance with our findings. *B. cereus* seems to be a common occurrence in spices.

Enzyme assays of the bacterial isolates

The sixteen bacterial isolates were assayed qualitatively for enzymes like catalase, amylase, pectinase, lipase, lignin peroxidase and laccase. Since the bacterial isolates originated from spices, and they may have key roles in degrading the spice samples, they were assayed qualitatively initially for the presence of some of these degradative enzymes, specifically, pectinase, lignin peroxidase, laccase and lipase, followed by quantifying the enzymes wherever they are present. The enzyme assay (qualitative) data have been presented in Table 3. Ten out of the sixteen bacterial isolates are catalase positive, five are negative and one demonstrated variable result. All the isolates except one are able to produce amylase. None of the isolates produce lipase. Eleven isolates are positive for laccase, while four isolates each for pectinase and lignin peroxidase are found to be positive. Four isolates were chosen that produces these degradative enzymes and the enzyme activity were quantified, the data is graphically illustrated in Fig. 3.

Enzymes like catalase and amylase production have been well documented in several bacteria isolated from various sources. Spice is no exception. Several different Gram positive rods, with a probability of belonging to the *Bacillus* genus will have both catalase and amylase production ability. Our study is also in line showing 62.5% of the isolates producing catalase and 94% producing amylase. Lipase production ability of bacteria has also been documented from a variety of sources by several researchers, but none from spices. Likewise, none of our isolates could produce lipase. The ability of fungi to degrade lignin and pectin has been well documented. But bacterial isolates, from spices, able to produce these enzymes, probably is new finding. Several workers have documented presence of pectinase, lignin peroxidase and laccase from bacteria isolated from different sources. Presence of laccase in *Bacillus* species have been reported (Franc *et al*. 2001; Canas *et al*. 2007). Nascimento and Silva (2008) reported presence of lignin peroxidase in *Streptomyces* species. While pectinase producing microbes are more prevalent in fruits and vegetables, lignin peroxidase and laccase, both being enzymes responsible for lignin degradation, are prevalent in plant material. Since spices are of plant origin, it thus becomes pertinent to assay for these enzymes in the isolates that we obtained from spices. As expected, 69% of the bacterial isolates tested positive for laccase and 25% isolates for lignin peroxidase and pectinase each. Pectinase activity was found to be highest for JWG2 isolate while laccase was highest for JPK2 and DPG4 and lignin peroxidase were evident for JPK1, JPK2, JPR3 and DWG1 among tested isolates.

Table 1. Colony characteristics of the bacterial isolates on Nutrient Agar and Chrome Agar.

Sample/Media	No. of colonies	Isolate	Size	Colour/ Chrome Agar	Shape,Form and Opacity
JWK (NA)[a]	2	NA[b]	Medium	Creamy white	Entire, smooth, translucent
JWK(Chrome)	62	JWK1	Large	White with blue zone	Undulate, smooth, translucent
		JWK2	Small	Deep blue	Entire, smooth, translucent
		JWK3	Small	Turquoise	Entire, smooth, opaque
		JWK4	Small	Creamy white	Entire, rough, translucent
JWG(NA)	3	NA	Small	White	Entire, smooth, translucent
JWG(Chrome)	48	JWG1	Small	Creamy white	Entire, rough, translucent
		JWG2	Small	Creamy white with zone	Entire, smooth, translucent
JWR(NA)	2	NA	Small	White	Undulate, rough, translucent
JWR(Chrome)	57	JWR1	Small	Off white	Undulate, rough, opaque
		JWR2	Small	Creamy white	Entire, rough, translucent
		JWR3	Small	White	Entire, smooth, translucent
		JWR4	Large	Off white	Undulate, smooth, opaque
JWC(NA)	5	NA	Medium	Creamy white	Entire, smooth, translucent
JWC(Chrome)	31	JWC1	Large	Creamy white	Undulate, rough, translucent
		JWC2	Small	Deep blue	Undulate, smooth, opaque
		JWC3	Small	Turquoise	Undulate, rough, translucent
		JWC4	Medium	Greenish blue	Undulate, rough, translucent
JPK(NA)	343	NA	Medium	Off white	Undulate, rough, opaque
JPK(Chrome)	451	JPK1	Small	Creamy white	Undulate, rough, opaque
		JPK2	Large	Creamy white	Undulate, smooth, opaque
JPG(NA)	4	NA	Large	Off white	Entire, smooth, translucent
JPG(Chrome)	28	JPG1	Small	Deep blue	Entire, smooth, opaque
		JPG2	Small	Creamy white	Undulate, rough, opaque
		JPG3	Small	Turquoise	Entire, smooth, translucent
JPR(NA)	12	NA	Medium	Yellowish white	Entire, smooth, translucent
JPR(Chrome)	37	JPR1	Small	Deep blue	Entire, smooth, opaque
		JPR2	Small	Creamy white	Entire, rough, translucent
		JPR3	Small	Creamy white with colorless zone	Entire, rough, translucent
DWK(NA)	382	NA	Medium	Off white	Undulate, rough, opaque
DWK(Chrome)	486	DWK1	Large	Turquoise with reddish zone	Undulate, rough, opaque
DWG(NA)	3	NA	Small	Creamy white	Entire, smooth, translucent
DWG(Chrome)	37	DWG1	Small	Pale yellow	Entire, smooth, translucent
		DWG2	Small	Turquoise	Entire, smooth, translucent
		DWG3	Small	White	Entire, rough, translucent
DWR(NA)	53	NA	Small	Yellow	Entire, smooth, opaque
DWR(Chrome)	73	DWR1	Large	White with blue pigment	Undulate, rough, opaque
		DWR2	Small	Deep blue	Entire, smooth, opaque
		DWR3	Small	Purple	Entire, smooth, translucent
DWC(NA)	73	NA	Medium	Creamy white	Undulate, smooth, opaque
DWC(Chrome)	47	DWC1	Large	Greenish blue	Undulate, rough, translucent
		DWC2	Punctiform	Creamy white	Entire, rough, opaque
		DWC3	Punctiform	Deep blue	Entire, smooth, opaque
DPK(NA)	9	NA	Small	Creamy white	Entire, rough, opaque
DPK(Chrome)	45	DPK1	Medium	Creamy white with blue pigment	Undulate, smooth, opaque
		DPK2	Medium	White	Undulate, rough, opaque
DPG(NA)	3	NA	Small	Off white	Entire, rough, opaque
DPG(Chrome)	41	DPG1	Small	Creamy white	Undulate, rough, opaque
		DPG2	Medium	Creamy white	Entire, rough, opaque
		DPG3	Small	Off white	Entire, rough, opaque
		DPG4	Small	White	Undulate, rough, opaque
DPR(NA)	27	NA	Large	White	Undulate, rough, opaque
DPR(Chrome)	77	DPR1	Small	White	Entire, rough, opaque
DPC(NA)	8	NA	Medium	White	Undulate, rough, opaque
DPC(Chrome)	56	DPC1	Small	Blue with white zone	Undulate, rough, opaque

[a]NA – Nutrient agar medium ; [b]NA - Not Applicable (Colonies from Nutrient Agar were not used as less numbers and varieties were obtained here when compared to Chrome agar, thus no isolate numbering)

Table 2. Probable identification of the 16 bacterial isolates through Gram staining and colony colour on Bacillus HiChromeAgar

Isolate	Gram's Character	Colony colour on Bacillus Hicrome agar	Probable Identification
JWK3	Gram positive, rods	Blue	*Bacillus cereus*
JWG2	Gram positive, rods	Light pinkish	*Lysinibacillus fusiformis*
JWR4	Gram positive, rods	Greenish yellow	*Bacillus megaterium*
JWC4	Gram negative short, rods	Not applicable	NO GROWTH
JPK1	Gram positive, rods	Dark green	*Paenibacillus alvei*
JPK2	Gram positive, rods	Turquise blue	*Bacillus mycoides*
JPG2	Gram positive, rods	Pinkish	*Lysinibacillussphaericus*
JPR3	Gram positive, rods	Dark green	*Paenibacillus alvei*
DWG1	Gram positive, rods	Pale greenish yellow	*Bacillus subtilis*
DWC1	Gram positive, rods	Turquise blue	*Bacillus mycoides*
DWC2	Gram positive, rods	Peach to brown	*Paenibacillus larvae*
DPK2	Gram positive, short rods	Dark green	*Bacillus licheniformis*
DPG1	Gram positive, rods	Peach to brown	*Paenibacillus larvae*
DPG2	Gram positive, rods	Peach to brown	*Paenibacillus larvae*
DPG3	Gram positive, rods	Pale greenish yellow	*Bacillus subtilis*
DPG4	Gram positive, rods	Greenish yellow	*Bacillus megaterium*

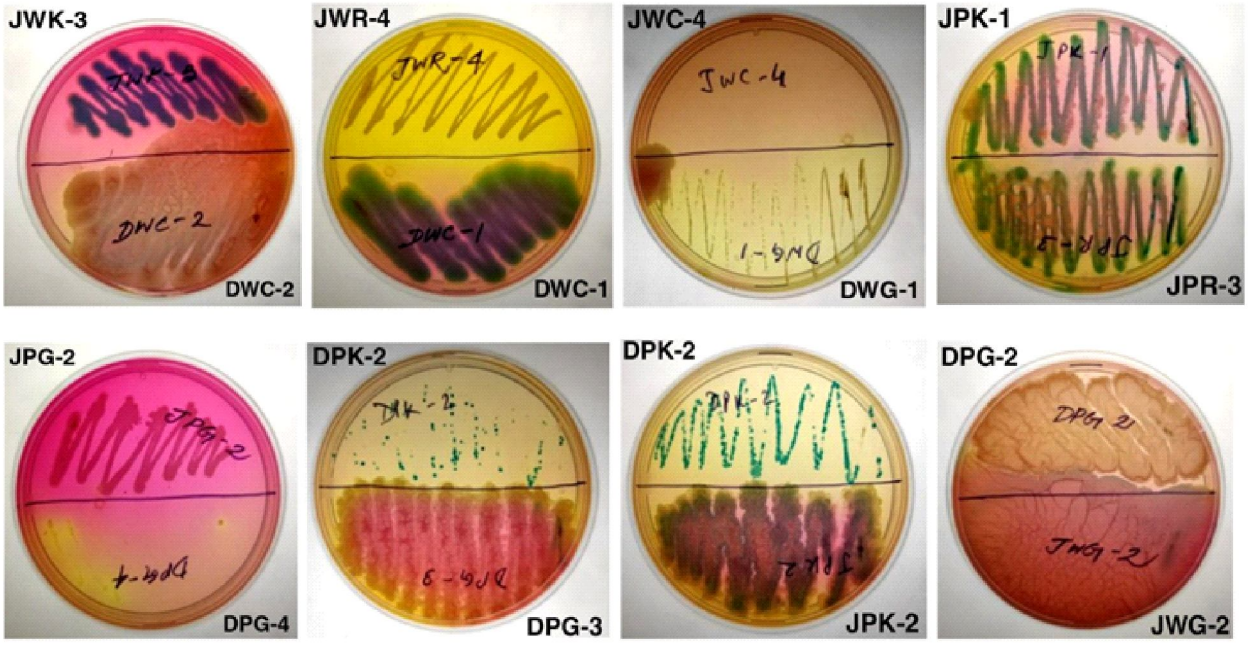


Fig. 2. Growth characteristics of the 16 selected bacterial isolates on Bacillus HiChrome Agar. Colony colour of individual isolates are specified in Table 2.

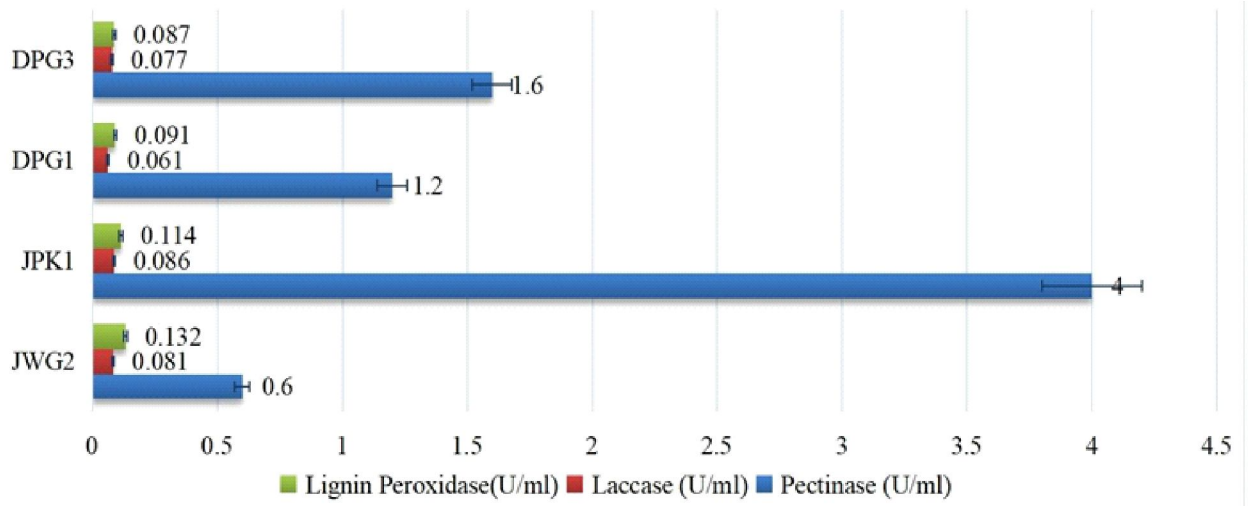
Molecular Sequencing of some isolates

The bacterial isolates have showed the presence of degradative enzymes like amylase, pectinase, lignin peroxidase and laccase. Hence four selected bacterial isolates showing potential degradative ability and the only Gram negative rod were identified by 16s rRNA sequencing. The bacterial isolates were identified as *Bacillus cereus, Bacillus tequilensis, Bacillus subtilis, Bacillus anthracis*, *Acinetobacter baumanii*. Identification of the 5 selected isolates by16S rRNA sequencing and their

Table 3. Enzyme production ability of the 16 bacterial isolates

Isolates	Catalase	Amylase	Pectinase	Laccase	Lignin Peroxidase	Lipase
JWK3	+	+	-	-	-	-
JWG2	+	+	++	+	-	-
JWR4	++	+	-	-	-	-
JWC4	+	-	-	+	-	-
JPK1	+	+	+	+	+	-
JPK2	+	+	-	++	+	-
JPG2	+ +	+	-	-	-	-
JPR3	+	+	-	+	+	-
DWG1	+	+	-	+	+	-
DWC1	-	+	-	-	-	-
DWC2	-	+	-	+	-	-
DPK2	-	+	-	+	-	-
DPG1	-	+	+	+	-	-
DPG2	-	+	-	-	-	-
DPG3	-/+	+	+	+	-	-
DPG4	+	+	-	++	-	-

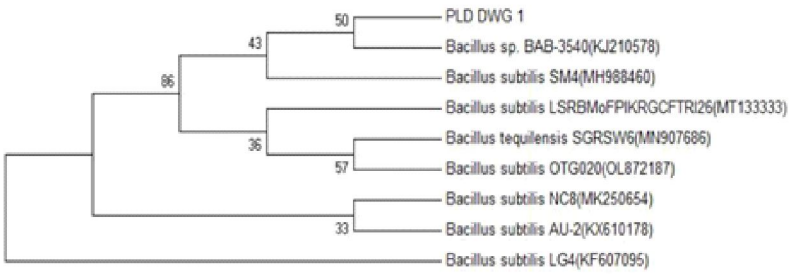
(++) indicates higher enzymatic activity of the isolates



3. Graphical Illustrations showing enzyme (pectinase, laccase, lignin peroxidase) activities of the 4 selected bacterial isolates (DPG3, DPG1, JPK1, JWG2)

Table 4 : Identification of the selected isolates by16S rRNA sequencing

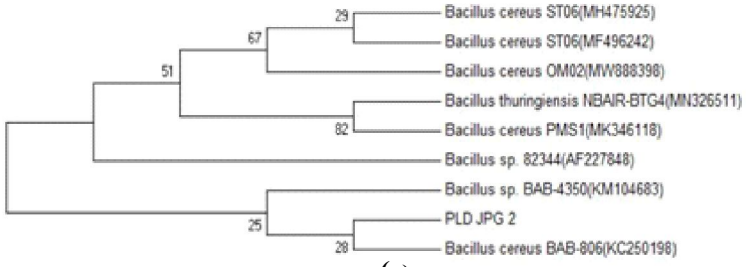
Isolates	Identification	Date of Publication	Accession No.
JWR4	*Bacillus tequilensis*	30.08.21	MZ903539
JWC4	*Acinetobacter baumanii*	30.08.21	MZ902933
JWK3	*Bacillus anthracis*	03.05.22	ON375335
JPG2	*Bacillus cereus*	03.05.22	ON369556
DWG1	*Bacillus subtilis*	03.05.22	ON369373



(a)

	1	2	3	4	5	6	7	8
1. PLD DWG 1								
2. Bacillus subtilis NC8(MK250654)	623.000							
3. Bacillus subtilis SM4(MH988460)	623.000	4.000						
4. Bacillus subtilis LSRBMoFPIKRGCFTRI26(MT133333)	624.000	2.000	2.000					
5. Bacillus sp. BAB-3540(KJ210578)	622.000	4.000	4.000	3.000				
6. Bacillus tequilensis SGRSW6(MN907686)	626.000	4.000	4.000	2.000	5.000			
7. Bacillus subtilis AU-2(KX610178)	623.000	0.000	4.000	2.000	4.000	4.000		
8. Bacillus subtilis OTG020(OL872187)	624.000	3.000	4.000	2.000	3.000	2.000	3.000	
9. Bacillus subtilis LG4(KF607095)	623.000	0.000	4.000	2.000	4.000	4.000	0.000	3.00

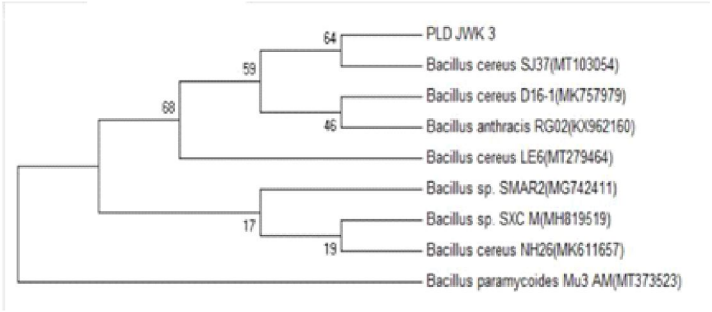
(b)



(c)

	1	2	3	4	5	6	7	8	9
1. PLD JPG 2									
2. Bacillus sp. BAB-4350(KM104683)	588.000								
3. Bacillus cereus BAB-806(KC250198)	588.000	0.000							
4. Bacillus thuringiensis NBAIR-BTG4(MN326511)	593.000	5.000	5.000						
5. Bacillus cereus PMS1(MK346118)	593.000	5.000	5.000	4.000					
6. Bacillus cereus OM02(MW888398)	590.000	2.000	2.000	5.000	4.000				
7. Bacillus sp. 82344(AF227848)	589.000	1.000	1.000	6.000	4.000	1.000			
8. Bacillus cereus ST06(MH475925)	590.000	2.000	2.000	5.000	4.000	0.000	1.000		
9. Bacillus cereus ST06(MF496242)	590.000	2.000	2.000	5.000	4.000	0.000	1.000	0.000	

(d)



(e)

	1	2	3	4	5	6	7	8	9
1. PLD JWK 3									
2. Bacillus sp. SMAR2(MG742411)	567.000								
3. Bacillus sp. SXC M(MH819519)	567.000	0.000							
4. Bacillus cereus NH26(MK611657)	567.000	0.000	0.000						
5. Bacillus cereus SJ37(MT103054)	566.000	4.000	4.000	4.000					
6. Bacillus cereus LE6(MT279464)	567.000	1.000	1.000	1.000	3.000				
7. Bacillus paramycoides Mu3 AM(MT373523)	567.000	0.000	0.000	0.000	4.000	1.000			
8. Bacillus cereus D16-1(MK757979)	566.000	2.000	2.000	2.000	2.000	1.000	2.000		
9. Bacillus anthracis RG02(KX962160)	566.000	2.000	2.000	2.000	2.000	1.000	2.000	0.000	

(f)

Fig 4 (a-f). Molecular phylogenetic identification of the bacterial isolates (a) DWG1, (c) JPG2, (e) JWK3 using 16S rRNA sequence analysis and their distance matrices (b, d,f)

NCBI Accession No. have been presented in Table 4. Phylogenetic identification of three isolates (DWG1, JPG2, JWK3) following the maximum likelihood method and Distance Matrices of these three isolates have been illustrated in Fig.4(a-f).

Conclusion and Future Prospect

The microbial plethora obtained from two most common Indian spices, namely cumin and coriander, have brought into light the existence of both harmful and beneficial bacteria. On repeated isolation from 16 different cumin and coriander varieties, 40 different bacterial types were obtained. Colony morphology study allowed selecting 16 dominant bacterial isolates for further studies. Gram staining showed majority to be Gram positive rods, hence growth in differential chromogenic media allowed for a probable identification. Different species of *Bacillus* and *Paenibacillus* were identified (probable). Enzyme assays showed the isolates to be capable of producing degrading enzymes like amylase, pectinase, laccase and lignin peroxidase. Five isolates were identified by the 16s R RNA sequencing and found to be *Bacillus cereus, Bacillus tequilensis, Bacillus subtilis, Bacillus anthracis* and *Acinetobacter baumanii*. Further studies are underway to bring out the beneficial aspects of other isolates lacking the degradative enzymes. These studies will throw some light on the good and bad abilities of spice microflora which is definitely in the dark.

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