

## Supplementary Materials

### A. Different tests for identification of bacteria

**Gram's staining:** This test categorizes organisms into two large groups: Gram-positive and Gram-negative. In this test, there are basically four steps: primary staining with crystal violet to a heat-fixed smear, followed by the addition of mordant (Gram's iodine), rapid decolorization with alcohol or mixture of alcohol and acetone, and finally counterstaining with safranin. Gram-positive bacteria appear as purple, and Gram-negative bacteria appear as pink or red.

**Spore staining:** This staining helps to determine whether the bacterium is a spore producer or not. The staining method uses malachite green as the primary stain and safranin as the counterstain. If spores are present, they appear as bright green, while vegetative cells appear as brownish red to pink.

**Catalase test:** This test determines whether the bacterium contains a catalase enzyme or not. For this, the bacterium inoculum is taken with the non-iron loop and smeared on the surface of the glass slide. Then, 2-4 drops of hydrogen peroxide are added to the smear. If bubbles are observed, the bacterium is catalase-positive; otherwise, it is regarded as catalase-negative.

**Oxidase test:** This test determines the presence of cytochrome oxidase enzyme. For this, an inoculum of pure bacterial culture is added to the surface of the test strip, impregnated with the reagent. Purple color observed within 5-10 seconds indicates that the bacterium has the oxidase enzyme.

**Methyl red–Voges Proskauer (MR-VP) test:** This consists of two tests.

1. The MR test is useful for determining the fermentation pathway for glucose utilization.
2. If the bacteria use a mixed acid fermentation pathway, glucose is fermented and different organic acids are produced. This results in the color of the medium changing from yellow to red.
3. If the bacteria use 2,3 butanediol fermentation pathways, glucose is fermented to produce 2,3 butanediol and the pH is increased, making it alkaline, and the color does not change.
4. The VP test determines the presence of acetoin or precursor of 2,3 butanediol. After  $\alpha$ -naphthol and potassium hydroxide are added to the medium, shaken vigorously, and set aside for few minutes, observation of a brownish-red to pink color denotes that acetoin is present in the culture, while a brownish-green to yellow color denotes an absence of acetoin.

**Indole test:** This test helps to determine whether a bacterium can break tryptophan into indole or not. For this, the bacterium is inoculated into tryptone broth and incubated for 24 hours. Then, five drops of Kovac's reagent is added to the medium. A cherry red ring at the surface of the medium denotes the positive test. Sometimes orange color, which is the precursor of indole, is observed.

**Citrate test:** This test is used to identify whether the organism has the ability to use citrate as a source of carbon. When the bacteria are grown on the citrate agar, bacteria having the citrate permease enzyme can use the citrate and break down the ammonium salts into ammonia. The ammonia results in an increase in the pH of the medium, changing the color of the medium from green to blue. The color change is due to the bromothymol blue indicator, which changes color to blue at alkaline pH levels.

**Sulfur, indole motility (SIM) test:** Some bacteria can utilize tryptophan and produce indole, ammonia, and pyruvic acid, and some bacteria can hydrolyze sulfur-containing amino acids or compounds to produce hydrogen sulfide.

In this test, bacteria are stabbed onto the medium with sulfur-containing compound and iron salts and incubated at 37 °C overnight.

1. If any black precipitate or color is observed in the medium, it indicates that the bacteria hydrolyzed sulfur-containing compound into visible ferric sulfide.
2. If there is a hazy growth observed around the stabbed area or all over the medium, it indicates that the bacteria are motile.
3. If the color changes to red when Kovac's reagent is added to the medium, it indicates that amino acid-like tryptophan is converted into indole, turning the color red.

Here, three properties of bacteria (sulfur production, motility, and indole production) are checked.

**Triple sugar iron (TSI) test:** This test determines whether the bacteria can utilize one or all saccharides (glucose, lactose, and sucrose). Glucose is a monosaccharide, and lactose and sucrose are disaccharides. For this, slants with the pH indicator phenol red are used. The bacteria are stabbed and streaked on the medium and incubated.

1. If the color of the slant/butt is red/orange, it indicates that the bacterium only utilizes peptone.
2. If the slant/butt color is red/yellow, it indicates that the bacterium only utilizes glucose.
3. If the color of slant/butt is yellow/yellow, it indicates that the bacterium utilizes glucose plus lactose and/or sucrose present in the medium.

If any bubbles or black precipitate or color are observed, it indicates that the bacterium is a gas and hydrogen sulfide producer.

**DNase test:** The test determines whether the microorganism can hydrolyze DNA or not. For this, the bacteria are grown in a DNase agar plate, which is pale green. If a colorless zone is observed around the colony, it denotes that the bacterium produces DNase.

**Urease test:** This test determines an organism's ability to hydrolyze urea and produces ammonia and carbon dioxide. Due to ammonia production, the pH of the medium changes to alkaline and the color changes from yellow to pink.

**Starch hydrolysis test:** This test helps identify whether the bacteria can hydrolyze starch as a carbohydrate source. The bacteria secrete an exoenzyme that hydrolyzes the starch by breaking down the bonds between the glucose molecules of starch.

For this, the bacteria are streaked on a starch agar plate and incubated at 37 °C overnight. Then iodine is added over the growth. If the bacteria hydrolyze the starch, there will be a clear zone around the bacterial growth; otherwise, the agar will remain a dark brown or blue/black color.

**Gelatin hydrolysis:** This helps to recognize whether the bacterium can liquefy gelatin or not.

For this, the bacteria are stabbed into the nutrient gelatin medium and incubated for 24 hours at 37 °C. If the medium is liquefied and any drops can be seen, the test is considered positive.

**Lactose fermentation test:** This test determines whether the bacteria can utilize lactose as a carbon source.

For this, the bacteria are inoculated in medium containing lactose and incubated at 37 °C for 24 hours. A color change in the media denotes a positive result.

**Violet red bile test:** This test is used to identify whether the bacterium is of the Enterobacteriaceae family. On the basis of lactose fermentation, they are classified into lactose fermenter and non-lactose fermenter. Lactose fermenters exhibit a pink to red color, while non-lactose fermenters possess colorless or transparent colonies.

**Amylase test:** This test is used to identify bacteria that can hydrolyze starch using amylase enzyme. The iodine is flooded over the colonies in an agar plate containing starch. A clear zone around the colony denotes the bacterium has the amylase enzyme.

**Capsule staining:** This staining is performed to identify whether the bacteria are capsule producers or not. For this staining, a smear is made in a slide with a drop of crystal violet. After drying of the smear, it is rinsed with 20% CuSO<sub>4</sub>, air dried, and observed under a microscope. A clear zone around the microbes and a violet background represents the presence of capsules.

**Biofilm test:** Bacteria were grown overnight in broth media at 30 °C and diluted to 1:100 w/v in medium with 2% glucose. Then about 200µL bacterial suspension was transferred into 96-well microtiter plates and incubated at 30 °C for 24 hours. The plates with the bacteria were washed twice with phosphate buffer saline and dried in inverted position. Subsequently, the adherent bacteria were fixed with 95% ethanol and stained with 1% w/v crystal violet for 5 mins. Again, the plate was washed with distilled water and air dried, and the optical density of each well was measured at 570 nm. The interpretation of the results was as follows: OD<sub>570</sub> ≥ 1: highly positive; 0.1 ≤ OD<sub>570</sub> < 1: moderately to weakly positive; and OD<sub>570</sub> ≤ 0.1: negative.

**Antibiotic susceptibility test:** The bacteria were cultured at 35 °C for 16 hours, and 200 µL bacterial suspension was evenly spread on the Muller-Hinton agar plate (MHA) with a sterile disposable spreader and left to dry for 20 minutes. The seven different antibiotic discs used in this experiment were ampicillin (10 µg), bacitracin (10 µg), erythromycin (15 µg), chloramphenicol (30 µg), tetracycline (30 µg), penicillin (10 µg), and novobiocin (10 µg). The antibiotic discs were gently pushed onto the agar plate with sterile forceps to ensure proper contact before incubation. Then the plates were incubated at 35 °C for 24-48 hours. The diameters of the inhibited zone were measured and interpreted according to the measurements provided by the Clinical and Laboratory Standard Institute (CLSI) guidelines.

**Table S1:** Antibiotic susceptibility ranges for different antibiotics as CLSI

Antibiotics	Diameter of inhibition range in mm		
	Resistant	Intermediate	Susceptible
Ampicillin (10µg)	≤11	12≤DI<13	≥14
Bacitracin(10µg)	≤6	7≤DI<10	≥11
Erythromycin(15µg)	≤13	14≤DI<22	≥23
Tetracycline(30µg)	≤14	15≤DI<18	≥19
Chloramphenicol(30µg)	≤12	13≤DI<17	≥18
Penicillin(10IU)	≤28	-	≥29
Novobiocin(30µg)	≤12	13≤DI<15	≥16

[DI: Diameter of inhibition]

## B. Composition of media

### Pectinase screening media

Ammonium sulphate	2 g/L
Yeast extract	1 g/L
Na <sub>2</sub> HPO <sub>4</sub>	6 g/L
KH <sub>2</sub> PO <sub>4</sub>	3 g/L
Citrus pectin	5 g/L
Agar	20 g/L

### Nutrient agar

Peptone	0.5% (w/v)
Beef extract/ yeast extract	0.3% (w/v)
NaCl	0.5% (w/v)
Agar	1.5% (w/v)
pH	6.8

### **Pectinase production media**

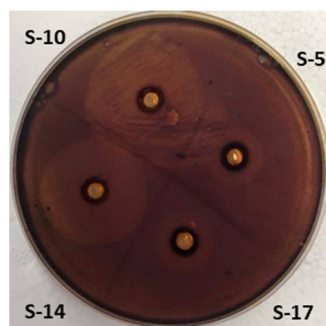
Yeast extract	0.3% (w/v)
K <sub>2</sub> HPO <sub>4</sub>	0.2% (w/v)
KH <sub>2</sub> PO <sub>4</sub>	0.2% (w/v)
K <sub>2</sub> NO <sub>3</sub>	0.2% (w/v)
Citrus Pectin	1% (w/v)

### **Luria-Bertani (LB) broth**

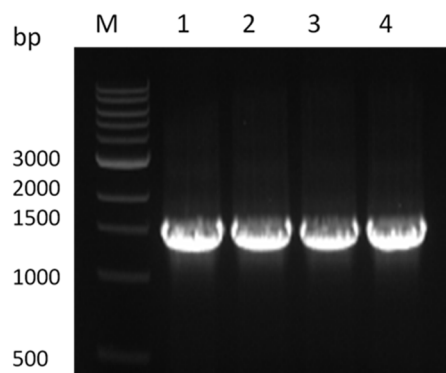
Yeast extract	1% (w/v)
Peptone	1% (w/v)
NaCl	0.5%(w/v)

## **C. Supplementary Figures**

**Figure S 1:** Screening of pectin hydrolysis of isolates.

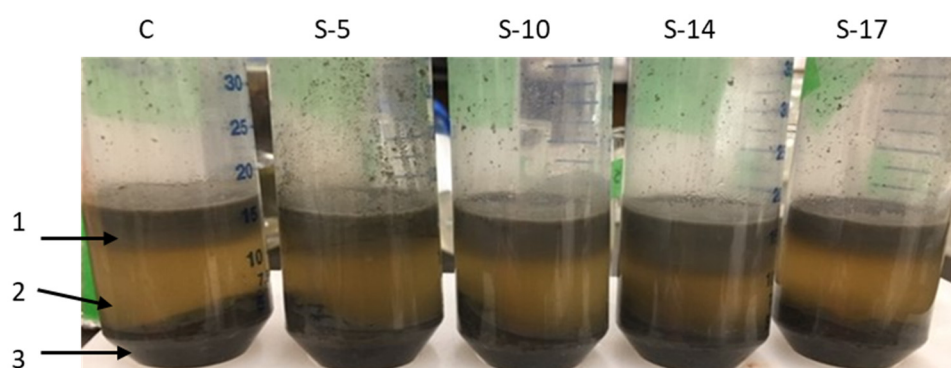


**Figure S2:** PCR results of 16s rDNA for the isolates after PCR in gel.



[M: DNA marker, 1,2,3,4: 16s rDNA of isolates]

**Figure S3:** Oil extraction from sesame seeds with enzyme extracts of isolates.



[C: control, S-5, S-14, S-10, S-17: isolates, 1: emulsified oil, 2: skim, 3: residue]