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Media used

For isolation, screening and maintenance of cellulose degrading bacterial strains: Nutrient agar (Peptone, 5 g; Beef extract, 3 g; Sodium Chloride, 5 g; Agar, 15 g; Distilled water, 1000 ml; pH 7.0); Carboxy Methyl cellulose (CMC) agar (CMC, 10 g; Dipotassium hydrogen phosphate, 1 g; Potassium dihydrogen phosphate, 1 g; Magnesium sulphate, 0.2 g; Ammonium nitrate, 1 g; Ferric chloride, 0.05 g; Calcium chloride, 0.02 g; Agar, 20 g; Distilled water, 1000 ml; pH 7.0); Cellulose Congo-red agar media (Dipotassium hydrogen phosphate, 0.5 g; Magnesium sulphate, 0.25 g; Cellulose, 2 g; Congo red, 0.2 g; Gelatin, 2 g; Agar, 15 g; Distilled water, 1000 ml; pH 6.8).

For biochemical and physiological characterization: Fermentation broth (Peptone, 5 g; Beef extract, 3 g; Lactose, 5 g; Glucose, 5 g; Sucrose, 5 g; Sodium chloride, 15 g; Phenol red, 0.018 g; Distilled water, 1000 ml); Simmon's citrate agar (Ammonium dihydrogen phosphate, 1 g; Dipotassium hydrogen phosphate, 1 g; Sodium chloride, 5 g; Sodium citrate, 2 g; Magnesium sulphate, 0.2 g; Bromothymol blue, 0.08 g; Agar, 15 g; Distilled water, 1000 ml); Tryptone broth (Tryptone, 10 g; Sodium chloride, 55 g; Calcium chloride, 1 ml; Distilled water, 1000 ml); MR-VP broth (Peptone, 7 g; Potassium phosphate, 5 g; Dextrose 5 g; Distilled water, 1000 ml); SIM agar (Peptone, 30 g; Beef extract, 3 g; Ferrous ammonium sulphate, 0.2 g; Sodium thiosulphate, 0.025 g; Agar, 3 g; Distilled water, 1000 ml).

Chemicals used

For isolation and screening of cellulose degrading bacterial strains: Gram's iodine solution, Iodine, 1 g; Potassium iodide, 2 g; Distilled water, 300 ml).

For biochemical and physiological characterization: MR indicator (Methyl red, 0.04 g; Ethyl alcohol, 40 ml; Distilled water, 60 ml); VP reagent I (Napthol, 5 g; Ethanol, 95 ml); VP reagent II (Potassium hydroxide, 40 g; Distilled water, 100 ml); Kovac's reagent (p-Dimethylaminobenzaldehyde, 5 g; Amyl alcohol, 75 ml; Conc. Hydrochloric acid, 25 ml), Catalase reagent (Hydrogen peroxide, 3 g, Distilled water, 100 ml).

Isolation and puri cation of the bacterial and fungal strains: e samples were serially diluted in sterilized normal saline and the aliquots of vefold of the soil solutions were plated on the sterilized solidi ed Nutrient Agar medium and Potato Dextrose Agar medium in the petri plates in aseptic condition. e Nutrient Agar plates were incubated at 37°C for 24 hours for the growth of bacteria and the Potato Dextrose Agar plates were incubated 26°C for 2 to 3 days. e di erent colonies of bacteria and fungi thus obtained were puri ed by single streak method and screened for their cellulolytic activities.

Screening of cellulose degrading microorganisms: e pure fungal cultures were allowed to grow on CMC Agar plates at 26°C for 5 days. CMC Plates streaked with pure bacterial colonies were incubated at 37°C for 5 days to allow the secretion of cellulase and degradation of cellulose present in media in the form of CMC. A er incubation CMC agar medium was ooded with an aqueous solution of Grams iodine for 10 minute to visualise the hydrolysis zone. e Grams iodine solution was then poured o . e clear zone was observed around the colonies. e strains showing a clear zone due to utilisation of CMC were selected as potential cellulolytic strains for further study.

Characterization of selected isolates on the basis of cultural, morphology and biochemical tests: e selected bacterial and fungal strains were culturally characterized by observing the colour, texture and margin of the colonies on Nutrient Agar medium and Potato Dextrose Agar medium. Morphological characterization was done by Gram's staining in case of bacterial strains and lacto-phenol staining for the fungal strains. en bacterial slides were observed under 100X and the fungal slides were observed under 40X magni cation of the research microscope. Further the selected bacterial isolates were subjected to biochemical tests as per Bergey's Manual of Systematic Bacteriology [7] like carbohydrate fermentation, catalase production, indole production, citrate utilization, MR-VP reaction, hydrogen sulphide production, Growth in 7% NaCl, etc.

Identi cation of bacterial and fungal isolates: Results of the Biochemical test were fed into the ABIS online bacterial identi cation tool for identi cation [8]. While the fungal isolates obtained were identi ed on the basis of cultural and morphological characterization.

Results and Discussion

Isolation and puri cation of the microbial strains from the collected soil samples

23 bacterial colonies were obtained on Nutrient Agar and 12 fungal colonies plates by plating the aliquots of vefold serially diluted decaying samples. e colonies were puri ed by single streak method on Nutrient Agar and Potato Dextrose Agar plates.

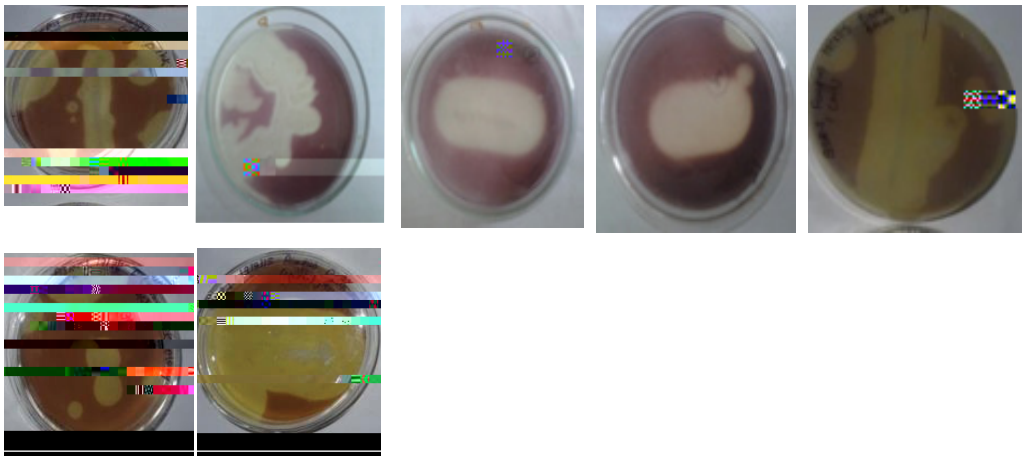

Figure 2: &0& DJDU PHGLXP ÀRRGHG ZLWK DQ DTXHRXV VROXWLRQ RI *UDPV LRGLQH VKRZLQJ FOH

Strain Number	Width of Zone in cm (Z)	Width of Culture in cm (C)	(Z:C ratio)
S1	0.6	0.3	2:1
S2	1.0	0.5	2:1
S3	0.6	0.4	3:2
S4	1.7	0.8	~2:1
S5	0.6	0.4	3:2
S6	0.6	0.4	3:2
S7	0.9	0.3	3:1

Table 1:

Isolates	Characteristics	
	Gram's Reaction	Shape
1	Positive	Coccus in Chains
2	Positive	Bacilli (Short Rods)
3	Positive	Bacilli (Short Rods)
4	Positive	Bacilli (in Chains)
5	Positive	Bacilli (Short Rods)
6	Positive	Bacillus Spore Formers
7	Positive	Bacilli in Chains

Table 5: Morphological characteristics of Bacterial Strain S1-S7.

Strain	Characteristics
1	Single- celled spores (conidia) in chains developing at the end of sterigma arising from the terminal bulb of the conidiophores, the vesicle; long conidiophores arise from a septate mycelium
2	Single- celled spores (conidia) in chains developing at the end of sterigma arising from the terminal bulb of the conidiophores, the vesicle; long conidiophores arise from a septate mycelium
3	Single- celled spores (conidia) in chains developed at the end of sterigma arising from the metula of the conidiophores; branching conidiophores arise from a septate mycelium
4	Single cell spores in chains developing of the end of sterigma arising from the medulla of the conidiophores, branching conidiophores arising from a septate mycelium.

Table 6: Morphological characteristics of Fungal isolates F1-F4 on Potato Dextrose Agar.

Biochemical tests	1	2	3	4	5	6	7
* O X F R V H I H U P H Q W D W L R Q	+	+	+	+	+	+	+
Sucrose fermentation	-	-	-	-	-	-	+
Lactose fermentation	-	-	-	-	-	-	-
MR-VP	-	-	-	-	-	-	--

of the selected bacterial isolates and fungal isolates has been tabulated in Tables 5 and 6 respectively.

Biochemical characterization of selected bacterial isolates

Strains 1-7 were characterized biochemically and the results were recorded in Table 7.

Identi cation of bacterial and fungal isolates

e results of the biochemical test were fed into the ABIS online bacterial identi cation tool. According to the identi cation so ware which the bacterial isolates were S1 as Bacillus subtilis ~98% (acc: 30%), S2 as Bacillus licheniformis ~ 99% (acc: 32.53%), S3 as Streptococcus ~97 (acc: 20%),S4 as Bacillus smithii (99%), S5 as Bacillus rmus (99%), S6 as Brevibacilus laterosporus (98%) and S7 as Pseudomonas chlororaphis (75%), however, the 16s rRNA sequencing has to be performed for con rmation of the bacterial isolates [9,10]. e fungal isolates were Aspergillus niger, Penicillium sps., Aspergillus avus, Rhizopus sps.

Conclusion

Bacteria are well known agents of decomposition of organic matter in general and of cellulosic substrates in particular [9]. As bacteria, can utilize wide range of cellulosic wastes, therefore, interest in the search for cellulase producing novel bacterial species is increasing. Such habitats which are rich in cellulosic substrates are the best sources

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