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ISOLATION, IDENTIFICATION, AND CHARACTERIZATION OF MICROORGANISMS FROM INDUSTRIAL DAIRY WASTE AND EVALUATION OF ITS BIODEGRADABILITY

RANJEET KUMAR[1]*, YOGITA MANDAVI[2], KUNAL JOSHI[1], SAPNA JOSHI[1] AND DEEPIKA BISWAS[1]

[1]Department of Biochemistry, Combined Institute of Medical Science and Research, Dehradun, India
[2]Department of Biotechnology, Sant Gahira Guru University, Sarguja, C.G., India

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Abstract– Microscopic organisms, commonly referred to as microbes, are organisms that are not visible to the naked eye. While some of these microorganisms can lead to diseases, numerous beneficial ones play essential roles in various domains. These advantageous microorganisms are pivotal in ecological systems, contributing to processes like biodegradation and bioremediation. They also find significant applications in diverse industries such as agriculture, food production, and the development of probiotics. This research aims to investigate the potential discovery of various biodegradable microorganisms capable of breaking down organic waste and similar materials. The focus is on finding effective ways to manage these materials in the context of global warming and related environmental concerns. The isolation of microorganisms involved the collection of samples from dairy industry effluents from dairy farms of Dehradun, Uttarakhand. Six isolates were screened following sample collection and examination. To distinguish the gathered microorganisms, various analyses were conducted. These included testing the isolates on nutrient agar at room temperature for 24 hours, with observations made on characteristics such as size, shape, color, margin, height, opacity, and stability. Morphological features like gram nature and motility were also examined. Subsequent to physical-biochemical characterization, tests for catalase, oxidase, and indole citrate were conducted. Biochemical tests were carried out to assess extracellular enzymatic activities, including caseinase and starch hydrolysis. Additionally, carbohydrate utilization tests were performed for lactose, maltose, inositol, xylose, among others. Out of the 06 isolates, two exhibited protease production and were selected for further investigation. Furthermore, two isolates demonstrated effective antibacterial activity and susceptibility to different antibiotics.

INTRODUCTION

In some developing countries, the major part of the dairy waste comes under the unorganized sector. In India 85% of dairy sector comes under unorganized sector, which lacks technology and advanced facilities for the treatment of dairy waste (Garcha, *et al.,* 2016). Food industry has an greater impact on the environment in comparison to other industries due to the excess utilization of water and resources and the greater production of waste and effluents, among which dairy industry is the leading industry (Ahmad *et al.,* 2019). The effluents generated by the dairy industry have a high organic load, having high levels of chemical oxygen demand (COD), biochemical oxygen demand (BOD), oil & grease and nitrogen and phosphorus (Singh *et al.,* 2014). To tackle the problem of dairy waste various steps are taken like Activated Sludge Process, which is one of the most used technology (Shivsharan *et al.,* 2013). Significant environmental costs have been caused by the large volumes of waste that have been released into the environment as a result of growing industry and urbanization. Microorganisms, including bacteria and fungi, have been used to break down pollutants (Dhall *et al*., 2012). Due to their wide pH range, high organic content, and relatively high temperature, dairy effluents need to be specially

([1]Assistant Professor)

treated to prevent or lessen environmental harm. Biological, physico-chemical, and mechanical methods are used to treat dairy wastewater. Manufacturing processes account for the majority of wastewater. About 50–80% of the water used in the dairy industry is contaminated, including from sanitary operations; the remaining 20–50% is only marginally clean (Slavov *et al.,* 2022). Dairy wastewater disposal is a challenging task that cannot be ignored. The government and environmentalists are looking for sustainable, low-cost, and successful wastewater treatment and recycling techniques. In developing countries like India, physical-chemical methods of treating wastewater are intrinsically costly and cannot be applied to all sectors of the economy (Garcha *et al.,* 2016). The wastewater includes biodegradable and organically-based dissolved carbohydrates, proteins, and fats (Priyadharshini *et al.,* 2016). The mineralization of organic matter by microorganisms (such as bacteria, fungus, and archaea) is known as biodegradation, and it ultimately yields carbon dioxide and water under aerobic conditions. Biotransformation takes place when mineralization is incomplete, producing both organic and inorganic metabolites, and transformation products (Singh and Sharma, 2008). The biodegradation of dairy effluent by native microorganisms is a practical, cost-effective, and environmentally responsible method. It includes a range of fungal strains such as *Alternaria species*, *Fusarium species*, and *Aspergillus species*, and bacterial strains such as *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Lactobacillus species*, *Staphylococcus aureus,* and *Enterococcus species*(Al-Wasify *et al.,* 2017). Through enzymatic or metabolic mechanisms, microorganisms alter the material. Growth and co-metabolism are its two pillars. The only source of carbon and energy needed for growth is an organic contamination. This process results in the organic pollutants being destroyed (mineralized). When a growth substrate which acts as the primary source of carbon and energy is present, co-metabolism refers to the metabolism of an organic material. Microorganisms that aid in biodegradation include bacteria, yeast, and fungi, to name a few (£awniczak *et al.,* 2020). Studies have demonstrated the effectiveness of *Pseudomonas aeruginosa* in breaking down complex organic chemicals present in dairy waste using its enzymatic machinery. The enzymes that *Pseudomonas aeruginosa* generates can break down the proteins, lipids, and lactose found in dairy waste. These enzymes include lipases, lactases, and proteases (Sandaruwani *et al.,* 2018). This work led to the formulation of a bacterial consortium that includes *B. licheniforms*, *S. marsescens*, *P. aeruginosa*, and *S. aureus*, which can be used to treat wastewater or industrial effluent that has high lipid content. Accordingly, this study aims to isolate, identify, and characterize the microorganisms in dairy waste water and evaluating their biodegradation potential.

METHEDOLOGY

Collection of samples: The dairy waste water sample were collected from dairy farms in the Dehradun region, Uttarakhand, on 7th April 2023 in a sterile glass bottle, maintaining the aseptic conditions and were stored at 4 °C after labeling. The collected wastewater sample was brought to the laboratory for the isolation of wastewater bacteria.

Isolation and Screening of Bacteria

For the isolation of bacteria and for achieving the pure culture the serial dilution of the sample was performed, where the sample was diluted till 10^{-5} dilutions and CFU was calculated. Each dilution was then spread over the nutrient agar plates, by spread plate technique and was than kept for the incubation at 37 °C overnight (Sanders, 2012).

For further identification of bacteria, the colonies grown overnight were sub cultured over the nutrient agar plate by streak plate technique. Different colonies are streaked separately in separate plates and were incubated for 24 hours at 37 °C (Sanders, 2012).

Gram Staining of the isolated bacterial colonies

For the further characterization of the sub cultured bacteria the bacterial colonies were gram stained and examined for its morphology, i.e. Gram positive or gram-negative bacteria, size, shape, arrangement etc. (Panicker *et al.,* 2023).

Biochemical analysis of isolated bacterial colonies

Once the morphology of the bacteria was determined, then biochemical analysis of the bacterial colonies was performed. Based on variations in the metabolic activity displayed by several species of bacteria, biochemical tests are employed for microbiological identification. Each colony was examined for different biochemical tests, i.e. Catalase test, Oxidase test, Citrate test, MR-VP test, Indole test, and Mannitol motility test (Shoaib *et al.,* 2020).

Determination of the moisture content and pH of the dairy waste sample

Together with the bacterial screening of the waste sample, the determination of moisture content and pH of the waste sample was done. For this the soaking filter paper was used to hold water samples from recently obtained dairy, and the starting weight was noted. Following that, the samples were air-incubated at 110 °C in a hot air incubator. Until the samples' weight became constant, they were weighed several times. It was established how much moisture the samples contained, computed using the following AWPA-provided formula (Singh, 2015).

MC (%) =W–w/w × 100

Where- MC= Moisture content
W= Initial weight
w= Final weight

The pH was determined with the help of pH strip.

Antibacterial activity test

After all these analyses the antibacterial activity test was performed by agar well diffusion method by some modification in the methodology of Balouiri, *et al.,* 2016. In this the agar plate was first spread by the pathogen (*K.pneumoniae*), and then the wells were made, these wells were then inoculated by the isolated bacteria at different concentration and were incubated at 37 °C for 24 hrs (Singh, 2015).

Antibiotic susceptibility test

For Antibiotic Susceptibility test the disc diffusion method was performed. In which the agar plates were made and the bacterial colony was spread over the agar and antibiotic discs were placed and was kept for the incubation at 37 °C overnight (Khan, *et al.,* 2019).

Extracellular Enzyme Production

Starch hydrolysis test

After the completion of the screening of the bacterial isolates their extracellular enzyme production activity was screened, which was done by performing starch hydrolysis test and casein hydrolysis test. In starch hydrolysis test the starch agar plates were prepared and was streaked with the isolated bacteria and kept for the incubation at 37 °C for 24 hours. After the incubation the plates were flooded by the iodine solution the color change of the colonies was observed (Lal *et al.*, 2012).

Casein hydrolysis test

Similarly, casein hydrolysis test was also performed, in which the casein agar plate was prepared and streaked with the single loop full colony and was kept for the incubation for 24 hours at 37 °C. After the incubation the plates were examined for the casein agar transparency due to casein breakdown (Dawadi *et al.,* 2021).

Biodegradation potential activity by bacterial strains of dairy wastewater by weight loss method

Using the weight loss strategy, the ability of microorganisms to break down waste was investigated. To prepare the culture, a 24-hour-old bacterial culture was placed in tubes with 10 ml of nutrient broth and incubated at 37 degrees Celsius for 24 hours. Samples of beer and dairy waste were gathered in sterile litter containers while maintaining aseptic conditions. In the laboratory, the gathered samples were autoclave sterilized for 15 minutes at 121 °C. After sterilizing the litter, 3 cc of the bacterial broth culture was added, and everything was thoroughly mixed. Following sterilization, the waste samples were weighed, infected with the isolated bacteria, and stored at 37 °C for five, nine, and eighteen days, respectively. Additionally, the appropriate weight was being taken. The weight loss was computed, and the bacteria's degradation efficiency was ascertained using the same data (Singh, 2015).

We conducted all of the tests in triplicate using this procedure, and the most meaningful findings have been chosen and displayed.

RESULTS

Isolation of bacteria from dairy waste water sample

In this work, samples of dairy wastewater from the Dehradun region were characterized and bacterial colonies were extracted.

Serial dilution and spread plate method

The serial dilution method was used to isolate each colony after the number of colonies was lowered. Over 250 colonies (25.0×10^{-5} CFU/ml), 41 colonies (4.1×10^{-6} CFU/ml), and 32 colonies (3.2×10^{-7} CFU/ml) were isolated from dilutions 10^{-3}, 10^{-4}, and 10^{-5}, respectively, in a sample of dairy wastewater as shown in Table 1. Colonies are uncountable at the dilution 10^{-2}. The colonies are white, cream, light green, and light yellow in color depicted in Fig. 1.

The formula used for CFU:

$$\text{CFU/ml} = \frac{\text{No. of Colonies} \times \text{Dilution Factor}}{\text{The volume of Culture Plate in ml}}$$

Identification and characterization of bacteria

Phenotypic characterization

The investigation focused on the Gram reaction, colony shape, and cell size of bacteria that were isolated from samples of dairy effluent. In addition, the bacteria's size, shape, color, opacity, and mucilage were used to identify them microscopically, which is listed in Table 2.

Six samples from a dairy wastewater were placed on different NAM plates, which were consistently maintained at 4 °C. The morphological characteristics of the colonies were observed after dairy effluent samples were placed on NAM plates. DW1 is a large, mucoid, cream-colored, smooth surface; DW2 is a moist, spherical, opaque, smooth surface; DW3 is a small, smooth, circular, opaque, and cream surface; DW4 is a small, circular, smooth, and opaque surface; DW5 is a small, spherical, brown, and smooth surface; and DW6 is a small, smooth, circular, and opaque surface, shown in Fig. 2.

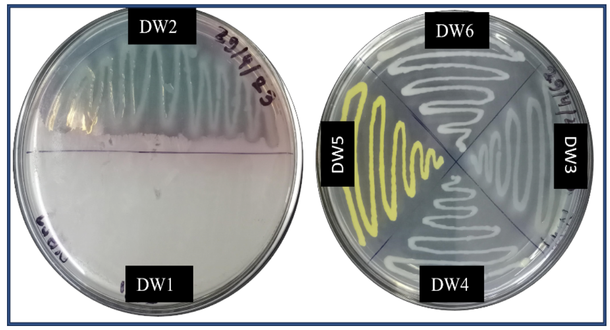

Fig. 2. Phenotypic characterization of isolates from dairy wastewater sample on NAM by streak plate method

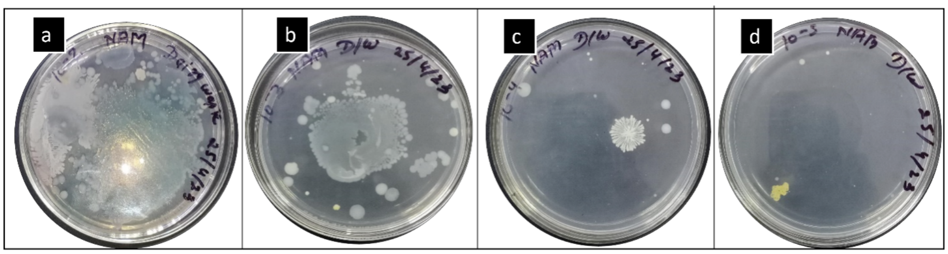

Fig. 1. Isolation of bacteria from dairy wastewater sample on NAM by spread plate method

Table 1. Serial dilution of the dairy waste sample and calculation of its CFU

Sample	Dilution	No of Colonies	CFU
Dairy wastewater sample	10^{-1}	Uncountable	Infinite
	10^{-2}	Uncountable	Infinite
	10^{-3}	250	25.0×10^{-5}
	10^{-4}	41	4.1×10^{-6}
	10^{-5}	32	3.2×10^{-7}

Table 2. Showing Phenotypic character of different isolates of Dairy wastewater sample.

S.No.	Isolates no.	Colony morphology
1	DW1	Large, mucoid, cream, and smooth
2	DW2	Moist, spherical, opaque, and smooth
3	DW3	Small, smooth, circular, opaque, and cream
4	DW4	Small, circular, smooth, and opaque
5	DW5	Small, spherical, mucoid, and cream
6	DW6	Small, circular, smooth, and opaque

Identification of Isolated Bacteria by Gram Staining

All six isolates from dairy waste were gram-negative rods and cocci depicted in Table 3. The majority of these bacterial cells were found in pairs as shown in Fig. 3.

Table 3. Showing the result of Gram staining of different isolates (Dairy wastewater)

S.No.	Isolate No.	Stain	Shape
1.	DW1	Gram Negative	Rod Shaped
2.	DW2	Gram Negative	Rod Shaped
3.	DW3	Gram-positive	Rod Shaped
4.	DW4	Gram Negative	Rod Shaped
5.	DW5	Gram Negative	Coccus
6.	DW6	Gram Negative	Rod Shaped

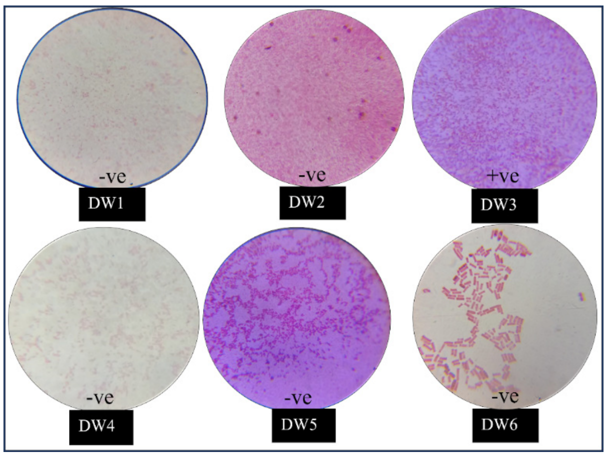

Fig. 3. Gram staining of Dairy wastewater sample isolates under 45X.

Characterization of bacteria using biochemical tests

Different biochemical analysis was performed over the all 6 samples.

Catalase Test

Positive responses were seen in all isolates (shown in the Fig.4) from the dairy wastewater samples, indicating the presence of active catalase enzymes that can break down hydrogen peroxide into oxygen and water and produce an observable effervescence.

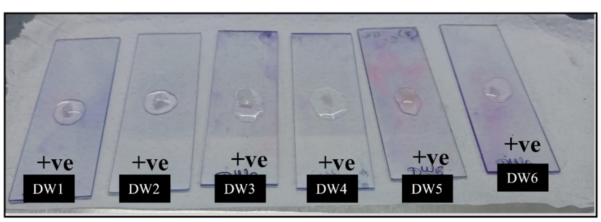

Fig. 4. Catalase test of Dairy wastewater sample

Oxidase Test

One isolate tested positive and five of the dairy samples tested negative as represented in the Fig.5, demonstrating the presence of Cytochrome C Oxidase in the microbial community.

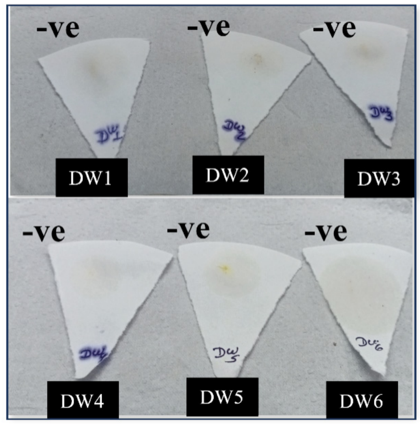

Fig. 5. Oxidase test of Dairy wastewater sample

Indole Test

As a result of the indole test, it was discovered that the isolates of dairy wastewater samples DW1 and DW3 were indole positive; that is, the culture had broken down the tryptophan in the medium and, as a result, there was no colure change upon adding Kovac's reagent, which appeared as a thin yellow layer on top of the medium. Conversely, DW2, DW4, DW5, and DW6 were indole negative; these isolates of dairy wastewater sample DW1 and DW3 were found to be indole positive (Fig. 6).

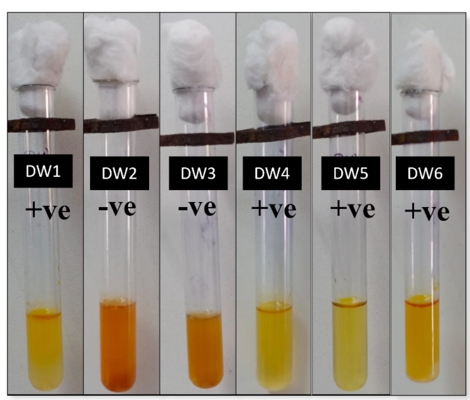

Fig. 6. Indole test of Dairywastewater sample

Citrate Test

To find out if the organism could use citrate as its only supply of carbon, the citrate test was carried out. The dairy wastewater sample's isolated DW1, DW2, DW3, DW4, and DW6 all tested positive as shown in Fig. 7.

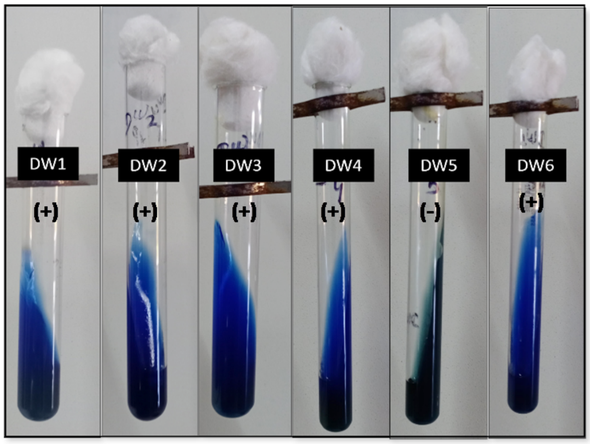

Fig. 7. Citrate test of Dairy wastewater sample

Methyl-Red Test

The bacterial culture in the dairy wastewater sample underwent a Methyl Red (MR) test; the results shown in DW2 and DW6 show a positive outcome. This indicates that the bacteria are able to ferment glucose in a mixed acid. Additionally, DW1, DW3, and DW5 show a negative outcome as in the Fig. 8.

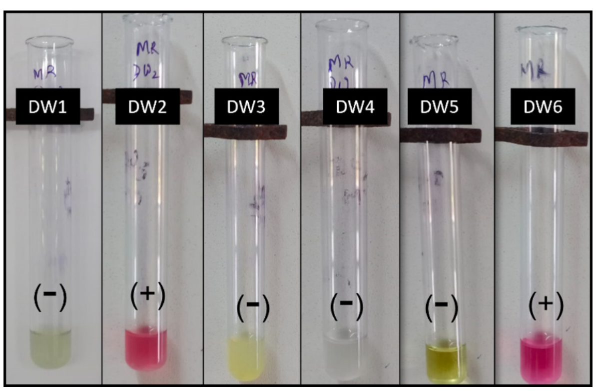

Fig. 8. Methyl-Red test of Dairy wastewater sample

Voges-Proskauer Test

The Voges-Proskauer (VP) test was performed on bacterial cultures of dairy wastewater, and samples DW1, DW4, and DW6 were positive, while samples DW2, DW3, and DW5 were found to be negative shown in Fig. 9.

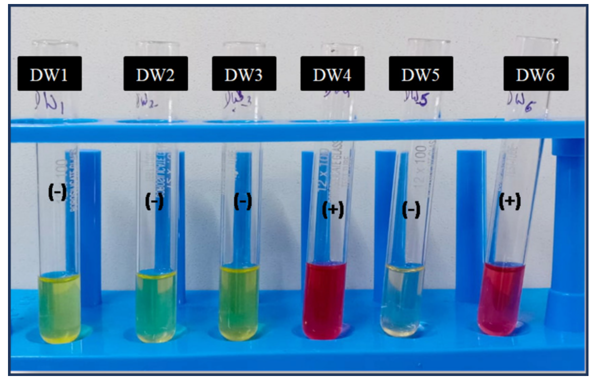

Fig. 9. Voges-Proskauertest of Dairy wastewater sample

Motility Test

Negative test was shown in all six samples of dairy wastewater as shown in Fig. 10.

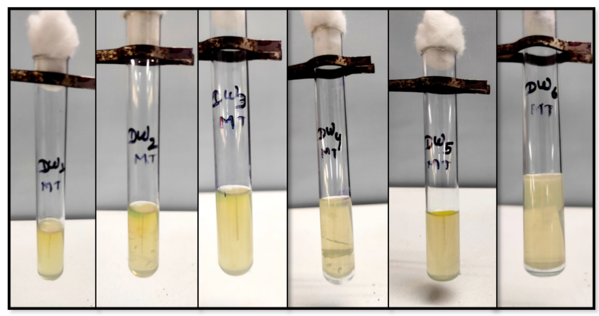

Fig. 10. Motility test of Dairy wastewater sample

Determination of Moisture content (%) and pH of wastewater sample

Shocked Filter Paper's Initial Weight of Dairy wastewater is 2.67g.

$W = 2.67g$

$w = 1.03g.$

Moisture Content $= 2.67-1.03/1.03\times100$

$MC = -97.33\%$

$pH = 7$

Table 4. Weighed multiple times after 2-2 minutes (in grams)

S.No.	Dairy wastewater weight (g.)
1.	2.67
2.	1.94
3.	1.44
4.	1.05
5.	1.03
6.	1.03
7.	1.03

Biodegradation potential activity by bacterial strains of dairy wastewater by weight loss method

Waste degradation potential of selected bacterial sample

The study found that DW4 and DW6 of dairy wastewater had the most degradation potential as depicted in Fig. 11. These strains may also produce a lot of enzymes, which enhances their capacity for breakdown. When the waste is broken down by bacteria, the litter becomes lighter. In the current decomposition research, the treated litter's weight decreased as the bacteria broke it down and converted it into smaller molecules. As the process went on, waste samples' percentage of weight reduction increased (shown in Table 5).

Antibacterial Activity Test

The findings of the antibacterial activity test were positive shown in Table 6. Different concentrations

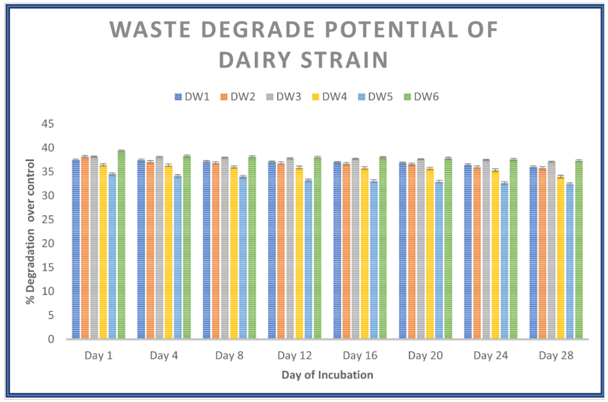

Fig. 11. Waste Degrade Potential of Dairy Strain

Table 5. Waste Degradation Potential of Dairy Waste Isolated strain

S. No.	Dairy wastewater strain no.	1	4	8	12	16	20	24	28
1.	DW1	37.50	37.45	37.19	37.10	36.99	36.87	36.50	36.06
2.	DW2	38.18	37.06	36.85	36.80	36.69	36.57	35.97	35.78
3.	DW3	38.18	38.10	37.94	37.84	37.74	37.62	37.49	37.09
4.	DW4	36.43	36.37	36.00	35.90	35.79	35.68	35.32	34.00
5.	DW5	34.55	34.13	33.98	33.20	33.06	32.93	32.65	32.43
6.	DW6	39.37	38.31	38.11	38.03	37.97	37.79	37.54	37.33

Table 6. Antibacterial Activity Data table of Dairy wastewater isolated strains

	Antibacterial Activity (Zone of Inhibition)			
Concentration Name of Dairy wastewater strain	DW4	*Klebsiella pneumoniae* Activity	DW6	Activity
10	26	Resistance	8	Sensitive
13	22	Resistance	7	Sensitive
16	21	Resistance	11	Sensitive
19	19	Resistance	10	Sensitive
22	18	Resistance	22	Resistance
25	18	Resistance	26	Resistance
28	20	Resistance	21	Resistance
31	21	Resistance	23	Resistance
34	13	Sensitive	21	Resistance
37	12	Sensitive	19	Resistance
40	20	Resistance	11	Sensitive
43	26	Resistance	20	Resistance
46	26	Resistance	18	Resistance
49	23	Resistance	18	Resistance
52	22	Resistance	20	Resistance
55	22	Resistance	22	Resistance
58	26	Resistance	0	Sensitive
61	25	Resistance	0	Sensitive
Control	25	Resistance	23	Resistance

of bacterial cells were utilized to test against *Klebsiella pneumoniae*. The target bacterium, *Klebsiella pneumoniae*, is effectively inhibited from growing and spreading by the potent antibacterial capabilities of the strain under test (Fig. 12). The production of antimicrobial

Compounds seem to be a typical occurrence for most microorganisms.

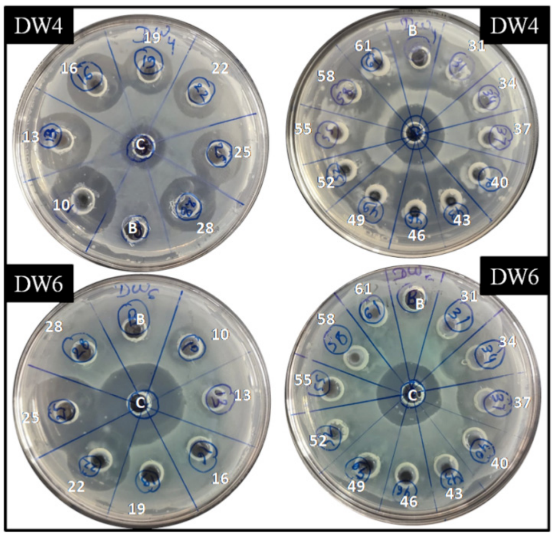

Fig. 12. Antibacterial activity results of DW4 and DW6 of dairy wastewater

Antibiotic Susceptibility Test

Isolates from dairy waste water sample DW4 and DW6 were tested for antibiotic susceptibility and the results were recorded in Table 7 and 8. The bacterial strain tested positive for several antibiotics, indicating its antibiotic susceptibility. It demonstrated a high susceptibility to several widely used antibiotics, including erythromycin, amoxicillin, ciprofloxacin, and penicillin (Fig.13.). These results are critical because they help inform wise treatment choices and pinpoint susceptible medications that can be utilized to properly target and eradicate the infection.

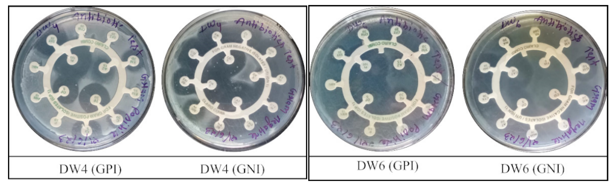

Fig. 13. Antibiotics results of isolates of dairy wastewater

Extracellular Enzyme Production

Significant enzymatic activity was found in our investigation's extracellular enzyme production data. This method was used to witness the robust extracellular enzyme synthesis in the microbial culture. Significant levels of enzyme release into the surrounding media were shown by enzymatic testing, indicating the potential for an efficient breakdown of complex organic compounds. These findings have enormous potential for a variety of biotechnological uses, such as the treatment of wastewater, bioconversion, and bioremediation. We conducted two enzymatic assays in this.

Starch hydrolysis test

In the starch hydrolysis test, we used two distinct isolates from dairy wastewater, DW4, and DW6. Their abilities to dissolve starch did not differ,

Table 7. Kirby-Bauer antibiotic susceptibility data results in GNI DISC for dairy wastewater

S. No.	Name of Antibiotics (GNI)	Zone of Inhibition			
		DW4	Susceptibility	DW6	Susceptibility
1.	Norfloxacin (NOR/10)	144	Resistance	-	-
2.	Aztreonam (AT/30)	22	Sensitive	10	Resistance
3.	Cefotaxime (CTX/30)	23	Sensitive	12	Resistance
4.	Ceftriaxone (CRO/30)	23	Sensitive	14	Sensitive
5.	Nalidixic acid (NA/30)	15	Sensitive	10	Resistance
6.	Nitrofurantoin (NI/300)	12	Resistance	-	-
7.	Cefuroxime (XM/30)	13	Sensitive	-	-
8.	Gentamycin (GM/10)	20	Sensitive	10	Resistance
9.	Amikacin (AK/30)	22	Sensitive	9	Resistance
10.	Ciprofloxacin (CI/5)	13	Resistance	22	Sensitive
11.	Ofloxacin (OF/5)	-		8	Resistance
12.	Ceftazidime (CAZ/5)	20	Sensitive	20	Sensitive
13.	Cefixime FIX/5)	23	Sensitive	-	-
14.	Cefdinir (CD/5)	22	Sensitive	-	-

Table 8. Bauer antibiotic susceptibility data results in GPI DISC for dairy wastewater

S. No.	Name of Antibiotics (GPI)	Zone of Inhibition			
		DW4	Susceptibility	DW6	Susceptibility
1.	Penicillin-G (P/10)	-	-	-	-
2.	Amoxicillin (AMX/10)	-	-	-	-
3.	Amoxicillin-Clavulanic (AMC)	-	-	-	-
4.	Co-trimoxazole (SXT/25)	21	Sensitive	11	Sensitive
5.	Cephalexin (CFM/30)	-	-	-	-
6.	Cefazolin (CFZ/30)	-	-	-	-
7.	Cefuroxime (XM/30)	13	Sensitive	-	-
8.	Erythromycin (EM/15)	-	-	-	-
9.	Chloramphenicol (C/30)	11	Resistance	-	-
10.	Ciprofloxacin (CI/5)	12	Resistance	-	-
11.	Ofloxacin (OF/5)	12	Resistance	-	-
12.	Piperacillin (PI/100)	-	-	-	-
13.	Azithromycin (AZ/15)	13	Sensitive	-	-
14	Tetracycline (TE/30)	14	Sensitive	-	-

according to the results shown in Fig. 14. The lack of a defined zone surrounding the development of any isolates on the starch agar plate, along with negative starch hydrolysis activity, suggests that the isolates were not amylase-producing bacteria.

Casein hydrolysis test

We used two samples DW4 and DW6 for casein hydrolysis test obtained from dairy wastewater. Both the isolates displayed a clear clearing zone around their development on the casein agar plate, a robust positive response, and significant proteolytic activity (Fig.15), which suggested the manufacture of protease enzymes. More characterization of these enzymes will yield useful knowledge and potential applications in biotechnology and industrial processes involving the degradation and utilization of proteins.

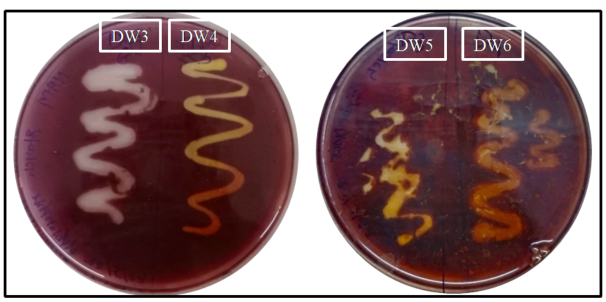

Fig. 14. Starch hydrolysis test result of dairy waste water

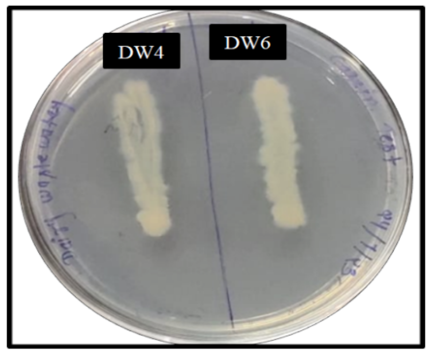

Fig. 15. Casein hydrolysis test result of Dairy wastewater

DISCUSSION

This study's primary goal was to evaluate the effectiveness of microbial degradation as a strategy for treating organic contaminants in wastewater. The purpose of this study was to examine how environmental influences affect the biodegradation process that occurs during wastewater treatment. The purpose of the study was to determine whether the chosen microbial strains could aid in the biodegradation of resistant substances found in wastewater.

This study involved the isolation and characterization of bacterial strains from dairy wastewater from the Dehradun region. Numerous physicochemical elements, such as medium, pH, temperature, incubation period, carbon supply, etc., affect bacterial development. Therefore, before mass-producing the bacteria for use as decomposers, it is imperative to investigate the various settings in which they formed.

Findings from multiple biochemical experiments conducted for this study. These investigations sought to determine how well microbial decomposition handled wastewater contaminated with organic pollutants. We also investigated the possibility that some microbial strains could enhance wastewater's ability to biodegrade compounds that are resistant. Using microbial degradation as a way to remove organic pollutants in wastewater, the gathered results demonstrated favorable trends in the methodology. But we also discovered a few methodological errors in the study that might have affected the results. We discovered several interesting similarities as well as some notable differences between the results of our biochemical tests and those of previous studies. Our findings are consistent with several other studies examining the efficiency of microbial degradation in treating organic-contaminant-polluted wastewater (Zaved *et al*., 2008).

Strong microbial activity is suggested by the data gathered, which raises the prospect of biodegradation results in the target material. Sensible color changes, gas production, and precipitate formation made the biodegradation process visible for the duration of the test. This study used the weight loss method to examine the biodegradation capacity. After a predetermined incubation period, we weighed the samples for our investigation. After incubation, the sample was rinsed with sterile water to get rid of the incubated microorganisms, so it could be compared to the results of the earlier study (Singh *et al.,* 2015).

Antibiotic compounds appear to be produced on a regular basis by a vast number of bacteria. In this study, three isolates exhibited antibacterial activity and produced positive results. Numerous antimicrobial compounds produced by members of the genus Bacillus have been identified as peptides, lipopeptides, and phenolic derivatives. The identification of newly discovered secondary metabolites with a range of biological actions in diverse situations has attracted a lot of attention in recent years (Joerger *et al.,* 2009).

Antibiotic susceptibility test results are examined and interpreted. Testing yielded valuable information about the effectiveness of several medications against target germs. The results of susceptibility tests indicate which drugs inhibited the bacterium's growth and which ones didn't. This data may help medical practitioner's combat antibiotic resistance and is essential for guiding the choice of the most effective antibiotic therapies in clinical situations. This pattern mirrored the findings of (Tiku *et al.,* 2016), who reported that 90% of the isolates were susceptible to antibiotics.

The extracellular enzyme assay results in this investigation show the variety of enzymatic capabilities of the isolated microbial strains. Protease activity in the majority of strains indicates that they are able to adapt to nutrient-rich conditions, like water that has been infused with organic debris. Because of these enzymes, bacteria can digest complicated substrates and get the nutrients they need to survive and develop. Protease enzymes were produced by 2 strains (Ramesh *et al.*, 2009).

CONCLUSION

Total 06 bacteria were isolated from dairy effluent in this investigation, and their morphological and biochemical characteristics were determined. We conclude that the reported bacteria are Bacillus species (DW4), and Enterobacter species (DW6) based on the results of the biochemical test. Two bacteria were found to be viable biodegraders following a test for biodegradation potential: DW4 and DW6 from dairy wastewater. It was discovered that DW4& DW6, were resistant to Klebsiella pneumonia and susceptible to antibiotics. In an enzyme production assay, the bacteria DW4 and DW6 produced protease. These bacteria could be used for various industrial purposes; further study is needed for the exploration of their other potential applications.

Conflict of interests

Author declares that there is no conflict of interest.

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