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Isolation of Microorganisms from Dairy Effluent

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Authors' contributions

This work was carried out in collaboration between all authors. Author VSS designed the study, wrote the protocol and the draft of the manuscript. Authors MW and MBK managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aim: Every Dairy industry has problems of effluent treatment. This can be revealed by effective treatment of the effluent. The effective treatment can be done by using microorganisms to stabilize the organic and inorganic load of the effluent. The aim of the present work is to study the dairy wastewater micro biota and to identify some new active strains which can bring about fast biodegradation of the organic compounds.
Study Design: Isolation and determination of bacteriological characteristics of the dairy effluents.
Methodology: Studies were carried out to isolate the microorganisms from collected effluent sample from the dairies under studies. Isolation of microorganisms was done by primary screening, Cultural Characterization, Biochemical characterization and Identified by using Bergey's Manuals of Systematic Bacteriology.
Results: During 2011-2013 from two different districts of Maharashtra (India), dairy industry effluents were collected for the isolation of micro organisms. Effluent samples were collected as per Jacksch and piper method and primary screening was done and totally 7 Isolates were screened out. These isolates were characterized on nutrient agar at room temperature for 24 hrs. Isolates were observed for the cultural characters like size, shape, colour, margin, elevation, opacity and consistency and morphological characters like Gram nature, sporulation, shape and arrangement of cells, motility etc. and were recorded. Physio-biochemical characterization was followed by biochemical tests for enzymatic activities like catalase, oxidase, nitrate reduction, urease, caseinase etc and carbohydrates utilization tests for lactose, maltose, inositol, xylose etc. performed to check their ability for

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metabolization. On the basis of these characteristics, isolates were identified by using Bergey's Manuals of Systematic Bacteriology. The identified Bacterial Isolates were of Genus Lactobacillus, Bacillus, Staphylococcus, Enterococcus, Listeria etc.
Conclusion: These Bacterial isolates have the ability to utilize the components like nitrate, starch, gelatin, sugars like sucrose, maltose, lactose etc. which was confirmed by the biochemical tests. Bacterial flora from the effluents can be identified and efficiently applied for the biological treatment of the dairy effluents.

Keywords: Dairy effluent; isolation and preliminary identification; microbiological and biochemical characterization; endogenous culture.

1. INTRODUCTION

Environmental pollution is one of the major ecological challenge, for the last few decades all over the world, in which developing countries are most affected. Major cause of the pollution is rapid industrialization, which is progressive at breakneck speed, and is suffocating the earth with the common problems of pollution. A 2007 study reports that the discharge of untreated sewage is single most important cause for pollution of surface and ground water in India. Among the major industries in India, dairy is one of the industries producing waste water rich in organic matter and thus leading to creation of odorous and high COD containing water [1]. The problem is not only that India lacks sufficient treatment capacity but also that the sewage treatment plants that exist do not operate and are not maintained [2]. The dairy industry on an average has been reported to generate 6-10 liters of waste water per liter of the milk processed [3]. It is estimated that about 2% of the total milk processed is wasted into drains [4]. Due to the high pollution load of dairy waste water, the milk-processing industries discharging untreated/partially treated waste water cause serious environmental problems [5-6]. In recent years, urban people are facing many problems and water pollution is one among them. Environmentalists and government are looking for efficient, cheap and long lasting solutions for waste treatment and recycling. Physico-chemical methods of waste water treatment are inevitably cost intensive and cannot be employed in all industries especially in developing countries like India. Hence, in recent years, the importance of biological treatment systems has attracted the attention of workers all over the world and has helped in developing relatively efficient, low cost waste treatment systems.

In order to design an efficient biological waste water treatment it is important to know the waste water microbiota composition and the biochemical properties correlated to the origin of pollutants, as well as the optimum metabolic activity and the physical-chemical conditions [7-8].The study of wastewater microbiota and to identify some new active strains adapted to the wastewater physical-chemical conditions, which metabolize organic compounds, similar to those which determine the pollution of wastewaters such as starch, casein, basic carbohydrates and lactic acid [9]. Microbial strains were identified for the rapid biodegradation of the organic compounds. In the present work bacteriological studies were carried out to isolate the microorganisms from dairy effluent samples.

2. MATERIALS AND METHODS

The designed experimental studies followed the methodology which represented in the Fig. 1.

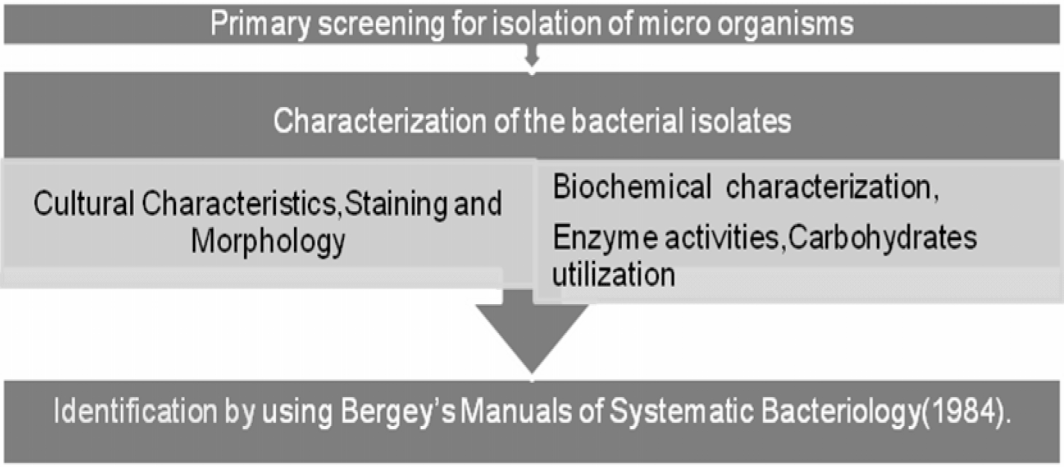

Fig. 1. Design of methodology

2.1 Primary Screening for Isolation of Micro Organisms

The effluent samples were collected from dairy 1 from Solapur at 75º East and 17º North and dairy 2 from Kolhapur at 74º East and 19º North of Maharashtra state in India. The samples were collected, in duplicate, in a clean sterile plastic container and stored at 4ºC until the analysis was carried out according to the standard methods of APHA [10] and Trivedy and Goel [11]. The experiments were carried out in five replicates and the mean value was considered. For primary screening, the dilutions of effluent samples were prepared in distilled water. Selective dilutions were spread on nutrient agar and incubated for 24 hours at room temperature. Isolated colonies were selected for the further studies.

2.2 Characterization of the Bacterial Isolates

Isolates were incubated on nutrient agar for 24 hours at room temperature and was characterized. Each of the isolates were observed for the cultural characters like size, shape, colour, margin, elevation ,opacity and consistency and morphological characters like Gram nature, sporulation, shape and arrangement of cells, motility etc. Biochemical characterization was followed by tests for enzymatic activities like catalase, oxidase, nitrate reductase, urease, caseinase etc. Carbohydrates utilization tests for various sugars like lactose, maltose, inositol and xylose were carried out. For characterization the methodologies were followed as per stander methods [12-16] as given in the Table 1. Microbial isolates were identified according to Bergey's Manual of Determinative Bacteriology [17,18].

Table 1. Methodology followed for biochemical characterization

Test	Procedure	Observation
Catalase	A loop full growth of each bacterial isolate from nutrient agar slant was stirred in 30.0 v/v hydrogen peroxide and observed for evolution of gas	Evolutions of gas indicate the presence of catalase whereas the negative test indicates the absence.
Gelatinase	Spot inoculation of the Bacterial isolate on sterile gelatin agar containing 0.4 % gelatin and incubated at 28ºC for 24 hours.	Gelatin hydrolyzing test indicate the appearance of clear zone after the addition of frazieres solution on the medium while the absence of clear zone on addition of frazieres solution indicate that the test is negative.
Nitrate Reductase	Bacterial isolates were to be inoculated in tubes containing sterile peptone nitrate broth and incubated for 24hours at room temperature.	Positive test show red colour formation after addition of sulphanilic acid - naphthyl-amine.
Urease	Slants of sterile Christensen's urea agar (1946) were inoculated with the bacterial isolates and incubated at 28ºC for 24 hours	Hydrolysis of urea was detected by the appearance of the pink colour in the medium and the absence of pink colour indicate negative test.
Oxidase	Colonies of the bacterial isolates are transferred by a glass rod on the filter paper strip moistened with freshly prepared 1% tetramethyl-1-p-phenylene diamine (TAPA) solution and observed.	Appearance of deep blue colour on the strip indicates the positive result for oxidase.
Starch hydrolysis	Spot inoculation of bacterial isolates on sterile starch agar containing 1% starch and incubated at 28ºC for 24 hours.	Lugols iodine when poured on the plates show zone of starch hydrolysis around the colonies for positive test.
Hugh and Leifsons (H&L)Test	The bacterial isolates were inoculated in to the two tubes of Hugh and Leifsons Medium (Aerobic and Anaerobic) in order to check oxidative and fermentative metabolism of sugar and incubated at 28ºC for 24 hours.	Observe for acid (yellow colour) and gas (bubble in durhum's tube) production on incubation of tubes for positive test.
Hydrogen Sulphide Production Test	The bacterial isolates were inoculated in to sterile standard thiosulphate iron agar stab medium and incubated at 28ºC for 24 hours.	Formation of black ferrous Sulphide precipitate in the medium indicated thiosulphate reduction and hydrogen sulphide production.
Carbohydrates Utilization	Peptone water is used as a basal medium for the tests. 5ml aliquots each to be taken to test the utilization of different carbohydrates. Tests were carried out for hexose sugar glucose; disaccharides like lactose, maltose and sucrose; sugar alcohols-mannitol and sorbitol; Sugar Vitamin – Inositol; Peptose – Xylose.	After incubation observe the tubes for acid and gas production for positive test.

3. RESULTS AND DISCUSSION

Characteristics of industrial wastewater vary greatly from industry to industry and within industries also. In the present studies effluent samples collected from dairy1 (Table 2) was studied for various physical characteristics such as Temp., pH, T.D.S.,T.S. & T.S.S. and the values indicated as 31,10, 515mg/lit,1603,1308mg/lit respectively. Mean values of chemical characteristics such as D.O., B.O.D., C.O.D., Sulphate, oil and grease and Chloride obtained are 1.3 mg/lit,516 mg/lit,1488 mg/lit,214 mg/lit,3 mg/lit and 122mg/lit, respectively. Effluent from dairy 2 (Table 2) showed mean values of physical characteristics such as Temp., pH, T.D.S.,T.S.S. & T.S. as 34, 9.8,1222mg/lit, 290 mg/lit,1837 mg/lit respectively and chemical characteristics such as D.O. B.O.D., C.O.D., Sulphate ,Oil & grease and Chloride indicated values,1.2 mg/lit,650 mg/lit,1448 mg/lit,223 mg/lit,2 mg/lit & 153mg/lit, respectively. Variations in the physical characters can be attributed to the treatment processes and locations of the dairies.

Table 2. Physico-chemical analysis of effluents from dairy industries

Parameters	Dairy 1	Dairy 2
Temperature(ºC)	34	31
P^H	9.8	10
D.O. (mg/lit)	1.2	1.3
B.O.D.(mg/lit)	650	516
C.O.D.(mg/lit)	1448	1488
T.D.S.(mg/lit)	1222	1308
T.S.S.(mg/lit)	290	515
T.S.(mg/lit)	1837	1603
Sulphate (mg/lit)	223	214
Chloride(mg/lit)	153	122
Oil & Grease(mg/lit)	2	3

Totally 7 Isolates were screened out from the effluents and were characterized on nutrient agar by incubating for 24 hours at room temperature. Each of the isolates was observed for the cultural characters like size, shape, colour, margin, elevation, opacity and consistency (Table 3). Similar results were reported by Vijayakumar *et. al.* [19]. They isolated different bacterial genera from dairy wastes for the biodegradation of dairy effluent and observed different *cyanobacteria* with different effluents. The colonies of the isolates on nutrient agar were circular to irregular shaped and of the size ranging from <1mm to 3mm. The colour of the colonies was dirty white and yellowish mostly. The colonies of all the isolates exhibited regular to irregular margins with mostly flat to convex elevation and moist, opaque, transparent, translucent consistency in nature.

The bacterial isolates on nutrient agar were stained for their morphological characters and the results are presented (Table 4). All seven bacteria isolated were Gram-positive in nature. Among that isolate number 2, 5, 6 and 7 were Gram-positive cocci and isolates 1, 3 and 4 were observed as Gram-positive rods. Isolates 1, 2 and 4 were observed as motile, while 3, 5, 6 and 7 were non-motile. Endospore production was showed by isolates 1, 4 and 6. Sreekumar *et.al* [20] have successfully isolated a new strain of spore forming Bacilli that is capable of fermenting lactose from dairy effluents.

Enzymatic activities were checked for catalase, oxidase, nitrate reduction, urease and caseinase (Table 5). These tests confirmed the ability of bacterial isolates to degrade various

substrates. Studies on enzymatic activities indicated the degrading capacity of these microbes and thus they can be utilized for the further treatment of dairy waste water. All isolates showed positive results for Hugh and Leifsons (H&L) test and nitrate reduction, except isolate 7. Isolate 1, 2, 3 and 7 showed positive test for catalase. Except isolate 2, other isolates indicated negative test for hydrogen sulphide production. Six of the isolates showed caseinase activity. Isolate 2 and 6 showed negative test for oxidase. Starch hydrolysis or amylase test indicated negative for isolate 4 while all others gave positive test. Urease tested positive for isolates 1, 3, 4, 5 and 7. Isolates 2 and 4 showed absence of gelatinase. Carbohydrates utilization tests for lactose, maltose, inositol, mannitol, xylose, glucose and sucrose were carried out (Table 6). Prakashveni and Jagadeesan [21] reported the isolation of *Lactobacillus* from dairy effluent.

Table 3. Colony characters of the bacterial isolates

Bacterial isolate	Size (mm)	Shape	Colour	Margin	Elevation	Opacity	Consistency
1	<1	Round	Dirty white	Regular	Slight convex	Opaque	Moist
2	1	Irregular	Golden yellow	Irregular	Flat	Transparent	Moist
3	2	Circular	White	Regular	Flat	Translucent	Moist
4	2	Circular	Milky white	Regular	Slight convex	Opaque	Moist
5	<1	Irregular	White	Entire	Flat	Transparent	Moist
6	2	Circular	White	Entire	Concave	Transparent	Moist
7	3	Irregular	Golden yellow	Regular	Convex	Transparent	Moist

Table 4. Staining and morphology of the bacterial isolates

Isolate No.	Gram character	Sporulation	Shape of cell	Arrangement of cell	Motility
1	Positive	+	Long rod	Long Chain	Motile
2	Positive	-	Cocci	Bunches	Motile
3	Positive	-	Short rod	Pairs & bunches	Non-Motile
4	Positive	+	Short rod	Long Chain	Motile
5	Positive	-	Cocci	Single	Non-Motile
6	Positive	+	Cocci	Single	Non-Motile
7	Positive	-	Cocci	Bunches	Non-Motile

Where " +": Spore producer ; " -" : Non- Spore producer.

Table 5. Enzymatic properties of the bacterial isolates

Isolate Number	Tentative identification
1	*Bacillus subtilis*
2	*Staphylococcus aureus*
3	*Corynebacterium kutscheri*
4	*Lactobacillus delbrueckii*
5	*Listeria monocytogenes*
6	*Enterococcus hirae*
7	*Enterococcus farcium*

Table 6. Utilization of carbohydrates by the bacterial isolates

Isolate No.	Catalase	Oxidase	H_2S	Nitrate reduction	H.L. test	Gelatinase	Urease	Amylase	Caseinase
1	+	+	-	+	+	+	+	+	+
2	+	-	-	+	+	-	-	+	+
3	+	+	+	+	+	+	+	+	+
4	-	+	-	+	+	-	+	-	+
5	-	+	-	+	+	+	+	+	-
6	-	-	-	+	+	+	-	+	+
7	+	+	-	-	+	+	+	+	+

Where "+": Positive test; -": Negative test.

Table 7. Tentative identification of bacterial isolate from bergey's manuals of systematic bacteriology

Isolate No.	Lactose	Sucrose	Glucose	Maltose	Mannitol	Inositol	Xylose
1	+	+	+	+	+	+	+
2	+	+	+	+	-	-	-
3	+	+	+	+	+	+	+
4	-	-	+	-	-	+	+
5	+	+	+	-	+	+	+
6	-	-	-	-	-	+	+
7	-	+	-	-	+	+	+

Table 7 represents the tentative identification of bacterial isolates by Bergey's Mannuals of Systematic Bacteriology. Phanse et al. [22] collected and studied soil samples, waste water samples (including industrial effluents, dairy waste, domestic sewage) and activated sludge samples from various sources and used for the isolation of bacteria and (PHA) Polyhydroxyalkanoic acids producing strains belonged to the genera- *Bacillus, Pseudomonas*, *Azotobacter* and *Staphylococcus.*

4. CONCLUSION

In any dairy plant, the quantity and characteristics of effluent is depending upon the extent of production activities. The present study indicates that isolated bacterial cultures from dairy effluent have the ability to utilize the components like nitrate, starch, gelatin, sugars like sucrose, maltose, lactose etc. These micro organisms can be efficiently applied for the biological treatment of the dairy effluent treatments. Micro organisms uses the organics in waste water as a food supply so that water pollution threats due to dairy effluents can be reduced through biological methods and this will enable the recycling of water.

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COMPETING INTERESTS

Authors have declared that no competing interests.

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