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Isolation, characterization and identification of microorganisms from unorganized dairy sector wastewater and sludge samples and evaluation of their biodegradability

S. Garcha[a], N. Verma[b,*], S.K. Brar[b]

[a] Department of Microbiology, Punjab Agricultural University, Ludhiana, India
[b] Department of Biotechnology, Punjabi University, Patiala, India

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ABSTRACT

In developing countries like India, the major part of the dairy sector is under the coverage area of unorganized sector, which lacks adequate treatment facility. In present investigation, the study was done to isolate most frequently occurring active strains adapted to the wastewater physical-chemical conditions and having good biodegradation potential. The 10 isolates were selected on the basis of their efficiency in reducing all the three pollution potential parameters i.e BOD, TSS and Oil and grease content. The identification of selected strains was done by 16 S rRNA sequencing. The maximum reduction in BOD_3 was shown by isolate no. 25 i.e 89.8% (90 mg/l). Isolate no. 4 and 25 were efficient in reducing the TSS content by 88.6%. Isolate no. 27 and 45 were more efficient in reducing the oil and grease content by 88.5% and 90% respectively.

1. Introduction

Dairy industries generate highly pollutant wastewater, characterized by high BOD (Biological Oxygen Demand), TSS (Total suspended solids) and Oil and grease content [1–3]. Wastewater with high organic load causes many ecological problems [4]. It shows adverse effects on both flora and fauna; its discharge to the land alters physical and chemical properties of the soil, thus reducing the fertility of land for crop production and its discharge to the water bodies may results in eutrophication, affecting the aquatic life and making water unfit for drinking [5–7]. Hence, the challenge for the safe disposal of the dairy wastewater cannot be ignored. Environmentalists and government are looking for cheap, efficient, effective and long lasting solutions for wastewater treatment and recycling. In developing countries like India, physico-chemical methods of waste water treatment are inevitably cost intensive and cannot be employed in all industries. Hence, in recent years, the biological treatment system has become popular and has helped in developing relatively efficient, low cost waste treatment systems [8]. In order to design an efficient biological waste water treatment it is important to know the microbiota composition of the wastewater and to identify the strains which metabolize organic compounds [9,10]. In India about 85% of the dairy sector is under the coverage of unorganized sector which lacks adequate treatment facility and management skills. Physico-chemical characteristics of the dairy wastewater generated by organized and unorganized sector exhibit huge variations. The wastewater generated by unorganized sector is rich in organic content. Its C:N ratio was calculated as 37.6 compared to the ratio of 11.9 of organized sector [11]. Variations in major pollution parameters of dairy effluent of both the sectors require appropriate treatment approaches for its safe disposal.

The present investigation was carried out to isolate the most frequently occurring and optimally performing microorganisms from dairy wastewater and sludge samples of unorganized sector and to test the bioremediation efficacy of the isolates by bioaugmenting them in dairy wastewater.

2. Material and methods

2.1. Sample collection

Five samples of dairy wastewater and five samples of dairy sludge were collected from unorganized dairy industry located in the districts of Patiala, Ludhiana, Shri Muktsar Sahib and Bathinda (Punjab, India) in dry plastic bottles which were rinsed with distilled water and then with dairy effluent. Physical properties like pH, temperature, odor, color were recorded at the site of sample collection. The pH was determined using a EI Deluxe pH meter - 101.

The sample was transferred to the laboratory immediately and stored at 4 °C to avoid any physical-chemical changes in the wastewater.

* Corresponding author.

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Table 1
Colony and morphological characteristics of bacterial isolates.

Culture No	Opacity	Size (Diameter 24 h of incubation) (mm)	Shape	Color	Margin	Elevation	Gram character	Shape of cell	Arrangement
1	Opaque	3	Irregular	White	Undulate	Raised	-ve	Rods	Pairs
2	Translucent	< 1	Round	White	Entire	Convex	+ve	Cocci	Pairs and Chains
3	Opaque	3	Irregular	Pale yellow	Filiform	Convex	+ve	Cocci	Chains
4	Opaque	2	Round	White	Undulate	Flat	-ve	Coccobacilli	Pairs
5	Transparent	< 1	Round	White	Entire	Flat	-ve	Rods	Chains
6	Transparent	3	Irregular	Golden yellow	Curled	Raised	-ve	Rods	Singly present
7	Translucent	2	Round	White	Entire	Flat	+ve	Rods	Present in group of 3
8	Transparent	< 3	Irregular	White	Undulate	Raised	+ve	Cocci	Singly present
9	Opaque	1	Round	White	Filamentous	Convex	+ve	Coccobacilli	Singly present
10	Transparent	1	Round	Pale white	Curled	Raised	+ve	Coccobacilli	Singly Present
11	Transparent	1	Round	White	Entire	Raised	+ve	Coccobacilli	Pairs
12	Opaque	2	Rhizoid	White	Undulate	Flat	-ve	Cocci	Pairs
13	Translucent	1	Irregular	White	Curled	Raised	-ve	Cocci	Chains
14	Opaque	1	Filamentous	White	Curled	Convex	+ve	Rods	Singly present
15	Transparent	< 3	Irregular	White	Undulate	Convex	+ve	Coccobacilli	Clusters
16	Opaque	< 3	Irregular	Golden yellow	Undulate	Raised	+ve	Coccobacilli	Pairs
17	Transparent	2	Rhizoid	White	Entire	Flat	-ve	Rods	Pairs
18	Opaque	< 3	Irregular	Grey	Undulate	Raised	-ve	Rods	Clusters
19	Opaque		Irregular	White	Entire	Raised	+ve	Coccobacilli	Singly presents
20	Opaque	1`	Round	White	Entire	Convex	+ve	Coccobacilli	Pairs
21	Transparent	1	Filamentous	Grey	Curled	Raised	+ve	Coccobacilli	Pairs
22	Opaque	2	Round	White	Filamentous	Raised	-ve	Cocci	Chains
23	Opaque	3	Rhizoid	Golden yellow	Undulate	Flat	-ve	Cocci	Pairs
24	Translucent	< 3	Irregular	White	Filamentous	Flat	-ve	Coccobacilli	Chains
25	Opaque	2	Round	White	Entire	Raised	+ve	Rods	Pairs
26	Transparent	< 3	Irregular	Pale White	Filamentous	Flat	+ve	Coccobacilli	Pairs
27	Opaque	< 3	Rhizoid	White	Entire	Raised	-ve	Rods	Chains
28	Transparent	< 3	Filamentous	White	Undulate	Flat	+ve	Coccobacilli	Pairs
29	Opaque	< 3	Filamentous	Pale white	Filamentous	Flat	-ve	Coccobacilli	Chains
30	Opaque	2	Rhizoid	White	Entire	Raised	-ve	Coccobacilli	Pairs and in Chains
31	Transparent	2	Round	White	Undulate	Flat	-ve	Rods	Singly and in pairs
32	Opaque	1	Irregular	White	Entire	Raised	+ve	Coccobacilli	Singly and in pairs
33	Opaque	< 3	Irregular	White	Undulate	Flat	+ve	Rods	Pairs
34	Opaque	2	Round	White	Entire	Raised	+ve	Rods	Pairs
35	Transparent	2	Transparent	Pale White	Udulate	Flat	-ve	Rods	Singly present
36	Opaque	< 3	Round	Pale White	Entire	Raised	-ve	Rods	Chains
37	Opaque	< 3	Irregular	White	Undulate	Flat	-ve	Rods	Singly present
38	Translucent	< 3	Irregular	White	Filamentous	Flat	+ve	Rods	Singly present
39	Opaque	2	Round	White	Entire	Raised	+ve	Coccobacilli	Pairs
40	Translucent	< 3	Irregular	White	Entire	Raised	+ve	Rods	Singly
41	Opaque	< 3	Irregular	White	Filiform	Raised	+ve	Rods	Singly
42	Opaque	< 3	Round	White	Filiform	Raised	+ve	Rods	Clusters
43	Opaque	< 3	Irregular	Pale White	Filiform	Raised	-ve	Rods	Clusters
44	Opaque	2	Round	White	Entire	Round	-ve	Rods	Clusters
45	Transparent	< 3	Round	Pale White	Entire	Round	-ve	Cocci	Pairs
46	Opaque	2	Irregular	White	Entire	Raised	+ve	Rods	Chains
47	Opaque	< 3	Irregular	White	Filamentous	Raised	+ve	Rods	Singly
48	Opaque	1	Round	White	Entire	Raised	+ve	Rods	Chains
49	Opaque	2	Irregular	White	Entire	Raised	+ve	Rods	Singly
50	Translucent	< 3	Irregular	White	Entire	Flat	+ve	Rods	Singly
51	Opaque	< 3	Irregular	White	Filamentous	Raised	+ve	Rods	Singly present and in Pairs
52	Opaque	< 3	Irregular	White	Filamentous	Raised	+ve	Coccobacilli	Singly present
53	Opaque	2	Round	Pale White	Filiform	Raised	+ve	Rods	Singly present
54	Transparent	< 3	Irregular	White	Undulate	Raised	+ve	Rods	Singly present
55	Transparent	< 3	Irregular	White	Undulate	Flat	+ve	Coccobacilli	Pairs
56	Opaque	1	Round	Pale White	Filamentous	Flat	+ve	Cocci	Singly present
57	Translucent	2	Irregular	White	Undulate	Raised	+ve	Coccobacilli	Pairs
58	Opaque	1	Round	White	Entire	Raised	+ve	Coccobacilli	Pairs
59	Opaque	< 3	Round	Pale White	Entire	Raised	+ve	Coccobacilli	Chains

2.2. Analysis of the dairy wastewater and sludge samples

Parameters of dairy waste samples analyzed included pH, color, temperature. BOD_3, Oil and grease and TSS (Total suspended solids) which were carried out as per standard procedure. Total suspended solids were determined by the equation TSS = TS (Total solids – TDS (Total dissolved solids). The Oil and grease content was determined by partition gravimetric method. The BOD was analyzed by titrimetric method [12].

2.3. Isolation of most frequently occurring micro-organisms from dairy sludge

Appropriately dilute sludge samples were plated onto Nutrient Agar