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BIODIVERSITY OF MICROBES IN DAIRY INDUSTRY EFFLUENT

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ABSTRACT
An investigation was carried out to assess the dairy industry effluent on microbial diversity viz. bacteria, fungi and cyanobacteria. One year study revealed that 10 species of bacteria, 12 fungi and 23 species of cyanobacteria were observed from the effluent stream. Among bacteria, *Pseudomonas* with two species and others with single each were recorded. *Aspergillus* was dominant (4 species) among the fungi with followed by *Penicillium* with two. Altogether 23 species of cyanobacteria belonging to seven genera were collected from the effluent stream. Among the genera, *Oscillatoria* dominated the effluent with ten species followed by *Phormidium* with 4 *Nostoc* with 4, *Anabaena* with 2 and *Aphanocapsa*, *Plectonema* and *Chlorogloea* with single species each. High amounts of phosphates and nitrates with sufficient amount of oxidizable organic matter, limited dissolved oxygen content and slightly alkaline pH were probably the factors favouring the growth of microbes especially cyanobacteria.

KEYWORDS: Dairy industry effluent, biodiversity, microbes, pollution.

INTRODUCTION
Discharge of urban, industrial and agricultural wastes has increased the quantum of various chemicals that enter the receiving waters which in turn alter considerably their physico-chemical characteristics thereby increasing eutrophication. Since effluents are rich in nutrients due to loading of organic wastes, they after ideal habitats for different microorganisms including algae, fungi and bacteria. A variety of algae are growing in these habitats. Algal growth in these habitats significantly influences the ecosystem (Sladeckova 1962). Some algal forms can be useful as indicators on which major water management practices, pollution studies and water quality analysis was carried out by Palmer (1969) and Schubert (1984). The importance of algal dynamics, particularly their response to environmental changes and nutritional fluctuation have been suggested in several studies (Frempong 1981; Tilman *et al.* 1982; Sudhakar *et al.* 1991). In recent years blue green algae have been drawing tremendous attention because of their ability to treat wastewater and improve water quality.

The milk house is a critical place on a dairy farm for maintaining sanitation to produce high quality milk. The milk house is where the milk is brought from the barn by pipeline, cooled and stored. A milk house may also have a utility room, storage room, or office space. Milk houses contain a bulk tank for storing the milk, a milk receiver jar where the pipeline empties, a filtration device, in-line cooling equipment, automatic cleaning controls, and a place to wash and store milking equipment. The walls and floor are cleaned daily to maintain proper sanitation for safely handling milk. Milking equipment and pipelines are cleaned after each milking. Bulk tanks are cleaned each time they are emptied, typically once a day or every other day. The typical milking equipment cleaning regime is usually has four cycles. Taking the above facts into consideration, a survey was undertaken in dairy effluent to explore the nature of microbial flora such as bacteria, fungi and cyanobacteria of dairy effluent.

MATERIALS AND METHODS
Effluent was collected from Dairy industry effluent, Thanjavur, Tamil Nadu, India for a period of one year (October 2015 to September 2016). Samples were collected in large sterilized container and brought to the laboratory. The effluent samples were filtered through cotton to remove suspended coarse particles before use. Population of bacteria and fungi were identified and isolated from the effluent samples by serial dilution technique. Bacteria were identified based on colony characteristics, Gram staining methods and by various biochemical studies as given by Bergey (1984). Fungi were identified by using standard manuals (Gillman 1957) and (Ellis 1971). Effluent samples were collected in duplicate from the station in pre-sterilized bottles. For cyanobacterial survey were selected along the effluent stream. Samples were collected from the places along with effluents in polythene bags. Standard

microbiological methods were followed for the isolation and identification of cyanobacteria (Desikachary 1959). Physico-chemical characteristics of effluent were done according to the standard methods (APHA 1981). Temperature and pH of the effluent were measured at the station itself.

RESULTS AND DISCUSSION

The effluent was slightly alkaline and contained high amounts of nitrate, nitrite and ammonia; total, inorganic and organic phosphate and calcium in all the three seasons (summer, winter and rainy) examined (Table 1). Values of dissolved oxygen (DO) were very low indicating highly obnoxious conditions. Though BOD and COD levels were high as per IS Standards. In general, the characteristics of effluent tested in all the three seasons were not varied much. Most of the parameters tested were slightly higher in summer than in winter and rainy seasons.

Bacteria isolated from the effluent were identified based on colony morphology, Gram staining, and various biochemical characteristics. The characteristics of isolated bacteria are given in the table 2. Totally ten different bacteria were isolated from the effluent sample. Jain *et al.* (2001) isolated three different bacterial strains from the distillery sludge, where as twelve different species of fungi belonging to eight genera were isolated from the dairy effluent (Table 4). Among the fungi recorded, *Aspergillus* was found to be dominant with four species viz., *A. flavus, A. fumigatus, A. luchensis* and *A. niger*. The genus *Penicillium* was represented by two species viz., *P. janthinellum* and *P. javanicum*. Similarly *Neurospora crussa* was not observed during rainy season. Kousar *et al.* (2000) isolated 23 species of fungi from dye effluent polluted habitat with *Aspergillus* as the dominant genus.

Altogether 23 species of cyanobacteria belonging to seven genera were collected from the effluent stream (Table 5). Among the genera, *Oscillatoria* dominated the effluent with ten species followed by *Phormidium* with 4 *Nostoc* with 4, *Anabaena* with 2 and *Aphanocapsa*, *Plectonema* and *Chlorogloea* with single species each (Table 5). *O. acuminata* and *O. pseudogeminata* were not observed in pre monsoon season. Similarly *O. terebriformis* was not observed in monsoon season. The rest of the species of *Oscillatoria* were noted in all the seasons. *Phormidium* with four species were reported in all the seasons. Of which the species of *Phormidium anamala* and *P. incrustatum* was the dominant one as it occurred in most of the months as compared to other species. Unicellular forms such as *Aphanocapsa pulchra* was also isolated in all the seasons, whereas *Chlorogloea fritschii* was reported in pre monsoon, post monsoon and monsoon seasons but not in summer. Many authors emphasize the importance of light, temperature, pH, carbon-di-oxide, organic matter, alkalinity, nitrates and phosphates as factors important in determining the distribution of cyanobacteria (Singh 1960; Philipose 1960; Venkateswaralu 1969b; Munawar 1970). In the present study, as a whole, conditions in the effluent appeared to be favourable for the cyanophycean members. The effluent had high oxidizable organic matter, nutrients such as nitrates and phosphates with high calcium content. Observations of Munawar (1970) suggest that Cyanophyceae grow luxuriantly with great variety and abundance in ponds rich in calcium. The present data also shows that the calcium is one of the factors for the growth of cyanophycear members. Dense cyanobacterial population was observed during summer in all the sites under study when the oxygen content was very low (Table 1). Similar observations were made by Rao (1953) and Venkateswarlu (1969b). They noted that oxygen deficiency favoured cyanobacterial growth. Dominant and persistent occurrence most of the species of *Oscillatoria* and *Phormidium* indicate their capacity to thrive in the type of man-made habitat. Moreover, these findings show that there are certain species of cyanobacteria which are tolerant to organic pollution and resist environmental stress caused by the pollutant.

The cheapest sources of nutrients for the mass culturing of cyanobacteria are undoubtedly sewage and other organic industrial wastes. The cyanobacteria that are isolated from an effluent stream could be grown on large scale in controlled waste stabilization ponds and thus pollution is taken care of to certain extent. Several investigators (Whitton 1975; Palmer 1980) have pointed out that the indicator species could be used to monitor pollution in controlled waste stabilization ponds. In this investigation, *O. animalis* was observed in all the months of all seasons and hence it could be treated as indicator species of dairy effluent. On the basis of this fact it is suggested that the indicator species could be used for pollution abatement programmes.

Table 1: Characteristics of effluent for the period from October 2015 to September 2016.

S.No.	Parameters	Summer	Pre monsoon	Monsoon	Post monsoon
1.	Temperature ^{0}C	19.35 ± 1.32	17.51 ± 0.57	16.90 ± 0.25	18.51 ± 0.36
2.	pH	7.54 ± 0.27	7.45 ± 0.12	7.38 ± 0.34	7.46 ± 0.17
3.	Total suspended solids	1087 ± 12.15	1065 ± 15.22	1013 ± 25.27	1055 ± 12.24
4.	Total dissolved solids	480 ± 13.41	442 ± 15.22	420 ± 17.45	438 ± 15.51
5.	Free carbon-di-oxide	20.5 ± 5.08	16.5 ± 2.14	15.2 ± 5.14	16.9 ± 3.21
6.	Carbonate	1.21 ± 0.24	1.87 ± 0.18	3.81 ± 0.27	1.92 ± 0.17
7.	Bicarbonate	68.7 ± 3.81	71.5 ± 6.27	75.7 ± 4.15	72.8 ± 5.22
8.	BOD	258 ± 4.51	233 ± 2.18	203 ± 3.47	235 ± 1.21
9.	COD	421 ± 5.14	382 ± 4.25	345 ± 8.71	391 ± 4.14
10.	Dissolved oxygen	3.15 ± 1.21	3.81 ± 1.15	4.12 ± 1.20	3.87 ± 1.19
11.	Nitrate	118.5 ± 5.47	113.3 ± 2.12	107.7 ± 4.17	114.8 ± 3.12
12.	Nitrite	75.71 ± 1.41	69.52 ± 2.16	67.24 ± 2.15	69.17 ± 3.17
13.	Ammonia	43.28 ± 3.22	41.12 ± 4.15	39.26 ± 4.28	42.13 ± 4.21
14.	Total phosphate	73.97 ± 5.04	77.98 ± 5.12	83.48 ± 5.12	76.56 ± 5.34
15.	Inorganic phosphate	37.82 ± 3.24	39.12 ± 3.37	41.21 ± 3.54	37.82 ± 3.15
16.	Organic phosphate	36.15 ± 3.15	38.86 ± 3.26	42.27 ± 3.24	38.74 ± 3.22
17.	Calcium	134.1 ± 2.41	129.5 ± 2.21	125.7 ± 2.34	130.3 ± 2.14
18.	Magnesium	112.7 ± 2.28	109.5 ± 2.15	105.8 ± 2.12	108.1 ± 2.11
19.	Chloride	65.12 ± 3.14	63.12 ± 3.12	60.8 ± 3.29	62.7 ± 3.37

Monsoon (Oct-Dec); Post-monsoon (Jan-Mar); Summer (Apr-June); Pre-monsoon (July-Sep)
*Except pH and temperature all values expressed in mg^{-1}.

Table 2: Bacterial flora observed from dairy industry effluent (October 2015 to September 2016).

S.No.	Name of bacteria	Summer	Premonsoon	Monsoon	Post monsoon
1.	*Escherichia coli*	+	+	+	+
2.	*Enterobacter aerogens*	+	+	+	+
3.	*Klebsiella pneumoniae*	+	+	+	+
4.	*Lactobacillus* sp.	+	+	+	+
5.	*Proteus vulgaris*	+	+	+	+
6.	*P. aeruginosa*	+	+	+	+
7.	*P. fluorescens*	+	+	+	+
8.	*Salmonella* sp.	+	+	+	+
9.	*Micrococcus* sp.	+	+	+	+
10	*Shigella sonnei*	+	+	+	+

+ : Observed in all the months.

Table 3: Fungal flora observed from dairy industry effluent (October 2015 to September 2016).

S.No.	Name of fungi	Summer	Premonsoon	Monsoon	Post monsoon
1.	*Aspergillus flavus*	++	++	++	++
2.	*A. fumigatus*	++	+	+	+
3.	*A. luchensis*	++	++	++	+
4.	*A. niger*	+++	++	++	+++
5.	*Verticillium* sp.	+	-	-	-
6.	*Saccharomyces* sp.	++	+	+	+
7.	*Helminthosporium*	+	+	+	+
8.	*Penicillium javanicum*	++	++	++	++
9.	*P. janthinellum*	++	+	+	+
10.	*Trichoderma viride*	++	+++	++	+++
11.	*Neurospora crassa*	+	+	-	+
12.	*Curvularia* sp.	+	-	-	-

+++ : Observed in three months
++ : Observed in two months only
+ : Observed in one month only
- : Not observed.

Table 4: Cyanobacterial flora observed from dairy industry effluent (October 2015 to September 2016).

S.No.	Name of cyanobacteria	Summer	Premonsoon	Monsoon	Post monsoon
1.	*Aphanocapsa pulchra*	+++	+++	++	+++
2.	*Anabaena fertilissima*	+++	+++	-	+++
3.	*A. flosaquae*	+++	+++	-	++
4.	*Chlorogloea fritschii*	-	+++	+++	++
5.	*Oscillatoria0acuminata*	++	-	+	++
6.	*O. animalis*	++	++	+	++
7.	*O. brevis*	++	+++	+++	+++
8.	*O. claricentrosa*	+	++	++	++
9.	*O. curviceps*	+++	+	-	+
10.	*O. guttulata*	+++	+++	-	+++
11.	*O. pseudogeminata*	+++	-	++	+++
12.	*O. salina*	+++	-	++	-
13.	*O. subbrevis*	++	+	++	+
14.	*O. terebriformis*	++	+++	-	+++
15.	*Phormidium anomala*	+++	+++	+++	+++
16.	*P. incrustatum*	++	+++	++	+++
17.	*P. papyraceum*	+++	+	-	++
18.	*P. tenue*	+++	++	++	++
19.	*Plectonema sp.*	+	-	+++	-
20.	*Nostoc communae*	+	-	++	-
21.	*N. calcicola*	+++	+++	++	++
22.	*N. paludosum*	+++	+++	++	+++
23.	*N. muscorum*	++	++	+	++

+++ : Observed in three months
++ : Observed in two months only
+ : Observed in one month only
- : Not observed.

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