Biological Forum – An International Journal **14(2): 937-939(2022)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Enumeration of Oleaginous Yeast from Dairy Environmental Samples

Rajasekhar P.[1*], Sachindra Babu A.[2], Ramachandra B.[3] and Prabha R.[4]
[1]Ph.D. Scholar, Department of Dairy Microbiology, Dairy Science College,
KVAFSU, Hebbal, Bengaluru (Karnataka), India.
[2]Dean, Dairy Science Collge, KVAFSU, Hebbal, Bengaluru (Karnataka), India.
[3]Associate Professor, Department of Dairy Microbiology,
Dairy Science College, KVAFSU, Hebbal, Bengaluru (Karnataka), India.
[4]Associate Professor & Head, Department of Dairy Microbiology,
Dairy Science College, KVAFSU, Hebbal, Bengaluru (Karnataka), India.

(Corresponding author: Rajasekhar P.)*
(Received 17 March 2022, Accepted 12 May, 2022)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: Dairy environmental samples like dung, feed, fodder, sewage, silage and soil samples were collected aseptically form KVAFSU dairy farm, Bengaluru-24, serially diluted and used malt extract agar adjusted to pH 3.5 for total yeast count while malt extract agar (100 ml) incorporated with 3.75 ml of 2 % Sudan black for identifying the oleaginous yeast colonies having black colour with incubation of 30°C for 5 days. The counts of total yeast ranged from 5.53 to 6.58 log_{10} cfu/g whereas 1.40 to 3.20 log_{10}cfu/g of oleaginous yeast was noticed among the dairy environmental samples. Soil sample showed more of both total yeast (6.58) and oleaginous yeast (3.20) with 48.63 % occurrence, while lowest oleaginous yeast of 1.40 log_{10}cfu/g accounting for 23.53 % was found in fodder. The significance of the present study is direct enumeration of oleaginous yeast from dairy environmental samples by adopting lipid stain Sudan black into agar medium where as many of the authors confirmed the presence of oleaginous yeast after making smear of colonies of yeast by staining using Sudan black and observing the cells for the presence of lipid under microscope.

Keywords: Oleaginous yeast, Malt extract agar, Dairy environment, Sudan black, Serial dilution, Asepsis, Soil.

INTRODUCTION

Diesel fuel is the common term for the distillate of crude fuel oil sold for use in motor vehicles that use the compression ignition engine named for its inventor. The major cause of increasing carbon dioxide concentration in the atmosphere is excessive consumption of fossil fuels, for example, petroleum, coal, and natural gases. Moreover, excessive consumption of fossil fuel causes not only global warming but also creating global economic problems. Biodiesel is an alternative to diesel obtained from plant, animals and limitations of these sources led to finding of oleaginous microorganisms that include bacteria and yeast. Oleaginous yeast have their own importance with respect to lipid accumulation and conversion to biodiesel. An attempt has been made in this research article to enumerate oleaginous yeast from dairy environmental samples.

Oleaginous yeast species are an alternative for the production of lipids or triacylglycerides (TAGs). These yeasts are usually nonpathogenic and able to store TAGs ranging from 20 % to 70 % of their cell mass depending on culture conditions. TAGs originating from oleaginous yeasts can be used as the so-called second-generation biofuels, which are based on non-food competing "waste carbon sources" (Lamers, 2016). Vickers *et al.* (2017) stated that oleaginous yeasts are advantageous because they can quickly grow to high densities with a high lipid content and their cultures can utilize a large number of renewable substrates and inexpensive materials like food wastes, make oleaginous yeasts economically interesting. Oleaginous yeasts predominantly belong to the genera *Rhodosporidium, Cryptococcus, Rhodotorula, Yarrowia, Lipomyces,* and *Trichosporon* (Athenaki *et al.,* 2018 and Blomqvist *et al*., 2018).

Yeast isolates obtained from soil (agricultural, orchards, milk collection area), plant surfaces (leaves, flowers and fruits), waste oils from traditional oil milling houses and dairy products (cheese, milk and yoghurt), fermented foods, samples from fruit surfaces, tabletop swabs, sugar cane juice, sago effluent were screened for lipid accumulation by staining the cells of using Sudan III or Sudan Black (Jiru *et al.,* 2016; Arous *et al.,* 2017a, Vincent *et al.,* 2018; Khobragade *et al.,* 2020). Few authors have used Nile red stain to screen the cells of isolated yeast colonies with the help of flourescent microscope (Patel *et al.,* 2019; Bardhan *et al.,* 2020).

MATERIALS AND METHODS

The samples such as - soil, dung, feed, fodder, silage and sewage water were collected from Department of Instructional Livestock and Farm Complex, College of Veterinary Science, KVAFSU, Hebbal, Bengaluru. All the samples collected were serially diluted in sterile phosphate buffer and pour plated the required dilutions using molten MEA medium adjusted to pH 3.5 using 10 % filter sterile lactic acid and incorporated Sudan black (0.08 %) of 0.2 ml maintained at 47 º - 50 ºC of 10-15 ml added, mixed gently and incubated after solidification at 30 ºC for 5 days for general yeast and oleaginous yeast, respectively. The black coloured (due to Sudan black that colours the lipid accumulating yeast colonies) non-cottony colonies obtained on the MEA agar were counted and expressed as log_{10}cfu/g. Statistical analysis (Anova) was carried out to identify the statistical significance among the samples and counts of yeast.

RESULTS AND DISCUSSION

Among the dairy environmental samples such as dung feed, fodder, sewage, silage and soil, soil had higher viable oleaginous yeast (3.20) on Sudan black incorporated malt extract agar (MEA) visible as black colonies (Fig. 1). Out of total yeast of 6.58 log_{10} cfu/g in soil sample, 3.20 were lipid accumulating yeast termed as oleaginous yeast of 48.63 % and fodder revealed lowest oleaginous yeast of 1.40 log_{10} cfu/g (23.53 %) out of 5.95 viable yeast counts (Table 1). The high oleaginous counts in soil may be due to oil spillage in dairy farm that would have reached the soil as vehicular movements are more in the farm. As fodder is green, ability of plant cells to hold the lipid may be less and hence low oleaginous yeast counts. Among the dairy environmental samples, when compared with total yeast count, oleaginous yeast presence in per cent among silage, dung, feed and sewage were 47.02, 36.73, 33.11 and 31.86, respectively (Fig. 2). Statistically significant difference (P = .05) did not occur in general yeast count and oleaginous yeast count among the dairy environmental samples. The present study enumerated the oleaginous yeast through Sudan Black stain incorporated media by obtaining black coloured colonies.

Many authors have carried out the isolation of oleaginous yeast but there is lack of data with respect to the counts of oleaginous yeast from samples as all of them used staining of yeast colonies and through staining of cells only confirmed lipid accumulation either using Sudan Black or Sudan III or Nile Red lipid stains. Out of 40 yeast isolates by Pan *et al.* (2009); Jiru *et al.* (2016); Vincent *et al.* (2018); Khobragade *et al.* (2020); Planonthand Chantarasiri (2022) applied Sudan III or Sudan Black staining to yeast cells and could able to confirm 20(50 %), 18 (5.3 %), 21 (100 %), 6 (27 %) and 6 (37.5 %) yeast isolates accumulated lipid through bright field microscope respectively. While Ashika *et al.* (2017); Bardhan *et al.* (2020); Chomchuen *et al.* (2021) confirmed 3 (100 %), 17 (100 %) and 8 (20.51 %) oleaginous yeast isolates for intracellular lipid using Nile red under fluorescent microscope, respectively.

(A) Yeast colony **(B)** Oleaginous yeast colony

Fig. 1. Yeast colony & Oleaginous yeast.

Table 1: Enumeration of oleaginous yeast from dairy environmental samples.

Sr. No.	Source	Total yeast count	Oleaginous yeast count (Per cent)
		log_{10}cfu/g or ml	
1.	Dung	5.99 ^{a}	2.20 ^{a} (36.73)
2.	Feed	6.04 ^{a}	2.00 ^{a} (33.11)
3.	Fodder	5.95 ^{a}	1.40 ^{a} (23.53)
4.	Sewage	5.65^{a}	1.80 ^{a} (31.86)
5.	Silage	5.53^{a}	2.60 ^{a} (47.02)
6.	Soil	6.58^{a}	3.20^{a} (48.63)
CD (P=.05)		**1.14**	**1.97**

Note:

– Medium used - Malt Extract Agar(MEA) incorporated with and incubation at 30 ºC for 5 days

– CD – Critical Difference

– All values are average of three trials

– Different superscripts within the column indicate significant difference

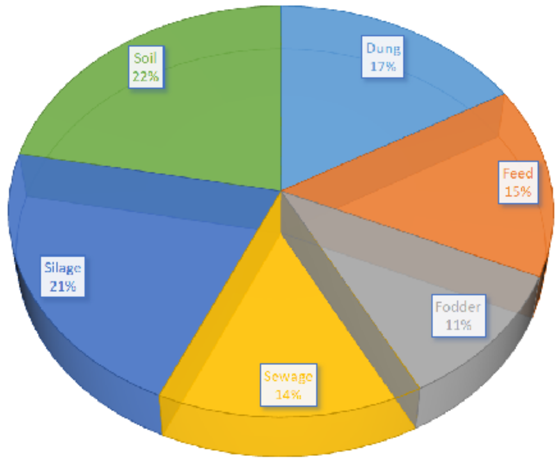

Fig. 2. Per cent occurrence of oleaginous yeast in dairy environmental samples.

CONCLUSION

The present study thus help to enumerate as well isolate directly the oleaginous yeast that accumulate lipid from any type of samples making the isolation process easy and use of Sudan black in laboratory is helpful as Nile red, a fluorescent dye requiring proper disposal.

REFERENCES

Arous F., Azabou, S., Triantaphyllidou I.E., Aggelis, G., Jaouani, A., Nasri, M., Mechichi, T. (2017a). Newly isolated yeasts from Tunisian microhabitats: lipid accumulation and fatty acid composition. *Eng. Life Sci., 17*: 226–236.

Ashika, S., Kiruthika, T., Ashokkumar, K., Suraj, H.M. and Sivakumar, U. (2017). Oleaginous Yeast from Sago Waste Water: Screening and Characterization of *Candida trophicalis* for Biolipid Production. *Madras Agric. J., 104*(7-9): 288-291.

Athenaki, M., Gardeli, C., Diamantopoulou, P., Tchakouteu, S. S., Sarris, D., Philippoussis, A., and Papanikolaou, S. (2018). Lipids from yeasts and fungi: physiology, production and analytical considerations. *Journal of Applied Microbiology*, *124*(2): 336–367.

Bardhan, P., Gupta, K., Kishor, S. Pronobesh, C., Chayanika, C., Eeshan, K., Vaibhav, V. G. and Manabendra, M. (2020). Oleaginous yeasts isolated from traditional fermented foods and beverages of Manipur and Mizoram, India, as a potent source of microbial lipids for biodiesel production. *Ann Microbiol., 70*: 27.

Blomqvist, J., Pickova, J., Tilami, S.K., Sampels, S., Mikkelsen, N., Brandenburg, J., Sandgren, M., and Passoth, V. (2018). Oleaginous yeast as a component in fish feed. *Scientific Reports, 8*: 15945.

Chomchuen, S., Arkornnak, M. and Amornrattanapan P. (2021). Isolation and Identification of Oleaginous Yeasts from Soils at Bang Phra Reservoir, Chonburi Province. *Burapha Sci. J*., *26*(2): 886 - 906

Jiru, T. M., Dawit, A., Nicholas K., Carolina, P. and Marizeth, G. (2016). Oleaginous yeasts from Ethiopia.*AMB Expr 6*: 78.

Khobragade, C.N., Shweta, R. G., Vinod, B. B. and Marathe. N.B. (2020). Isolation and characterization of oleaginous yeasts from dairy waste.*Indian J Dairy Sci., 73*(3): 236-241.

Lamers, D., Van, B. N., Martens, D., Peters, L., Van De Z. E., Jacobs, V. D. N., Wijffels, R.H., and Lokman, C. (2016). Selection of oleaginous yeasts for fatty acid production. *BMC Biotechnol.*, *16*: 45.

Pan, L., Yang. D., Li S., Wei Li., Chen, G. and Liang. Z. (2009).Isolation of the Oleaginous Yeasts from the Soil and Studies of Their Lipid-Producing Capacities. *Food Technol. Biotechnol.*, *47*(2): 215–220.

Planonth, S. and Chantarasiri, A. (2022). The oleaginous yeast *Pichia manshurica* isolated from Lansium domesticum fruit in Thailand and its fatty acid composition of single cell oil. *J. Biolog. Diver*, *23*(2):

Vickers, C., Williams, T., Peng, B., and Cherry, J. (2017). Recent advances in synthetic biology for engineering isoprenoid production in yeast. *Curr. Opin. Chem. Biol*. *40*: 47–56.

Vincent, M., Hung, H.C., Baran, P.R.N., Azahari, A.S and Adeni D.S.A. (2018). Isolation, identification and diversity of oleaginous yeasts from Kuching, Sarawak, Malaysia. *Biodiversitas*, *19*(4): 1266-1272.

How to cite this article: Rajasekhar P., Sachindra Babu A., Ramachandra B. and Prabha R. (2022). Enumeration of Oleaginous Yeast from Dairy Environmental Samples. *Biological Forum – An International Journal*, *14*(2): 937-939.