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Preparation of Direct Vat Set Cultures from Domestic Lactic Cultures

Deepa B.G.[1]*, Manjunatha H.[2], Akshaykumar[3], Basavabharati[3] and Madhusudan N.M.[3]
[1]Department of Dairy Microbiology, Dairy Science College, Hebbal, Bangalore (Karnataka), India.
[2]Dean, Dairy Science College, Mahagaon Cross, Kalaburagi (Karnataka), India.
[3]Assistant Professor, Department of Dairy Microbiology,
Dairy Science College, Mahagaon Cross, Kalaburagi (Karnataka), India.

(Corresponding author: Deepa B.G.)*
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ABSTRACT: Dahi is an important fermented milk product. Liquid culture is normally used in the preparation of Dahi where microbial contamination with sequential propagation of liquid culture are the noted limitations. Dried and frozen cultures show lower viability and in order to overcome these problems, Direct Vat Set cultures are slowly introduced in the commercial sector. The present study was carried out to develop Direct Vat Set Dahi cultures through solid sate fermentation technique. Dahi samples of 5 each were collected from houses and local markets of Bengaluru. That showed total lactic bacterial viable count ranging from 5.36 to 7.04. Among 72 lactic isolates obtained. lactobacilli predominated (38) followed by leuconostoc (18); lactococci (9) and streptococci (7). The lactic cultures that set the milk with higher direct microscopic counts in sterile skim milk were *Leuconostoc* sp. Leu6: *L. lactis* ssp. *lactis* Lc1*: L. fermentum* Lb8*: S. thermophilus* St3 especially at the ratio of 0.15 %: 0.3 %: 0.5%: 1 %. The growth of mixed dahi culture on sterile black gram dhal with 1% skim milk powder, 10% tomato juice and 70% moisture as solid substrate medium showed 9.42 log_{10}cfu/g at 24 hrs of incubation. SSF Dahi culture or DVS Dahi culture at ambient and refrigeration temperature revealed viable count reduction in total lactic count by 4.5 log and 3.5 log by the end of 70 days and 60 days of storage respectively. Dahi prepared from refrigerated stored DVS culture scored better than Dahi prepared out of ambient temperature stored DVS culture.

Keywords: Lactic acid bacteria, Dahi, DVS cultures, Liquid cultures and solid state fermentation.

INTRODUCTION

Dairying has played a prominent role in strengthening of the India's rural economy. It has been recognized as an instrument to bring about socio-economic transformation. The white revolution has transformed India's dairy industry (Elsborg, 1997).

Milk and milk based products have been a good source of nutrition to human health. India has emerged as the largest milk producing country in the world with present level of annual milk production estimated as 221.01 million tonnes. According to the DAHD's latest annual report (Department of animal husbandry and dairying, 2021), about 46% of milk is consumed unprocessed in households, with the remaining 34 %, 10%, and 10% consumed by the unorganised, cooperative, and commercial sectors, respectively. However, due to abundant milk production, organised and unorganised dairy sectors, as well as sweet makers, convert a large portion of milk into Traditional Indian Dairy Products (TIDP) (Sindhav *et al*., 2020). These are produced with approximately 50-55 % of the milk produced in India. It shows the importance of traditional milk products in our country's economy. Indigenous milk products have influenced the people's economic, social, cultural, nutritional, and religious status. Simple technology, minimal investment, low cost of production, simple infrastructure, low operational overheads, and, most importantly, high profit margins and established markets are all factors that contribute to the potential of these traditional dairy products resulting in enormous production volumes (Patel and Bhadania 2012).

Dahi is a major fermented milk product which plays an important role in human diet in India and neighbouring countries. It is made from cow milk or buffalo milk or combination thereof where part of lactose has been converted into lactic acid by lactic acid bacteria. Dahi has the same nutritional value as that of milk from which it is made and easily digestible than milk, as 60% of lactose is fermented and protein is partially hydrolyzed by the starters used in the preparation (Esayas *et al.*, 2003). At home, Dahi is prepared by boiling of milk, cooling to room temperature, inoculating with previous batch of dahi and keeping at room temperature overnight. The microflora of dahi is not well defined usually a heterogenous group of organisms which include *Lactococcus lactis* ssp. *lactis*, *Lactococcus lactis* ssp. *cremoris*, *Lactococcus lactis* ssp. *diacetylactis*, *Streptococcus thermophillus*, *Lactobacillus delbrueckii* ssp. *bulgaricus*, *Lactobacillus*

helveticus, Lactobacillus plantarum etc. (Shekar and Mariappan 2007).

The traditional method for the production of starters is through propagation of liquid culture that involves various stages such as preparation of stock, mother, working and finally bulk culture. This method of propagation is material and time consuming along with labour oriented. 'Bulk starter' in liquid form was used to inoculate the milk in the manufacture of various fermented milk products such as dahi, yoghurt, buttermilk, cheese and other fermented products. Liquid cultures if not propagated properly may be contaminated with spore forming bacteria, coliforms and yeasts so on (Forouhadeh *et al.*, 2010).

Over the past 10-15 years, the use of starter cell concentrates designated as either Direct Vat Set (DVS) or Direct Vat Inoculation (DVI) cultures have increasingly being used in order to overcome the problems encountered in liquid starters, particularly in small plants, to replace bulk starter in cheese and fermented milk manufacture. (Hansen *et al.*, 2002). DVS can be used directly as inoculation in heat treated cooled milk in fermentation vat. The terms DVI and DVS are used interchangeably. In addition to these high activity cell concentrates, lower activity commercial cell concentrates have been used for many years to inoculate milk for bulk starter preparation, and in the manufacture of 'long set products' that require extended incubation (Mullan, 2006).

Solid State Fermentation (SSF) technique has been widely used in the preparation of fermented foods, enzymes, organic acids, fungal biomass and enriched animal feeds which involves the controlled growth of microorganisms on solid substrate. Solid State Fermentation (SSF) has been defined as "the fermentation process occurring in the absence of free water, so as to contain large number of viable cells in concentrated form" (Ganina *et al.*, 2005).

SSF cultures if produced under strict hygienic conditions may provide an alternative method for the production of DVI cultures. SSF processes generally employ a natural raw material which may be inert (paddy husk, wheat bran) or nutritive (dhals, grams) as carbon and energy source. Solid substrate (matrix), however, must contain enough moisture (Garbal *et al.*, 2008). Depending upon the nature of the substrate, the amount of water absorbed could be one or several times more than its dry weight, which leads to relatively high water activity (a_w) on the solid or gas interface, in order to allow higher rate of biochemical processes. Low diffusion of nutrients and metabolites takes place in lower water activity conditions whereas compaction of substrate occurs at higher water activity. Hence, maintenance of adequate moisture level in the solid matrix along with suitable water activity are essential elements for SSF processes (Gilland *et al.*, 1985).

Solid substrates should have generally large surface area per unit volume. Smaller substrate particles provide larger surface area for microbial attack but pose difficulty in aeration or respiration due to limitation in inter-particle space availability. Larger particles provide better aeration or respiration opportunities but provide lesser surface area. In bioprocess optimization, sometimes it may be necessary to use a compromised size of particles for the reason of cost effectiveness (Pandey and Ashok 2007).

None of the Indian companies are producing DVS cultures. Production of DVS culture through SSF technique may be a break through in starter culture propagation. Keeping in view the above facts, an attempt has been made in the present investigation.

MATERIALS AND METHODS

Maintenance of Lactic acid bacteria. Stock lactic acid cultures were maintained in a yeast glucose agar stab (0.75% of agar) and subcultured once in 21 days. Working lactic cultures were maintained in a yeast glucose broth and sterile skim milk, which were stored in refrigerator and subcultured once in a week.

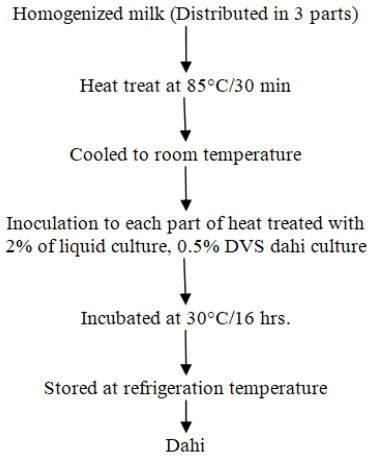
Isolation of Lactic acid bacteria. Domestic and market samples of curd were collected in a sterile sample bottle. Sample of 11g were weighed separately under aseptic condition on to the sterile aluminum foil and transferred to 99ml of diluent bottle and mixed by rotating by placing on working bench to get 10^{-1} dilution and using this, required dilutions were prepared. Appropriate dilutions were transferred to four sets of Petri plates. To the first set of Petri plate sterile, molten Neutral Red Chalk Lactose Agar (NRCLA) maintained at 50°C was added, to the second set of Petri plates, Yeast Glucose Agar (YGA) was added, to the third set of Petri plate, Sucrose Agar (SA) was added, to the fourth set of Petri plate, Rogosa Agar (RA) was added, mixed thoroughly and allowed to solidify. First dilution was lab pasteurized (63°C/30min), cooled to room temperature and required dilutions were prepared using sterile pipettes, 1ml quantity of required dilutions were transferred to label Petri plates and sterile molten YGA maintained at 50°C was poured, mixed thoroughly and incubated at 37°C. Neutral Red Chalk Lactose Agar, Sucrose Agar plates were incubated in anaerobic jar at 30°C for 24 - 48hours. Yeast Glucose Agar, Rogosa Agar plates were incubated in anaerobic jar at 37°C for 24 - 48hours. After incubation pink coloured colonies in NRCLA plates, all the colonies in YGA plates and glistening colonies on Sucrose Agar, all the colonies on Rogosa Agar were counted. The counts were expressed as colony forming units per gram (cfu/g) by multiplying average count with dilution factor and then converted into $\log_{10}$ cfu/g of sample. The colonies were selected based on Harrison's disc. The isolates were purified by streaking on YGA for 3 times and purified isolates were maintained in Yeast glucose broth and stabs containing 0.75% of agar (Harrigan, 1998).

Growth study of Direct Vat Set Culture. Combination of isolates were inoculated at 1% into sterile black gram dhal medium (solid substrate medium) and incubated at 30°C. At every 6 hrs of incubation, DMC was determined up to 48 hrs. Based on the results of DMC, the period that showed a good biomass was selected for further study.

Production of DVS culture by SSF technique. Sterile black gram dhal medium was inoculated with liquid dahi culture, mixed thoroughly and incubated at 30°C

for a period that yielded a good biomass determined by DMC. After optimum incubation period, fermented dhal was spread on a sterile Petri plate aseptically, dried in BOD incubator at 20°C for 24 hrs and packed in polythene pouches and stored in refrigerator and ambient temperature (24°C)

Preparation of Dahi. The whole milk of Nandini homogenized milk was taken from the market, heat treated to 85°C for 30 min and cooled and divided into three parts inoculated with 2% of liquid culture and 0.5% of refrigerated DVS culture and room temperature stored DVS culture respectively. The inoculated milk was transferred to 50 ml sterile polypropylene cups and incubated at 30°C/18-20 hrs.



RESULTS AND DISCUSSION

Growth study of mixed Dahi Culture on sterile black gram dhal medium: *Leuconostoc* sp. Leu6: *Lactococcus lactis* ssp *lactis* Lc1: *Lactobacillus fermentum* Lb8: *Streptococcus thermophilus* St3were grown on sterile black gram dhal medium and incubated at 30°C for 48 hrs. The samples were drawn at every 6 hrs up to 48 hrs and DMC was determined.

DMC of mixed Dahi culture ranged from 6.02 to 9.42 log_{10}/g (Table 1). It is indicated that significant difference existed on incubation period on mixed Dahi culture at 30°C .

Sensory attributes, acidity and DMC of Dahi prepared from SSF Dahi culture or Direct Vat Set (DVS) Dahi culture: Dahi was prepared using 2% of liquid culture (C), 0.5% of refrigerated DVS dahi culture and ambient DVS dahi culture and after inoculation of culture, incubated at 30°C (Table 2) and served to panel of judges. Judges gave maximum score to dahi prepared from the refrigerated DVS dahi culture compared to ambient SSF culture. The stastical analysis indicated significant difference between liquid culture, refrigerated DVS dahi culture and ambient DVS culture Set I containing *Leuconostoc* sp. Leu6: *Lactococcus lactis* ssp *lactis* Lc1: *Lactobacillus fermentum* Lb8: *Streptococcus thermophilus* St3 in the ratio of 0.15 % : 0.3% : 0.5 % : 1% was inoculated into sterile skim milk and at every 6 hrs of interval at 30°C samples were drawn and subjected to DMC and acidity. Maximum of 8.68 was obtained at 24 hrs of incubation with acidity of 0.65% and later the DMC as well as acidity started decreasing, incubation period of 24 hrs was ideal for performance of dahi cultures.

Mixed Dahi culture was grown on black gram dhal medium as solid substrate at 30°C. For every 6 hrs of interval the sample was taken and subjected to DMC, at 24 hrs of incubation Dahi culture showed highest log count of 9.42.

Wong and Yalet (1999) found increase in viable count of *L.lactis* ssp. *lactis*, *Streptococcus thermophilus*, *Lactobacillus acidophilus* and *Lactobacillus bulgaricus* in nonfat milk medium to about 10^8 cfu/ml at 24 hrs of incubation.

Table 1: Growth study of Mixed Dahi Culture on sterile black gram dhal medium at 30°C.

Time (hrs)	DMC (log_{10}cfu/g)
0	6.02
6	7.25
12	8.44
18	8.80
24	**9.42**
30	8.51
36	8.01
42	7.87
48	7.35
CD ≥ 0.05	**0.1**

Note: Solid substrate consists of Black gram dhal, skim milk powder (1%), tomato juice (10%) and moisture (70%). — Dahi culture in solid substrate medium was incubated at 30°C for up to 48 hrs.

Table 2: Sensory attributes, acidity and DMC of Dahi prepared using SSF Dahi culture or Direct Vat Set (DVS) Dahi culture.

Dahi cultures	Sensory score out of 9 points	Acidity (%LA)	DMC (log_{10}cells/ml)
C	7	0.61	8.20
CR	**8**	**0.67**	**8.51**
CRT	6	0.58	8.25
CD ≥ 0.05	**0.8**	-	-

Note: C: Liquid culture, CR: Refrigerated DVS dahi culture, CRT: ambient DVS dahi Culture incubated at 30°C for 18 hrs.

CONCLUSIONS

Solid state fermented (SSF) Dahi culture or DVS Dahi culture was prepared by inoculating liquid culture of 2% level on to black gram dhal medium as solid substrate and incubated at 30°C for 24 hrs as it yielded higher DMC, dried at 20°C in BOD incubator for 18 hrs. The total lactic count of dried DVS culture was 9.75 log10/g.

FUTURE SCOPE

Dahi was prepared by using 0.5% of DVS Dahi culture which was stored at refrigeration and ambient temperature and 2% of liquid Dahi culture. The study revealed that dahi prepared by using refrigerated DVS Dahi culture was best accepted by the panel of judges.

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Conflict of Interest. None.

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