International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2018; 6(1): 1123-1125

Received: 21-11-2017
Accepted: 22-12-2017

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Growth study of solid state fermented lactic cultures

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Abstract
Various dhals such as raw Bengal gram dhal, roasted Bengal gram dhal, black gram dhal, green gram dhal, hyacinth dhal (Avarae bele), masoor dhal, red gram dhal, and soya bean dhals for growing lactic cultures, after screening for bacterial spores. Among dhals, screened black gram dhal showed lowest spore count of 1.52 $\log_{10}$ cfu/g. Hot air oven exposure of black gram dhal at 100^0C for 1 h and sterilization at 121^0C for 30 min completely destroyed the spores. Maximum viable counts of dahi 8.41 $\log_{10}$ cfu/g while yoghurt 8.98 $\log_{10}$ cfu/g and acidophilus cultures 9.47 $\log_{10}$ cfu/g were obtained when black gram dhal containing 1:0.8 of moisture was supplemented with 1% level of skim milk powder, ash gourd, carrot and tomato juices as they provided growth promoting agents. In order to find the optimum growth period for good biomass of lactic culture, 1% supplemented black gram dhal was inoculated with 1% dahi, yoghurt and acidophilus cultures respectively and incubated at optimum temperature of 30^0C in case of dahi culture, 37^0C for yoghurt and acidophilus. The viable counts obtained at every 6 h up to 48 h, revealed that, at 24 h of incubation the counts were maximum such as 8.51, 9.03 and 9.57 $\log_{10}$ cfu/g for dahi, yoghurt and acidophilus cultures, respectively.

Keywords: Black gram dhal, Solid state fermentation, Supplements, Lactic cultures

1. Introduction
Food is perishable commodity due to intrinsic factors such as nutrients, pH, moisture and others which make it feasible for the growth of microorganism. In order to increase the keeping quality, heat treatment is the most popular method followed by fermentation.
Fermentation is the process of transformation of simple raw materials into a range of value added products by utilizing, the phenomenon of growth and activities of microorganisms on various substrates. It is a natural way to preserve food and the fermented products are known to promote the consumers health. Lactic acid bacteria used as starter cultures in fermented milk products convert lactose in milk to lactic acid thus lowering pH which inhibits growth of spoilage bacteria and increases shelf life.
Fermentation may be classified based on the substrate such as submerged fermentation (SmF) and Solid state fermentation (SSF). SSF is the fermentation process taking place in the absence of free flowing water where solid material is substrate. Substrates used in SSF may be inert and nutritive ones. Solid state fermentation technique has been widely used in preparation of fermented foods, enzymes, organic acids, polysaccharides, biomass of lactic acid bacteria.
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. 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) [3].
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 moisture. 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 (aw) 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.

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) [4].

Materials and methods

Lactic acid bacterial cultures

The dahi culture consisting of *Lactococcus lactis* ssp. *lactis* and *Lactococcus lactis* ssp. *lactis* bv. *diacetylactis,* yoghurt culture that included *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Lactobacillus acidophilus* as probiotic culture, which had been maintained in sterile Yeast glucose chalk litmus milk (YGCLM) in the department of Dairy Microbiology, Dairy science college, KVAFSU, Hebbal, Bengaluru-24 were used in this study.

Collection and Screening of various dhals for aerobic spore counts

Dhals of 8 various types, commonly available in local market of Bengaluru such as raw bengal gram dhal, roasted bengal gram dhal, black gram dhal, green gram dhal, hyacinth dhal (avarae bele), masoor dhal, red gram dhal, soya bean dhal were purchased, cleaned to remove stones and unwanted plant materials and stored in a self-sealing polythene pouches.. To determine the extent of spores present in dhals, they were subjected to aerobic spore counts by plating method as per Harrigan (1998) [2].

Various sporicidal treatments to black gram dhal to use as solid substrate

Various treatments like dry heat treatment such as dry frying (5 min), exposure to microwave (1 min) and exposure to 100^0C for 1 h in hot air oven and wet heat treatments like hydration of dhal for 30 min, 12 h and 24 h, 0.01% and 0.05% treatment with hydrogen peroxide and tyndallisation (steaming for 3 successive days) were given to dhal to reduce aerobic spore count and after treatment analysed the treated dhal for aerobic spore count as mentioned in Harrigan (1998) [2].

Supplementation for treated black gram dhal for the growth of lactic cultures

Best sporicidal treatment to completely destroy aerobic spore was selected and then supplemented with skim milk powder, ash guard juice, carrot juice and tomato juice.

Preparation of ash guard juice, carrot juice and tomato juice

Ash guard, carrot and tomato were obtained freshly from local market. The edible portions were obtained, washed with potable water, grated, steamed for 15 min and mashed in clean, dry, mixer. The obtained puree was filtered through muslin cloth. After filtration the juices of ash guard, carrot and tomato were collected separately in a sterile conical flask.

Final supplementation to black gram dhal

The aerobic spore free black gram dhal was supplemented with each of SMP, ash guard juice, carrot juice and tomato juice at 0.5, 1, 1.5 and 2% level. The moisture maintained was 1:0.8 level, including volume of water in juices.

Growth study of SSF cultures on supplemented black gram dhal

The maximum growth period required for good biomass of lactic culture on supplemented black gram dhal was determined at optimum growth temperature for 48 h. At every 6 h interval, aseptically drawn samples of SSF dahi, yoghurt and acidophilus cultures were subjected for viability determination.

Determining the viability of lactic culture grown on supplemented black gram dhal

SSF cultures of dahi, yoghurt and acidophilus drawn at different growth periods were aseptically transferred to sterile pestle and mortar, triturated with sterile 99 ml of phosphate buffer separately, required dilutions were prepared and plated using yeast glucose agar for total lactic count. The plates were incubated at 300C for dahi culture and while in case of yoghurt and acidophilus cultures at 370C. The viable lactic counts were expressed as log10 cfu/g.

Statistical Analysis

The data was analyzed using R software [R. version 3.1.3 (2015-3-09), copyright © 2015, R foundation] for statistical computing both one way and two way Completely Randomed Design (CRD) which is the most appropriate for the study. Data on the response variables were collected for three replications for each of these treatments. ANOVA tables were prepared to analyse the data and where the F value was significant the critical difference was calculated and used to identify where significant differences existed and was indicated in the table use superscripts. The formula for the critical difference (CD) is

$$CD = \frac{\sqrt{2 \times MSS\ (E)}}{R}\ t\alpha$$

Where, MSS (E) = Mean Sum of squares of the error

r = number of replications

tα = table t value of the α level of significance

Results and discussion

Growth study of lactic cultures on supplemented black gram dhal

In order to find the optimum growth period for good biomass of lactic culture, 1% supplemented black gram dhal was inoculated with dahi, yoghurt and acidophilus cultures and incubated at their optimum temperature for 48 h. The viable counts obtained at every 6 h up to 48 h, revealed that, at 24 h of incubation the counts were maximum such as 8.51, 9.03 and 9.57 $\log_{10}$ cfu/g for dahi, yoghurt and acidophilus cultures, respectively (Table 1 and Fig 1). Later the lactic counts started decreasing on solid substrate. Acidophilus showed higher counts followed by yoghurt and dahi cultures at 24 h of incubation. Hence 24 hours of incubation at optimum temperature of lactic cultures was considered as optimum period for good biomass production, however further incubation reduced the growth due to the accumulation of lactic acid. The viable counts of dahi, yoghurt and acidophilus on supplemented black gram dhal showed significant difference at 24 h of incubation at $p \leq 0.05$.

In agreement with the present study, Prabha (1999) [5] reported that black gram dhal supplemented with 1% SMP with moisture of 1:0.8, when inoculated with *Bifidobacterium longum* PF1 and incubated at 37 ^{0}C, maximum viable count, was observed at 24 h of incubation and later the counts decreased when studied up to 48 h.

Ramachandra *et al*. (2008) [6] reported that 48 h of incubation at 37 ^{0}C was ideal for *Lactobacillus acidophilus* 111 on paddy husk supplemented with peptone (1%), yeast extract (1%), skim milk powder (1%), $MnSO_4$ (0.05%), black gram dhal (1%), tween 40 (1%) with count of 10 $\log_{10}$ cfu/g.

Deepa (2011) [1] grew dahi culture on black gram dhal containing 70% supplemented with 1% skim milk powder and 10% tomato juice at 30 ^{0}C for 24 h that showed maximum count of 9.5 $\log_{10}$ cfu/g when studied up to 48 h of incubation.

Table 1: Growth study of lactic cultures on supplemented black gram dhal

Incubation period (h)	Dahi culture	Yoghurt culture	*Lactobacillus acidophilus*
	Viable counts ($\log_{10}$ cfu/g)		
0	6.26^a	6.28^a	7.80^a
6	6.83^a	6.59^a	8.45^a
12	7.61^a	6.84^a	8.62^a
18	7.96^b	7.85^a	8.71^a
24	8.51^b	9.03^b	9.57^b
30	8.29^b	8.79^b	9.34^a
36	7.60^a	8.33^b	8.54^a
42	6.43^a	7.15^a	7.16^a
48	6.22^a	6.30^a	6.50^a
CD ($p \leq 0.05$)	1.50	1.80	1.60

Note

- The results were average of three trials.
- Same superscript indicate non-significance while different, indicate statistically significant difference at $p \leq 0.05$.
- Sterile black gram dhal with moisture of 1:0.8 supplemented with 1% each of SMP, ash guard juice, carrot juice and tomato juice was used for the study.
- Incubation of dahi culture was at 30 ^{0}C up to 48 h.
- Incubation of yoghurt and acidophilus culture was at 37 ^{0}C up to 48 h.

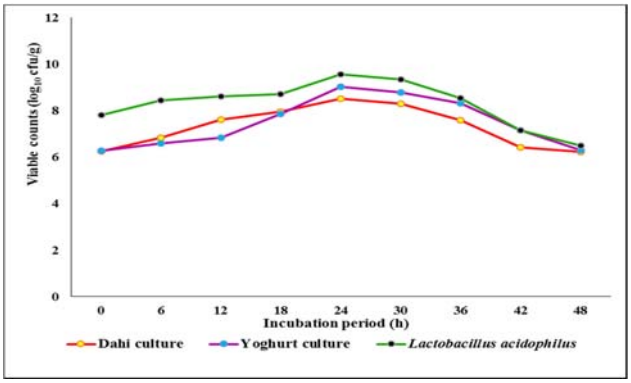

Fig 1: Growth study of lactic cultures on supplemented black gram dhal

Conclusion

The growth of SSF cultures were determined at 6 hourly interval up to 48 h. Dahi, yoghurt and acidophilus culture were inoculated at 1% level into sterile black gram dhal supplemented with 1% of each of skim milk powder, ash guard juice, carrot juice and tomato juice and then sample mixed properly and incubated at optimum temperature. Acidophilus showed higher counts followed by yoghurt and dahi cultures at 24 h of incubation. Hence 24 hours of incubation at optimum temperature of lactic cultures was considered as optimum period for good biomass production.

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