The Pharma Innovation Journal 2019; 8(9): 174-175

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2019; 8(9): 174-175

www.thepharmajournal.com
Received: 13-07-2019
Accepted: 15-08-2019

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Effective utilisation of whey for maintenance of lactic acid bacteria

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Abstract
Skim milk and commercial broth like MRS or M17 is commonly used in the starter culture industry for the propagation of starters, which makes them very costly. So the utility of whey for maintenance of starters was evaluated by estimating the growth rate of lactococci starters like *Lactococcus lactis* 676, *Lactococcus lactis* 621, *Streptococcus thermophilus* 199 and lactobacilli starters like *Lactobacillus bulgaricus* 253 and *Lactobacillus rhamnosus* 296. Seven types of whey media *viz*., whey (W), whey supplemented with 0.3, 0.5 and 1 per cent yeast extract (YE) or peptone (P) was prepared and the growth rate of starter cultures after 18 hours of incubation at optimum temperatures in these media was compared to that obtained in the control medium, skim milk. The results of statistical analysis showed that the whey supplemented with one percent yeast extract (1WYE) supports the growth rate of starter cultures to a significantly higher level when compared to all other treatment whey media and skim milk medium. The findings of the study revealed the successful use of whey based media for maintenance of lactic cultures at lower cost.

Keywords: Starter cultures, whey, yeast extract, peptone, growth rate

Introduction
Whey is a byproduct obtained from cheese and paneer industry as a result of coagulation of milk using rennet or citric acid. Majority of water soluble nutrients of milk like lactose, whey proteins, water soluble vitamins and minerals is present in whey. The global cheese whey production in 2008 is about 186 million tonnes per annum (Affertsholt, 2009) [1]. The European Union and United States produce 70 percent of whey in the world. Only about 50 percent of the total whey produced is processed (Baldasso *et al*., 2011) [2]. Minor portion of whey was used as feed additive but major part was considered as a waste. In developing countries the utilization of whey is the major problem, mainly due to improper marketing and utilization, less capital and poor infrastructure. Discarding of whey leads to loss of significant nutrients and energy as well a potent pollutant to the environment (Macwan *et al.*, 2016) [3]. These nutrients can be utilized effectively for the growth of starter cultures. Starter cultures are mainly lactic acid bacteria (LAB) cultures which act as the heart of fermented dairy products. Starter culture production is mainly in the hands of multinational companies and it is very costly due to the use of expensive media for fermentation, which makes it less affordable by the small scale entrepreneurs. About 30 per cent cost of microbial fermentation arises from the use of costly media for fermentation (Rodrigues *et al*., 2006) [4]. The objective of this study was to analyse the utility of whey for the maintenance of starter cultures which will lead to the cheaper production of starter cultures.

Materials and methods
Procurement of starter cultures
Five pure LAB cultures like *Streptococcus thermophilus* 199, *Lactobacillus bulgaricus* 253, *Lactobscillus rhamnosus* 296, Lactococcus lactis 676 and *Lactococcus lactis* ssp diacetylactis 621 were procured from National Collection of Dairy Cultures (NCDC), Karnal. These freeze dried cultures were further propagated in commercial broths like MRS (for lactobacilli) or M17 (for lactococci) broth and stored at 4 °C.

Preparation of wheybased media
Fresh skim milk was collected from University dairy plant, KVASU, Mannuthy. For coagulating the skim milk, rennet (0.003%) was added and incubated at 31 °C for 40 minutes.

The coagulum was cut and cooked, followed by the collection of whey.The collected fresh whey was divided into seven equal fractions and different types of whey based media were prepared by incorporating commonly available exogenous nitrogen sources like yeast extract or peptone at varying concentrations *viz*., Whey + 0.3% yeast extract (0.3 WYE), Whey + 0.5% yeast extract (0.5 WYE), Whey + 1% yeast extract (1 WYE), Whey + 0.3% peptone (0.3 WP), Whey + 0.5% peptone (0.5 WP), Whey + 1% peptone (1 WP), Whey alone (W). These media were autoclaved at 121 °C for 20 min and cooled. The clear fraction of autoclaved whey was taken for further study.

Estimation of growth rate of starters

Starter cultures were inoculated into the autoclaved skim milk (control media) and whey based media at 2 per cent level. These were further incubated for 18 hours at optimum temperatures for each culture and the growth rate of cultures was estimated as cell count by microbial plate count method (Wehr and Frank, 2004) [5] at suitable dilutions in MRS or M17 agar and expressed in log cfu/ml.

Result and Discussion

The growth rate of the starter cultures after 18 hours of incubation was estimated as cell count through plate count method and the results after statistical analysis is given in the table below. It is evident from the table that, when whey alone was used for the growth of organisms, growth rate was unsatisfactory. Whey supplemented with exogenous peptides like yeast extract or peptone gave a significantly higher growth rate when compared to unsupplemented whey medium. When compared to all other treatment media and skim milk control medium a significantly higher growth rate was obtained in whey incorporated with one per cent yeast extract (1WYE) for all the cultures under study. Highest growth was shown by *Lactococcus lactis* 676 in 1 WYE ie., 16.39 ± 0.009 log cfu/ml. A similar finding was obtained by Hsu *et al.* (2005) [6] that the production of β- galactosidase was highest, which indicates higher growth rate of cultures, while using yeast extract as the nitrogen source when compared to other growth promoters like casein and peptone.

For majority of the cultures used for the study, a higher growth rate was obtained in 0.5 WYE also when compared to the skim milk. The easily available nutrients in whey medium incorporated with yeast extract may be the reason for this increased growth. Even though milk is a rich growth medium; the low concentration of free amino acids and peptides in milk makes it inefficient to support the growth of LAB (Shihata and Shah, 2000) [7].

Cell count of starter cultures in log cfu/ml

Type of media	*S. thermophiles* 199	*L. bulgaricus* 253	*L. lactis* 676	*L. diacetylactis* 621	*L. rhamnosus* 296
Skim milk	9.62 ± 0.026^{b}	7.86 ± 0.007^{d}	15.83 ± 0.042^{d}	15.81 ± 0.037^{c}	7.52 ± 0.011^{f}
W	7.29 ± 0.022^{h}	6.61 ± 0.021^{g}	8.56 ± 0.019^{g}	8.67 ± 0.015^{g}	6.77 ± 0.010^{g}
0.3 WYE	8.47 ± 0.037^{d}	7.46 ± 0.006^{f}	15.95 ± 0.012^{bc}	15.87 ± 0.011^{b}	8.13 ± 0.008^{de}
0.5 WYE	8.56 ± 0.019^{c}	7.97 ± 0.008^{b}	15.97 ± 0.011^{b}	15.88 ± 0.005^{b}	8.23 ± 0.002^{b}
1 WYE	9.72 ± 0.008^{a}	8.05 ± 0.006^{a}	16.36 ± 0.009^{a}	16.10 ± 0.006^{a}	8.28 ± 0.006^{a}
0.3 WP	7.52 ± 0.011^{g}	7.43 ± 0.016^{f}	15.28 ± 0.005^{f}	15.11 ± 0.009^{f}	8.12 ± 0.001^{e}
0.5 WP	7.67 ± 0.007^{f}	7.67 ± 0.008^{e}	15.29 ± 0.005^{f}	15.25 ± 0.004^{e}	8.14 ± 0.006^{d}
1 WP	7.87 ± 0.002^{e}	7.90 ± 0.013^{c}	15.40 ± 0.010^{e}	15.32 ± 0.007^{d}	8.18 ± 0.004^{c}

Each value is a mean of six observations with SE

Means with different superscript in same column differ significantly ($p \leq 0.001$)

Conclusion

Among seven types of whey based media analyzed, whey incorporated with one per cent yeast extract (1 WYE) was selected as the optimized whey medium, based on the growth rate of starter cultures. Yeast extract concentration in whey lowered to 0.5 percent also gives acceptable growth rate when compared to the control skim milk. Thus the whey, which was considered as a waste, can be effectively utilized for the growth of lactic acid bacteria starter cultures, which can lower its cost of production and will become more affordable for small scale entrepreneurs in fermented dairy product sector.

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