AN INITIAL ATTEMPT TO DEVELOP A FAST SCREENING METHOD FOR LACTIC ACID BACTERIA CAPABLE OF DEGRADING PHYTIC ACID

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Introduction

Phytic acid (PA) which is known to reduce the absorption of essential nutrients from food, could be degraded by fermentation (Mahgoub and Elhag, 1998). Certain strains of lactic acid bacteria (LAB) are known to be more efficient in degrading PA (Shirai *et al.*, 1994). It will be useful to isolate such strains for future applications. A spectroscopic method is available for extraction and quantification of PA analysis (Mohamed *et al.*, 1986). In a previous study, this method was scaled down 10 fold (Karunaratne *et al.*, 2008). The present study was an initial attempt to develop a faster screening method in a liquid medium (eliminating the extraction step), for LAB capable of degrading phytic acid using the scaled down method.

Materials and Methods

Microorganisms were isolated from buffalo curd (Dept. of Animal Science, University of Peradeniya) and a commercially available yogurt. Streak plates (three per food source) were made on HYA agar (Anderson and McCann, 2008) and Nutrient agar (NA). The plates were incubated (at 37 °C for 72 h) and colonies were streaked onto agar slants of HYA in duplicate. While one slant was stored at 5 °C the other was used as a working culture. Colony characters were observed and both simple and Gram staining was carried out from smears made (at least three per plate) before observing under oil emersion (x 1000) using a light microscope.

For inoculating the isolated bacteria broth culture (100 ml) were made separately with or without PA. For this, a stock solution of PA was made using PA powder (Sigma chemical company, USA). The above broth cultures contained 75 ml HYA and 25 ml PA, or 75 ml HYA and 25 ml distilled water. The extraction step in the original method (Mohamed *et al.*, 1986) was omitted. Therefore 200 µl of broth culture, 300 µl of $FeCl_3$, 300 µl of TCA were mixed and heated in a boiling water bath (45 min) to obtain ferric phytate, which was subjected to a chromogenic reaction followed by measuring absorbance at 830 nm.

Results

While the colonies appeared within 24 h on HYA agar, growth was observed on both HYA agar and NA after 72 h. The colonies (about 0.5 - 1 mm in diameter) were white, spherical, gelatinous with entire margins.

There were comparatively higher number of cocci and a fewer number of bacilli per field of view and all were Gram positive bacteria. A higher but insignificant reduction (P=0.112)

of PA was observed in the broth culture inoculated with microorganisms from the commercial yogurt (Table 1). However a 21 % loss of PA during extraction was observed in the controls. Additionally it was noted that the growth of bacteria was inhibited in the presence of added PA.

Table 1. The initial and final (after 24 h incubation at 37 ^{0}C) concentrations of PA (in mol L^{-1}) in the broth cultures, of two different sources of bacterial colonies and the percentage reduction of PA

Source of the bacteria	Initial PA	Final PA	Percentage reduction
Curd	$2.1x10^{-5}$±0.1	$1.1x10^{-5}$±0.1	57±28 %
Yogurt (commercial)	$2.8x10^{-5}$±0.5	$4.1x10^{-6}$±1.2	85±7 %

There was no significant difference between percentage reduction at P=0.05
n=2 to 5

Discussion and Conclusion

All selected microorganisms showed better growth on HYA agar (a specialized medium for LAB) than NA. In yogurt, *Lactobacillus bulgaricus* (a bacillus) and *Streptococcus thermophilus* (coccus) are known to be present (Anderson and McCann, 2008). The bacteria were mainly cocci indicating its presence in higher numbers. All isolated organisms were Gram positive and both *S. thermophilus* and *L. bulgaricus* are Gram positive bacteria. Curd is known to contain several LAB (Dassanayake *et al.*, 1995). The reduction of PA after fermentation by LAB (by probable microbial phytase activity) from commercial yogurt and curd were 64 % and 36 % respectively (after correcting for the loss of 21 %).

This new approach to screen microorganisms capable of degrading PA eliminates sample preparation and extraction procedures required for PA analysis. However, the growth of microorganisms was partially inhibited in the presence of PA added to the medium and there was a 21 % loss of PA during extraction. In spite of these draw backs whether this method is suitable for screening, needs to be evaluated by comparing these results with a natural food system which will involve sample preparation and extraction.

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