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MICROBIAL POPULATION LOAD AND ENZYME PRODUCTION OF INDIGENOUSLY ISOLATED YEAST

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Abstract

Traditionally, yoghurt is in use for centuries because of its beneficial effects on human health. People and health practitioners have long been considering it as prophylactic and therapeutic agent for many gastrointestinal ailments primarily because of its microbial flora. Hence, the health benefits warrant the need to study microbial flora/natural contaminants of indigenously prepared yoghurt. In the present study, 75 yoghurt samples were collected from the retail outlets from all 18 towns of Karachi. Total yeast counts in terms of colony forming units (CFU) per gram were determined using selective media. Yeast load was also compared with bacterial load, particularly coliforms and enterococci in the samples under study. Several isolated yeast strains were screened for enzyme production that in future can be exploited in various industrial/health applications for instance amylase, beta-galactosidase, protease and lipase. Observations thus obtained were subjected to rigorous statistical analysis. The total yeast population in yoghurt samples was in between 45-2.5 x 10^7 CFU having population mean of 5 x 10^6 CFU compared to total bacterial counts (1.3 x 10^4 -7 x 10^7 CFU) with an average of 1.0 x 10^7 CFU. The estimated coefficients of variance (CV) exhibited by total bacterial and yeast counts were 160% and 45% respectively. Enzymatic screening results showed that 32% of yeasts were protease producer followed by lipase (8%) and β-galactosidase (7%). Interestingly no amylase activity was detected in yeast isolates. Laconically, data thus obtained showed more prevalence of bacteria in yoghurt compared to yeast. Beside useful microorganisms like *Lactobacillus* sp., yeast etc; some pathogenic organisms were also detected during sampling, which indicates malpractice in the preparation of yoghurt. Implication of enzymatic profile studies of the isolated yeast strains suggests that yoghurt yeast could be exploited as a source of industrially and therapeutically important metabolites

Introduction

In many modern societies, yoghurt constitutes a substantial proportion of total daily food consumption primarily because of its long history of proven health benefits and taste. Studies encompassing scientific parameters of yoghurt have proved it as the best source of natural probiotics (Guarner & Schaafsma, 1998). Yoghurt is generally defined as coagulated milk that results from the fermentation of lactic acid in milk by *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (Adolfsson *et al.*, 2004). In Pakistan, yoghurt production chiefly exploits traditional technologies, using yoghurt itself as a starter culture. However, industrial production of yoghurt is a controlled fermentation process both with reference to microbiological and physical conditions (White, 1995). Despite such arduous fencing, contamination owing to yeast is still one of the major limiting factors for shelf life and commercial value of yoghurt (Canganella *et al.*, 1998). On the other hand yeast are applied as starter cultures in cheese, bread, wine,

beer and other alcoholic fermentation products (Lowes *et al.*, 2000). Conversely, role of yeast in yoghurt spoilage has been well documented (Cappa & Cocconcelli, 2001). Since yeasts are considered as natural contaminant of yoghurt there are discrepancies in acceptable threshold of yeast load in yoghurt. For instance under good manufacturing practices (GMP), the final product should contain not more than one yeast CFU/g at the time of production (Suriyarachchi & Fleet, 1981) in contrast to this, other studies extended this limit to less than or equal to 50 CFU (Li & Li , 1998). Different surveys of retail marketed yoghurt revealed that samples could exhibit counts more than 10^5 CFU (Rohm *et al*., 1990; AL-Tahiri, 2005). The presence and predominance of certain yeast species in yoghurt is associated with their chemico-physical properties including their ability to ferment variety of sugars, and to produce several enzymes that hydrolyze milk sugars, fats and proteins (Fleet, 1992).

The mentioned enzymes have multi dimensional implications in pharmaceutical industries. This verity could be well exemplified as β-galactosidase produced by lactose fermenting yeast species of *Kluyveromyces* group is indeed being utilized for the treatment of lactose intolerance (Gekas & Lopez-Leiv, 1985). Furthermore, yeast isolates from yoghurt showed bactericidal activity against *Helicobacter pylori* suggesting its potential application to be used as probiotics (Oh *et al*., 2002). Briefly, yoghurt is considered as a potential source of different metabolites of biotechnological interest. Hence numerous studies have been undertaken to isolate and screen potentially important microbes in particular yeasts from yoghurt (Bialasiewicz *et al.*, 2001). This study is undertaken to determine the range of yeast population in yoghurt sold in different localities of Karachi city and their potential for the production of enzymes.

Materials and Methods

Sample collection: Seventy five yoghurt samples were collected in sterilized glass containers from retail outlets situated in different localities of Karachi mega city. All samples were stored in an icebox till further processing.

Sample processing: After uniform mixing each yoghurt sample was serially diluted by margin of 10 fold in 0.1% of sterile peptone solution. Each dilution (100µl) was plated over acidified YEPG (Yeast extract peptone glucose; pH 3.5) agar, plate count agar (Moreira *et al.*, 2001) and bile esculin media, Merck (Hussain *et al.*, 2007). YEPG Plates were then incubated at 28ºC upto 7 days, while plate count agar and bile esculin agar plates were incubated at 37ºC for 24 to 36 hours.

Enzyme based screening: Chromogenic substrate X-Gal (0.01%), casein (1%), Tween 20 & 80 (1%) and soluble starch (1%) were incorporated in YEPG media to screen β-galactosidase, protease, lipase and amylase production respectively.

Load determination: Non-filamentous large colonies on YEPG agar were considered as of yeast while black colonies on bile esculin agar belong to enterococci (Hussain *et al.*, 2007).

Table 1. Viability range, average & coefficients of variance of total colony count (CFU) and yeast counts (CFU) of yoghurt sold by retailers all over Karachi.

Microbial load	Range (CFU)	Average (CFU)	Coefficient of variance (%)
Total counts	1.3×10^4 - 7×10^7	1.0×10^7	160
Yeast counts	45-2.5×10^7	1.5×10^6	45

Table2. Total bacterial and yeast population in yoghurt samples (n = 75).

	Samples in range	
Count range (CFU)	Total counts	Total yeast counts
$1 < 10^2$	Not observed	2%
$10^2 < 10^3$	Not observed	3%
$10^3 < 10^4$	Not observed	5%
$10^4 < 10^5$	2%	15%
$10^5 < 10^6$	30%	40%
$10^6 < 10^7$	32%	31%
$10^7 < 10^8$	36%	4%

Enzyme screening: Colonies showing blue colour production on plates containing X-Gal were considered as positive for β-galactosidase production however, precipitates around colonies on casein and Tween 20 & 80 containing plates were indication of protease and lipase production. Appearance of colorless zones around colonies on starch containing plates after pouring iodine is indicative of amylase production.

Statistical analysis: All data were subjected to "Mini Tab" program for statistical analysis.

Results and Discussion

Total mesophilic aerobic bacterial and yeast counts of 75 yoghurt samples from 18 towns of Karachi revealed that majority of samples showed significantly higher bacterial and yeast counts (P = 0.05). The average bacterial counts in the yoghurt samples collected from Baldia, Landhi, Liaqutabad, Kaemari, Orangi, New Karachi, Layari, Saddar and Shah Faisal towns were in excess of 10^7 CFU. However, yoghurt samples from Bin Qasim, North Nazimabad and Gulshan-e-Iqbal towns exhibited bacterial counts closer to 10^5 CFU. The rest of 6 towns showed average bacterial counts of approximately 10^6 CFU. The average bacterial load in yoghurt samples from all over the towns ranges from 1.3×10^4 CFU to 7×10^7 CFU having average counts of 1.0×10^7 CFU (Table 1). In case of yeast the average counts in yoghurt samples from 18 towns were in the range of 10^5 CFU to 10^6 CFU (Fig. 1.). The mean population of yeast varies from 4.5×10^1 CFU to 2.5×10^7 CFU having an average of 1.5×10^6 CFU (Table 1). The averages of bacterial and yeast counts were significantly lower in contrast to the ranges reported by Zekai & Erdoğan (2003) but are comparable to the counts observed by Viljoen *et al*., (2003) and Rohm (1990). In contrast, AL-Tahiri (2005) observed 10^5 yeast CFU in locally marketed retail yoghurt which is in agreement with our observations.

Statistical analysis of the microbial counts revealed that 36%, 32%, 30%, and 3% of the samples possessed bacterial counts in excess of 10^7, 10^6, 10^5 and 10^4 CFU respectively (Table 2). The total yeast count in 40%, 31%, and 4% of the samples were in the ranges of 10^5,10^6 and 10^7 CFU respectively. In rest of the 25% of the samples, cumulative yeast population varied from $1.0 \times 10^2 - 1.0 \times 10^4$ CFU (Table 2). However, in another study, Green & Ibe (1987) found that 60% of retail marketed yoghurt samples possessed yeast counts in excess of 10^4 CFU. The average bacterial and yeast count in this study were significantly higher ($p>0.01$) compared to the counts reported by Moreira *et al.*, (2001).

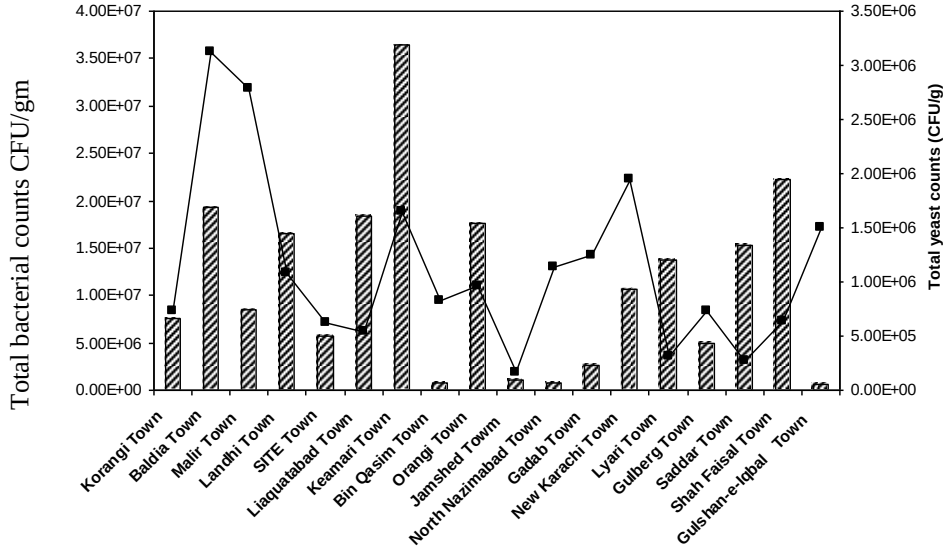

Fig. 1. Total bacterial and yeast counts of 18 towns of Karachi.
Key: squares represent bacterial load; bars represent yeast count

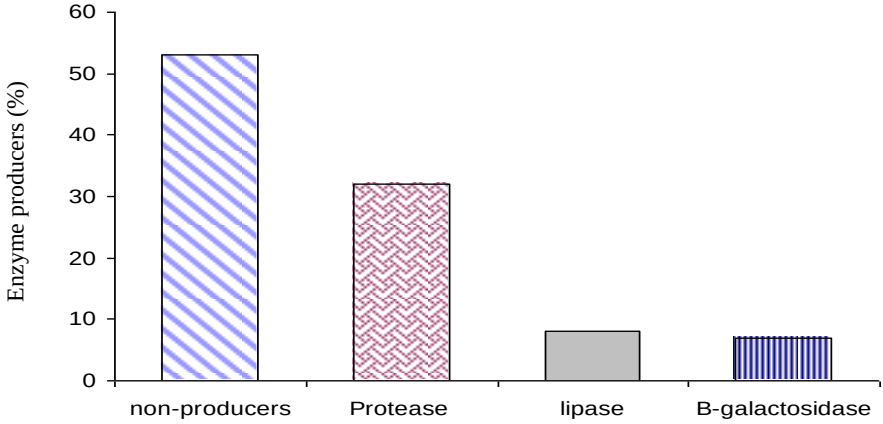

Fig. 2. Enzymatic profile of yeast isolates.

In this study yoghurt samples were randomly collected from vendor outlets. The collected samples showed a great degree of variance in terms of bacterial (CV = 160%) and yeast counts (CV= 45%) (Table 1). In Karachi, the variations in total counts are because of the use of traditional method and poor hygienic conditions that existed during manufacturing process and improper storage conditions. Similar observations were reported by Viljoen *et al*., (2003) as a result of manufacturing and storage malpractices.

Enzymatic screening of the isolated yeast strains revealed that protease enzyme producing yeasts constitute higher proportion in yoghurt, up to 32% than lipase and β-galactosidase producing yeasts which were present up to 8% and 7% respectively. However, amylase activity was not observed in any of the isolated yeast strain from yoghurt

(Fig. 2). These enzymatic activities of yeast are associated with adulteration of the yoghurt and concomitantly decreasing its shelf life (Mayoral *et al.,* 2005). The selected yeast strains isolated from yoghurt with different enzymatic potential can also be incorporated in a variety of dairy products in order to obtain their health benefits and bio-yoghurt is one of the good examples in this connection (Lourens-Hattingh & Viljoen, 2001).

Conclusion

Beside the presence of industrially important yeast strains having lipase, protease, and β-galactosidase activities the exceptionally high microbial counts in locally marketed yoghurt (from 18 towns of Karachi) suggests an improper handling of the product during and after the production. These problems are attributed to many factors such as use of contaminated starter cultures (12-24h old), poor hygienic conditions, especially sanitation of utensils used. This study can be helpful in diagnosing the problems of contaminated yoghurt which is unhealthy for the general population moreover, mass scale screening program should be undertaken to characterize the potential pathogens at the molecular level.

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References

Adolfsson, O., S.N. Meydani and R.M. Russell. 2004. Yoghurt and gut function. *Am. J. Clin. Nutr.*, 80: 245-256.

AL-Tahiri, R. 2005. A comparison on microbial conditions between traditional dairy products sold in Karak and Sam products produced by modern dairies. *Pak. J. Nutr*., 4: 345-348.

Bialasiewicz, D., M. Klosinski, K.B.P. Lekarskiej, Lodzal and Kosciuszki. 2001. Contamination of dairy products by yeasts-like fungi. *Wiad. Parazytol*., 47: 345-351.

Canganella, F., M. Ovidi, S. Paganini, A.M. Vettraino, L. Bevilacqua and L.D. Trovatelli. 1998. Survival of undesirable micro-organisms in fruit yoghurts during storage at different temperatures. *Food Microbiol*., 15: 71-77.

Cappa, F. and P.S. Cocconcelli. 2001. Identification of fungi from dairy products by means of 18s rRNA analysis. *Int. J. Food Microbiol.*, 19: 157-160.

Fleet, G. 1990. Yeasts in dairy products. *J. Appl. Bacteriol*., 68: 199-211.

Fleet, G. 1992. Spoilage yeasts. *Crit. Rev. Biotechnol*., 12: 1-44.

Fleet, G.H. and M.A. Mian. 1987. The occurrence and growth of yeasts in dairy products. *Int. J. Food Microbiol.*, 4: 145-155.

Gekas, V. and M. Lopez-Leiva. 1985. Hydrolysis of lactose: A literature review. *Process. Biochem*., 20: 2-12.

Green, M.D. and S.N. Ibe. 1987. Yeasts as primary contaminants in yoghurts produced commercially in Lagos, Nigeria. *J. Food Protect.*, 50: 193-198.

Guarner, F. and G.J. Schaafsma. 1988. Probiotics. *Int. J. Food Microbiol.*, 39: 237-238.

Hussain, M., S.A. Rasool, M.T. Khan and A. Wajid. 2007. Enterococci vs coliforms as a possible candidate of fecal contamination indicator: Base line data for Karachi. *Pak. J. Pharm. Sci*., 20(2): 107-111.

Li, F. and Y. Li. 1998. Study on the contamination level and the tolerable limit of mould and yeast in yoghurt. *Wei Sheng Yan Jiu.*, 27: 257-258.

Lourens-Hattingh, A. and B.C. Viljoen. 2001. Survival of dairy-associated yeasts in yoghurt and yoghurt-related products. *Food Microbiol.*, 19(6): 597-604.

Lowes, K.F., C.A. Shearman, J. Payne, K. Mackenzie, D.B. Archer, R.J. Merry and M.J. Gasson. 2000. Prevention of yeast spoilage in feed and food by the yeast Mycocin HMK *Appl. Environ. Microbiol*., 1066-1076.

Mayoral, M.B., R. Martin, A. Sanz, P.E. Hernandez, I. Gonzalez and T. Garcia. 2005. Detection of *Kluyveromyces marxianus* and other spoilage yeasts in yoghurt using a PCR-culture technique. *Int. J. Food Microbiol.*, 15; 105(1): 27-33.

Moreira, S.R., R.F. Schwan, E.P. de Carvalho and A.E. Wheals. 2001. Isolation and identification of yeasts and filamentous fungi from yoghurts in Brazil. *Braz J. Microbiol.*, 32: 117-122.

Oh. Y., M.S. Osato, X. Han, G. Bennett and W.K. Hong. 2002. Folk yoghurt kills *Helicobacter pylori*. *J. Appl. Microbiol.*, 93: 1083-1088.

Rohm, H., F. Lechner and M. Lehner. 1990. Microflora of Austrian natural-set yoghurt. *J. Food Protect*., 53: 478-480.

Suriyarachi, V.R. and G.H. Fleet. 1981. Occurrence and growth of yeasts in yoghurts. *Appl. Environ. Microbiol.*, 42: 574-579.

Viljoen, B.C., L.H. Analie, I. Bridget and P. Gabor. 2003. Temperature abuse initiating yeast growth in yoghurt. *Food Res. Int.*, 36: 193-197.

White, C.H. 1995. Manufacture of high quality yoghurt. *Cult. Dairy. Prod. J*., 30: 18-24.

Zekai, T. and K. Erdoğan. 2003. Physical, chemical, microbiological and sensory characteristics of some fruit-flavoured yoghurt. *YYU. Vet. Fak. Derg.*, 14: 10-14.

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