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Full Length Research Paper

Identification of *Lactobacillus* strains isolated from faecal specimens of babies and human milk colostrum by API 50 CHL system

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Lactic acid bacteria are very significant to human health due to the production of some antimicrobial substances and ability to inhibit pathogenic bacteria. Furthermore, the bacteria are also used as starter culture in various food production. In this study, *Lactobacillus* strains were isolated from 100 human milk colostrum samples and 50 faecal samples of 3 to 30-day old infants who were fed on only breast milk. The isolated *Lactobacillus* strains were futher identified by API 50 CHL systems as *Lactobacillus acidophilus* (20.0%), *L. acidophilus* -3 (10.0%), *Lactobacillus brevis* (30.0%), *Lactobacillus casei* (15.0%,) and *Lactobacillus plantarum* (25.0%) from colostrum, and *L. brevis* (41.2%), *Lactobacillus fermentum* (11.8%), *Lactobacillus reuteri* (5.9%), *Lactobacillus rhamnosus* (11.8%) and *L. plantarum* (29.4%) from faeces. In conclusion, the higher isolation rate of *Lactobacillus* spp. in infantile faecal specimens than in colostrum may indicate based on the sterility of colostrum.

Key words: *Lactobacillus*, MRS agar, API 50 CHL.

INTRODUCTION

The human intestinal tract is inhabited by different species of microorganisms; some of which are responsible for the microbial balance in the normal flora of healthy hosts, since the stability of intestinal system flora is dependent on the interactions between the beneficial and harmful microorganisms (Çakır, 2003). While these beneficial microrganisms like the lactic acid bacteria aid in the digestion of nutritional substances, they also prevent the effects of pathogenic micro-organisms due to certain inhibitory substances which they secret during digestion. These inhibitory substances include metabolites like organic acids, diacetyl, hydrogen-peroxide, acetoin, 2,3-butanediol, acetaldehyde, benzoate, bacteriolytic enzymes,bacteriocin, reuterin, etc (Chung et al., 1989; Vanderbergh, 1993; De Vuyst and Vandamme, 1994) display antagonistic activity towards many pathogenic microorganisms.

The consumption of soured milk can be traced back thousands of years but it was not until the beginning of the 18th century that the beneficial effects of fermented milks were given a scientific basis. Metcnikoff (1907) believed that the microflora of the lower gut was having an adverse effect on the host animal and proposed that fermented milks would reverse the effect and promote good health. He based this hypothesis on observations of Bulgarian peasants who consumed large quantities of soured milk and lived to a ripe old age. Without any real evidence he made the connection between the two observations. Subsequent experiment studies have suggested that there is at least some scientific foundation to the belief that fermented milks have a beneficial effect on the health of the consumer (Fuller, 1991).

Probiotics, are therefore, defined as live microorganisms that confer health benefits to the hosts (Salminen et al., 1993, 1998; Holzapfel et al., 1998, 2001; Madsen, 1999; FAO/WHO, 2001; Perdersen et al., 2004), and some lactic acid bacteria (LAB) species, which include the groups *Lactobacillus*, *Streptococcus*, *Pediococcus* and *Leuconostoc* have been reported as active probiotic candidates by several workers (Fuller, 1991; Ogunshe,

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2008). Fermented Milk Products (FMP) containing viable lactobacilli have been used by humans primarily as prophylactics and their use has been extended to intestinal infections (Ogawa et al., 2001; Ogunshe, 2006, 2008; Ogunshe and Olaomi, 2008). Studies, such as in the treatment of *Clostridium difficile* colitis with *Lactobacillus* have been done on humans (Hopkins and Macfarlane, 2003), while dietary lactobacilli have also been used for the treatment of infantile diarrhoea. Alm (1983), Ogunshe Olaomi (2008) and Ogunshe (2008) observed that the oral administration of *L. acidophilus* reduced the carrier time in children and in adults infected with *Salmonella*.

This study also tries to identify potential indigenous *Lactobacillus* probiotics isolated from 100 human milk colostrum and 50 infantile faecal samples obtained from breast-fed 3 to 30-day-old babies using the API 50 CHL system.

MATERIALS AND METHODS

Materials

This study was carried out between September 2008 and June 2009 in Konya Dr. Faruk Sukan Birth and Childen Hospital and Konya Province Control Laboratory, Turkey. 100 colostrum samples and 50 faecal samples of 3 to 30 day old breast-fed infants were obtained in sterile small jars from Konya Dr. Faruk Sukan Maternity and Children Hospital. Samples were transported within two hours to Konya Province Control Laboratory for microbiological studies.

Media and culture conditions

The colostrum and infantile faecal samples were plated on MRS (Man Rogosa Sharpe) agar supplemented wuth 0.25 % (w/v) L-cysteine (Sigma-Aldrich). One ml of each colostrum and facal samples (after diluting in proportion of 1/10 within MRD (maximum recovery diluent, Merck)) was plated on each MRS agar plate, followed by incubation under anaerobic conditions at 37°C for 48 h using GENbox anaerobic kit (Bio-merieux, Marcy l'Etoile, France). Obtained colonies were randomly picked from the primary plates after incubation and pure colinies identified with API 50 CHL system. Pure colonies were kept as stock and bench cultures in 15% glycerine and preserved in deep- freeze at -20°C.

API 50 CHL system

Fermentation of carbohydrates was determined using API 50 CHL, a standardized system, consisting of 50 biochemical tests for the study of carbohydrate metabolism by microorganisms. API 50 CH is used in conjunction with API 50 CHL medium for the identification of *Lactobacillus* and related genera strips according to the manufacturer's instructions (Biomerieux, Marcy l' Etoile, France) (Ghanbari et al, 2009). 10 ml of pure water was dispensed into the incubation box with the strip placed in the incubation box, after the bacterial cultures had been introduced into the API 50 CHL system in API 50 CHL medium (5 ml), in concentration 2 McFarland. The set-up system was then incubated at appropriate temperature of 35°C for 48 h, after the wells were filled with the bacterial suspensions by the line mark with the addition of mineral oil.

Bacterial strains from colostrum and faecal samples were identified based on the fermentation of carbohydrates. Identification tables were prepared as (+/-) according to colour change in evaluation of results of API strips reaction. Numerical profiles of strains were identified adding positive values in indicative table. Species designations were identified by evaluating with software identification apiweb™.

RESULTS AND DISCUSSION

A total of 20 (25.0%) *Lactobacillus* strains were isolated from 100 colostrum samples in this study. The *Lactobacillus* strains were identified by API 50 CHL systems, program identification apiweb™ as *Lactobacillus brevis* 6 (30.0%), *Lactobacillus plantarum* 5 (25.0%), *Lactobacillus acidophilus* 4 (20.0%), *Lactobacillus casei* 3 (15.0%) and *Lactobacillus acidophilus*-3 in 2 were identified by program identification apiweb™ (10.0%) (Table 1). As shown in Table 2, seventeen *Lactobacillus* strains were also isolated from the 50 infantile faecal samples in this study. They were identified as *Lactobacillus brevis* 7 (41.18 %), *Lactobacillus plantarum* 5 (29.4%), *Lactobacillus fermentum* 2 (11.8%), *Lactobacillus rhamnosus* 2 (11.76 %), *Lactobacillus reuteri* 1 (5.9%). The results obtained in this study also support previous ones in which recovery of similar *Lactobacillus* species from infantile faecal speciemns have been earlier reported (Gronlund et al., 1999; Ahrne et al., 2005).

Lactobacillus strains jist like many other bacterial species have been identified phenotypically with the use of biochemical characteristics and kits like API 50 CH system, and currently by molecular methods, such as with the use of PCR technique. This study identified some *Lactobacillus* strains with the use of the API 50 CH system as belonging to *L. Brevis, L. plantarum*, *L.fermentum*, *L. rhamnosus*, *L. reuteri* from infantile faecal samples. Similar groups of *Lactobacillus* strains had been earlier isolated from infantile faecal specimens from other countries (Apella et al., 1992; Nader de Macias et al, 1992; Ogunshe, 2008), although slight variation which may be due to fields and geographical locations could be observd.

Classically, searching for bacterial strains that are able to exert beneficial effects on human health has been focused on the natural inhabitants of our gastrointestinal tract, mainly being directed at members of the genera *Bifidobacterium* and *Lactobacillus*. In the last few years, a substantial body of scientific evidence suggests that other bacterial genera could rationally be screened for use as probiotics (SÃ¡nchez et al., 2010), thus the screening for likely probiotic *Lactobacillus* strains from human milk colostrum in this study. In contrast to breast milk, little is known about the bacterial composition of human colostrums (Jiménez et al., 2008), although few studies like that of Martín et al. (2010), reported that human breast-milk provides a rich source of commensal lactic acid

Table 1. Recovery rates of *Lactobacillus* strains from colostrum (n = 100) samples.

Species name	Strain number	Strain percentage(%)
Lactobacillus brevis	6	30
Lactobacillus plantarum	5	25
Lactobacillus acidophilus	4	20
Lactobacillus casei	3	15
Lactobacillus acidophilus-3	2	10
Total:5	20	100

Table 2. Recovery rates of *Lactobacillus* strains from faecal (n = 50) samples.

Species name	Strain number	Strain percentage(%)
Lactobacillus brevis	7	41.18
Lactobacillus plantarum	5	29.42
Lactobacillus fermentum	2	11.76
Lactobacillus rhamnosus	2	11.76
Lactobacillus reuteri	1	5.66
Total: 5	17	100

bacteria (LAB) to the infant during breastfeeding and stimulates abundant growth and colonization of these bacteria at mucosal surfaces in the infant gastrointestinal tract. *L. brevis*, *L. plantarum*, *L. acidophilus*, *L.casei* and *L. acidophilus*-3 were the *Lactobacillus* species obtained from the milk colostrum in this study.

It was found that there were no indications that the colostrum samples contained harmful bacteria in the study of Jiménez et al. (2008), while Martín et al. (2010) also documented that breastmilk confers critical nutritional and immunologic support to the developing newborn. The result findings of this present study conclude that beneficial *Lactobacillus* strains can be obtained from colostrum and infantile faecal specimens using API 50 CHL technique.

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