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Screening of Milk and Dairy Products for Isolation of Lipolytic Bacteria

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Twenty one samples of milk and milk products like milk, curd, paneer etc. were screened for presence of lipolytic bacteria and fungi by enrichment culture on Tributyrin agar medium. Of the hundred isolates only seven bacterial strains showed significant lipolytic activity. These isolates were purified and maintained on agar slant for further work. Study suggests that consumption of milk and milk products may be beneficial for lowering serum cholesterol levels in the body. Further these lipolytic stains can be used to produce lipase for industrial and pharmaceutical use.

Key words: Milk and Dairy Samples, Lipolytic activity, Enrichment, Lipase.

Lipolytic enzymes are currently attracting an enormous attention because of their biotechnological potential (Benjamin, S. *et al.*, 1998). Bacterial lipases have ability to catalyse entioselective reactions with a wide range of substrates. Lipases show stability over wide range of temperature and pH which make them very attractive alternative of various industrial processes (Lima *et al.,* 2003). Bacterial lipolysis is helpful for aroma and flavour development in cheese. Lipolytic activity has been reported in several bacteria, fungal species and actinomycetes naturally found in dairy products (Sztajer et al, 1988, Rapp and Backhaus, 1992, Wilhelm *et al.*, 1999, Hyri co kun *et al*., 2004, Grasian *et al.*, 2007).

Formulations prepared from lipolytic bacteria also have various industrial applications such as in detergent formulations, for synthesis of bio surfactants, in oleochemical, dairy and agrochemical, cosmetic industry, paper manufacture and pharmaceutical processing (Sharma et al, 2001). In addition to these, lipolytic lactic acid bacteria can also be used as probiotics to reduce serum triglyceride level and can prove beneficial for those people who produce inadequate pancreatic lipase (Roos *et al.*, 2000;

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Medina *et al.*, 2004).

Studies on lipolytic activity of some bacteria like in *Pseudomonas sp*. and *Bacillus sp.* has been done (Wilhelm *et al.,*1999; Rajendran *et al*.,2007; Droge *et al*.,2007; Blake *et al*., 1990; Eggret *et al*., 2001). Reports are also available on lipolysis shown by *Staphylococcus sp.* etc. (Gotz *et al*., 1998). Lactic acid bacteria are the naturally occurring bacterial flora of food products have been implicated in lipid degradation (De angelis *et al*.,1999; Marta *et al*., 2002; Matthews *et al*., 2004). However there are few reports of isolation of lipolytic bacteria from cheese, cow's milk etc. Camel milk has not been investigated for lipolytic bacteria.

In the present work 21 milk and dairy samples collected from commercial and domestic sources such as dairy, shop, home made products etc. screened for the presence of lipolytic bacteria.

MATERIAL AND METHODS

Collection of sample

Total 21 dairy samples viz. curd, cheese, milk (goat, buffalo, cow, camel) were used in this study (table 1). These samples were collected from domestic and commercial sources in presterlized ampoule from different regions of Udaipur and screened same day of collection.

Media used for enrichment and isolation of lipolytic bacteria

Enrichment medium contained 20 g/l (tributyrin / castor oil) as lipid substrate; 2.5 g/l K_2HPO_4; 1.3 g/l $(NH_4)_2$ SO_4; 0.5 g/l $MgSO_4$; 0.5 g/l yeast extract and 6.5ml of 200 g/l filter sterlized urea stock (added after autoclaving). pH of the medium was adjusted to 8.5 by addition of sterile 1:3 sodium carbonate: bicarbonate mixture (75: 25 g/l). Isolation of lipolytic bacteria was done on tributyrin agar (TBA) containing 10 g/l tributyrin (lipid substrate); 5 g/l peptone; 3 g/l yeast extract; 20 g/l agar. The pH of media was adjusted to 7.5.

Enrichment of samples and isolation of lipolytic bacteria

For the enrichment of dairy samples modified method of Vargas et al, 2004 was used. In which suitable dilution of collected samples was inoculated into 25 ml of enrichment media and incubated in an orbital shaker at 220 rpm at 37°C for 24h . After incubation the appropriate dilutions of enrichment culture were streaked on tributyrin agar media and incubated for 2-3 days at 37°C in a B.O.D incubator. Pure cultures of bacterial colonies showing significant activity were developed in nutrient broth and the cell free extract was screened for lipolyic activity. 24 h culture of purified lipolytic isolates was centrifuged at 5000 rpm for 10 minutes to obtain clarified supernatant. This supernatant was filtered through 0.45 µm membrane filter to obtain cell free extract. 100 µl of this cell free extract was filled into 12 mm wells in tributyrin agar plates which were subsequently incubated at 37 °C for 24h. A clear zone around the well indicated lipid hydrolysis. The size of clear zone was measured with the help of Hi- Antibiotic zone scale-C. Nutrient broth was used as negative control and pancreatic lipase as positive control.

RESULTS AND DISCUSSION

Various plate assay methods using selective media have been described for detection of lipolytic activity in microorganisms. These methods include incorporation of fluorogenic substrate like methyl umbeliferyl in plating media (Kim *et al.*,1986), incorporation of indicator dye like methyl red, phenol red, rhodamine B or Victoria blue B in media (Samad *et al.*, 1989), use of TBA media (Blake *et al.*, 1996) etc. The basic principle behind these methods is the hydrolysis of lipid substrate present in the media by the microbial enzyme which can be observed as a clear zone around the bacterial colonies or wells containing cell free extract. The size of zone of clearance indicates the magnitude of activity. In the present study tributyrin was used as a lipid substrate to detect lipolytic activity by plate agar assay method.

100 bacterial and fungal isolates were obtained from the 21 samples used for the study (Table 2) of which MRL01, MRL06, MRL05, MRL04 A, MRL06, MRL11B and MRL12 isolated from sample 5, 10, 15 and 17 showed significant lipolysis. Among these isolates, strain MRL01, MRL04A and MRL06 A isolated from cheese, camel milk and cow milk respectively showed highest activity whereas least activity was shown by MRL06 and MRL11B strains isolated from cow and goat milk. Results also indicate that lipolytic activity of crude lipase prepration of MRL 01,

Table 1. List of samples screened for isolation of lipolytic bacteria

S.No.	Name of sample	Region from where sample procured
1	Goat milk I	Ghorana (Udaipur, Raj.)
2	Cow milk I	Vashisth dairy farm (Udaipur,Raj.)
3	Cow curd I	Vashisth dairy farm (Udaipur,Raj.)
4	Salted butter I	Amul (Udaipur, Raj.)
5	Mozzarella Cheese I	Amul (Udaipur, Raj.)
6	Goat milk II	Kantariya road (Jhadol,Udaipur, Raj.)
7	Goat milkIII	Ghorana (Jhadol, Udaipur, Raj.)
8	Goat milk IV	Kherwada (Udaipur, Raj.)
9	Goat milk V	Sandol mata (Udaipur, Raj.)
10	Goat milk VI	Palia kheda (Bari, Udaipur, Raj.)
11	Goat milk VII	Gariyon ka kuda (Udaipur, Raj.)
12	Buffalo milk I	Sindhu (Manduthal, Udaipur, Raj.)
13	Buffalo milk II	Sindhu (Manduthal, Udaipur, Raj.)
14	Cow II	Chirwa (Udaipur, Raj.)
15	Cow III	Visenwas (Khameli, Udaipur, Raj.)
16	Curd II	Saras dairy (Udaipur, Raj.)
17	Camel milk	Nathdwara (Udaipur,Raj.)
18	Pasteurised milk	Saras dairy (Udaipur,Raj.)
19	Paneer	Panchwati dairy (Udaipur, Raj.)
20	Cow milk IV	Mawali (Udaipur, Raj.)
21	Goat milk VIII	Mawali (Udaipur, Raj.)

Table 2. Comparison of bacterial lipolysis in dairy samples collected from various region of Udaipur (Rajasthan)

S.No.	Dairy sample	Extent of lipolysis	Name of isolates
1	Sample 1	NS	NI
2	Sample 2	NS	NI
3	Sample 3	NS	NI
4	Sample 4	NS	NI
5	Sample 5	S	MRL 01, MRL 05
6	Sample 6	NS	NI
7	Sample 7	NS	NI
8	Sample 8	NS	NI
9	Sample 9	NS	NI
10	Sample 10	S	MRL 11B,12
11	Sample 11	NS	NI
12	Sample 12	NS	NI
13	Sample 13	NS	NI
14	Sample 14	NS	NI
15	Sample 15	S	MRL 06 A,06
16	Sample 16	NS	NI
17	Sample 17	S	MRL 04 A
18	Sample 18	NS	NI
19	Sample 19	NS	NI
20	Sample 20	NS	NI
21	Sample 21	NS	NI

*NS- Not significant; NI- No isolates; S - Significant

Table 3. Zone of hydrolysis produced by lipolytic isolates on tributyrin agar plate

S.No.	Lipolytic isolate	Size of zone of hydrolysis* (mm)
1	MRL 01	12
2	MRL 06	7
3	MRL06 A	13
4	MRL 05	10
5	MRL 04A	12
6	MRL 11B	5
7	MRL 12	10
8	Control	N.D.
9	Pancreatin	15

* Average of three replicates
N.D. Not determined

MRL 06A, MRL 04A is comparable with that of standard and hence there is a possibility that pure lipase prepration might have greater activity than pancreatin.

One way annova of results performed by using statistical analysis software origin 40. Analysis showed at p value 0.05 results are significant.

Lipolytic activity has been reported in several bacteria naturally found in many dairy products such as *Lactobacillus, Lactococcus, Streptococcus, Pseudomonas, Achromobacter, Enterobacter* etc. *(*Kamaly *et al*,1990, Woo *et al*, 1984, Ren *et al*, 1988 and Thomas *et al*,1963). Lipase enzyme responsible for lipolytic activity of bacteria may be present extracellularly, intacellularly or it may be cell membrane bound (Mourey., 1981, Lee and Lee., 1989 and Large *et al*., 1999). This enzyme hydrolyzes the lipid into fatty acids and glycerol. Hydrolysis of tributyrin results in development of a zone of clearance in the medium. Presence of extracellular lipase in cell free extract is therefore possibly responsible for the lipolytic activity of the isolates (Kalogridou-Vassiliadou,1984).

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