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Identification of lactobacilli at genus level by using PCR

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Abstract
Lactic acid bacteria (LAB) are gram positive non sporulating, Microaerophilic bacteria whose main fermentation product from Carbohydrate is lactates. The LAB especially the genera Lactococcus, Lactobacillus, Leuconostoc, Streptococcus, Bifidobacteria and Pediococcus, Have been traditionally used as starter cultures for the fermentation of foods and beverages because of their contribution through flavor and aroma development and to spoilage retardation. Lactobacilli are found in milk, although their numbers are few and in certain cases even their absence has also been reported (Hill and Thornton, 1958). It is very difficult to isolate lactobacilli from milk as they are outnumbered by other microorganisms. Thus rendering most of the normal nutrient media unsuitable for their isolation.
With the advent of molecular biology techniques, it is now possible to classify bacteria by comparing their genomes. Genotyping has many advantages over traditional typing procedures the rRNA locus is a genetic unit of broad evolutionary interest (Cedergen *et al*., 1988). In prokaryotes two additional assumptions are basic for the validity of this approach, namely that lateral gene transfer has not occurred between rRNA genes and that the amount of evolution or dissimilarity between rRNA sequences of a given pair of organisms is representative of the variation by the corresponding genomes (Rochelle, 1992). In present investigation 25 isolates alleged to be lactobacilli were recovered from BCP-MRS agar plates streaked with milk samples which was collected from NDRI, Karnal campus. These isolates on the basis of Grams staining and catalase test were subjected to PCR by using Lb1 and Lb2 23s rRNA lactobacilli specific PCR primers. Tll the isolates produce a PCR amplified product of 194 bp specific for lactobacilli species on agarose gel which are consistent with the earlier observations of Suja (2003).

Keywords: lactobacilli, development, microorganisms

Introduction
Lactobacilli are gram positive, catalase negative, non-spore forming rod shaped bacteria that produce lactic acid as the major end product of fermentation they have complex nutritional requirements regarding to be supplied with carbohydrates fatty acids or fatty acid esters. Salts nucleic acid derivatives and vitamins lactobacilli are found in milk although their numbers few and in certain cases even their absence has also been reported (Hill and thronton, 1958) [3]. It is very difficult to isolate lactobacilli from as they are outnumbered by other organisms, thus rendering most of the normal nutrient media unsuitable for their isolation (Chalmers, 1962) [2].
Lactobacilli constitute an important group of organisms which considerable improve the nutritional and therapeutic value of fermented foods. These organisms play an instrumental role in numerous fermentation processes in a variety of foods including dairy products, baked products and alcoholic beverages. The economic importance of lactobacilli clearly demands the development of a new technology to identify lactobacilli at DNA level. With the advent of molecular biology techniques, it is now possible to classify bacteria by comparing their genomes. Genotyping has many advantages over traditional typing procedures which includes

1. DNA can always be extracted from bacteria so that all strains are type able.
2. Analytical strategies for the Genotyping methods are similar and can be applied to DNA of Any source.
3. In general, genomic DNA is a stable characteristic and its composition is independent of cultural conditions or methods pf preparation.
4. It allows for statistical data analysis and is amenable to automation.

In present investigation we isolated lactobacilli from milk samples and all the samples were subjected to grams staining and catalase test, the purity of the isolates as a lactobacilli were ascertained by using PCR techniques.

NDRI, Karnal campus the samples were collected in sterile 10 ml capacity screw capped glass tubes after takings appropriate precautions as recommend by ISI (IS,1977).

One ml of raw milk were added into 9 ml of litmus milk which was incubated overnight at 37 ºC and plated on BCP-MRS agar. Two types of colonies were observed, yellow colonies were picked up from the plates after an incubation period 24 hrs at 37 ºC. totals of 25 isolated were picked up into MRS broth and further purified by repeated sub culturing. The purity of all bacterial isolates was always ascertained prior to use by Grams staining, cell morphology and catalase test. These isolates as a lactobacilli were then identified by using Lb1 and Lb2 primers (23s rRNA) specific to lactobacilli. The genomic DNA from the test cultures grown in MRS broth was extracted by following the method of Pospiech and Neikmann (1995) method.

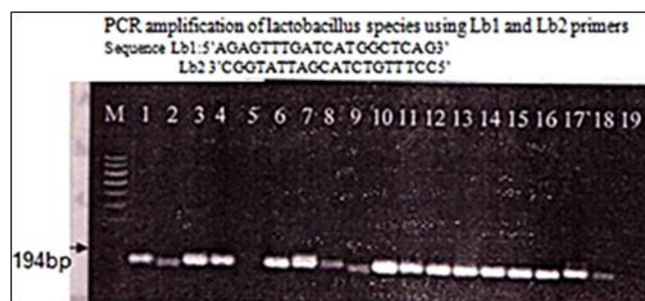
The PCR assay basically involves these steps viz. setting up of PCR reaction initial denaturation 95 ºC for 5 min, denaturation at 94 0 C for 30 sec, renaturation at 56 ºC for 1 min, synthesis at 72 ºC temp for 1 min and extension at 72 ºC for 10 min by using MgCl2, DNTPs 0.2ul, primer Lb1 and Lb2 2mM, taq DNA polymerase 2.25 units and template DNA about 100ng to 1µg. The PCR amplified products obtained with different templates were electrophoresed on 2% agarose gel and examined under UV (302 nm, photo dyne) and photographed using a Polaroid camera with Polaroid type 55 sheet film.

Results and Discussion

The role of LAB in human nutrition and health is well documents as those food grade bacteria have traditionally been use for various purposes. Among these LAB lactobacilli enjoy a special status, on account of their extensive use in food pharmaceutical and dairy industry as well. The commercial value of lactobacilli can be enhanced considerably by their genetic manipulation. The prerequisite for applying gene cloning technique to lactobacilli, is the development of method to identify lactobacilli at genus level (Vaishali *et al*., 1992) [10].

Lactobacilli are found in milk, although their numbers are few and in certain cases even their absence has also been reported (Hill and Thornton, 1958) [3] In present study, 25 isolates alleged to be lactobacilli were recovered from MRS agar plates streaked with milk samples pre enriched for 24 hrs in litmus milk and MRS broth for 24 hrs. Malton and Sandin (1986) [5] also developed a selective medium for Gram positive bacteria which was prepared using Ellikers lactic agar as the basal medium supplemented with 1% Tween 80.

The identity of the isolates which was recovered from yellow colonies as a lactobacilli was determined by Gram staining and then ascertained by PCR by using Lb1 and Lb2 set of primers specific to lactobacilli. All the isolates produce a PCR amplified product of 194 bp specific for lactobacillus species on agarose gel. The rRNA genetic locus is a unit of broad evolutionary interest in prokaryotes (Cedergren *et al*., 1988) [1]. Two additional assumptions are basic for the validity of this approach, namely that lateral gene transfer has occurred between r RNA genes and that the amount of evolution or dissimilarity between r RNA sequences of a given pair of organism is a representative of the variations by corresponding genomes (Rochelle, 1992) [7]. Constituent results of 194 bp PCR product (specific for lactobacillus species on agarose gel), indicates that an important feature of the 16 s RNA molecule in its use as a universal standard parameter for phylogenic interferences is the relative case of sequence alignment. Thus, a myriad studies have exploited the features of 16 sRNA for speciation identification of lactobacilli at molecular level. It is evident that the resolving power of 16 sRNA sequences is limited when closely related species are being inspected.



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