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ISOLATION, SCREENING AND EVALUATION OF ANTIOXIDANT PROPERTY OF *Lactobacillus* SPECIES FROM GOAT MILK

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Abstract: This investigation was performed with few *Lactobacillus* isolates obtained from goat milk. They were screened for radical scavenging effects using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical. The radical scavenging activity value of an isolate *Lactobacillus* LB 36 was estimated as 57.28% (v/v). Carbohydrate fermentation and physiological characterization of isolate LB 36 matched with *Lactobacillus casei* as per Bergey's manual of systematic bacteriology. Our results revealed that *Lactobacillus* species particularly *Lactobacillus casei* can be used in food formulations or cosmetics for prevention of oxidative stress related diseases.

Keywords: Goat milk, *Lactobacillus* species, antioxidant property.

Introduction

Free radicals and active oxygen have been recognized as an important factor in the pathogenesis of several human diseases. Excessive amounts of reactive oxygen metabolites (ROM), generated through normal reactions within the body during respiration in aerobic organisms, can cause cellular damage, which, in turn, promotes chronic diseases. To neutralize the oxidant molecules, antioxidant enzymes form the biological antioxidant barrier. Antioxidant defenses are considered to be important in the maintenance of human health and disease prevention. *Lactobacillus* species which are "generally regarded as safe" (GRAS) status have the potency to produce such enzyme. Because of such physiological versatility, *Lactobacillus* species have drawn the attention of many researchers worldwide in order to formulate several potential drugs with nutraceutical supplement properties, health promoting functional foods or other pharmaceutical products (Rochat et al 2005; Driscoll et al 2002).

Material and methods

Isolation and propagation of *Lactobacillus* isolates from goat milk

The milk sample was processed in the laboratory for preliminary isolation of the bacterial culture. One mL of dairy sample was mixed well in nine mL of saline water to make an initial dilution (10^{-1}). The suspensions were used for making suitable serial dilutions up to 10^{-8} by

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transferring 1mL into 9mL of sterile saline water and pour-plated aseptically using MRS agar and plates were incubated at 37 ^{0}C for 48 h. After incubation period, bacterial cultures were randomly picked from master plates on the basis of their colony characteristics. Morphologically different colonies were streaked on MRS agar plates. Pure cultures of various bacteria were preserved in MRS slants and maintained in glycerol stock at 4 ^{0}C.

For preliminary identification of *Lactobacillus* species, the pure cultures were again screened as per Bergey's Manual. It mainly includes Gram's reaction, spore formation, catalase and oxidase test.

Antioxidant assay (2, 2-Diphenyl-1-Picryl-Hydrazyl (DPPH) scavenging assay)

The 1,1-Diphenyl-2-picrylhydrazyl free radical (DPPH) is a rapid, simple, widely used and inexpensive method to evaluate antioxidant activity of foods (Brand-Williams et al 1995). This assay measures the ability of a sample to donate hydrogen to DPPH radical (Huda-Faujan et al 2007). DPPH radical solution (0.004%, w/v, Sigma Aldrich) in 95% ethanol was prepared. Cell free supernatant of all isolated *Lactobacillus* species were added to 2 ml of DPPH (0.4 mM) solution. When DPPH interacts with an antioxidant that can donate hydrogen, it is reduced, and this decreases the absorbance; readings at 517 nm were recorded using a UV-Vis spectrophotometer. Ethanol was used as a blank, while DPPH solution in ethanol served as control. The antioxidant activity was expressed as percentage of DPPH activity using the following formula:

$$\text{DPPH activity } (\%) = \frac{\text{Absorbance of blank } - \text{ Absorbance of sample}}{\text{Absorbance of blank}}$$

Identification and biochemical characterization of potent isolate

The most useful test for the determination of strain differences is carbohydrate fermentation. Fermentation capacity of different carbohydrates like arabinose, cellobiose, esculin, D-fructose, galactose, maltose, mannose, melibiose, raffinose, sorbitol, sucrose etc were determined by using API 50 CH kit (Biomeriux, France) as per manufacturer's instruction and following Bergey' s manual of systematic bacteriology (1994).

Result and analysis

Preliminary isolation and morphological characterization of *Lactobacillus* isolates

A number of 110 isolates were obtained from goat milk samples. Out of which, 48 isolates were identified as *Lactobacillus* species in their preliminary characterization performed as per Bergey's manual. After all preliminary characterization performed as per Bergey's manual, the isolates were reported as gram positive, short rod diplobacillii, catalase negative,

non spore forming and non motile. The isolates give small colonies of approximately 1mm of diameter with whitish and circular appearance on MRS pour plate. Similar result was also observed by (Agrawal and Prakash, 2013), who isolated 13 lactic acid bacteria (LAB) from dairy products and identified as *Lactococcus* sp. by morphological characterization.

Antioxidant peptide assay (DPPH assay)

DPPH is a compound that possesses a proton free radical and this feature of DPPH was used to determine its proton-radical scavenging action. In the present work, out of all isolates, culture supernatant of *Lactobacillus* LB 36 showed highest radical scavenging activity (57.28%). It proved that LB 36 has high potential for inhibition of oxidative stress.

Biochemical analysis of LB 36

Lactobacillus LB 36 was able to ferment different kinds of sugars including D- fructose, galactose, maltose, mannose etc as per API 50 CH kit (Table I). From sugar fermentation analysis, the isolate was preliminary identified as member of the *Lactobacillus* spp., namely as *Lactobacillus casei* (with a 98% similarity to the reference).

	Strain LB 36		**Control (*L. casei* ATCC 9595)**	
Test name	**24hrs.**	**48hrs**	**24hrs.**	**48hrs**
control	_	_	_	_
Glycerol	_	_	_	_
Erythritol	_	_	_	_
D-arabinose	_	_	_	_
L-arabinose	_	+	_	+
D-ribose	+	+	+	+
D-xylose	_	_	_	_
L-xylose	_	_	_	_
D-adonitol	_	_	_	_
Mythyl-BD-xylopyranoside	_	_	_	_
D-galactose	+	+	+	+
D-glucose	+	+	+	+
D-fructose	+	+	+	+
D-mannose	+	+	+	+
L-sorbose	_	_	_	_
L-rhamnose	_	_	_	_
Dulcitol	_	_	_	_
Inositol	_	_	_	_
D-manitol	+	+	+	+
D-sorbitol	_	+	+	+
Methyl-aD-mannopyranoside	_	_	_	_
Methyl -aD-	_	+	_	+

glucopyranoside				
N-acetylglucosamine	+	+	+	+
Amygdalin	+	+	+	+
Arbutin	+	+	+	+
Esculin ferric citrate	+	+	+	+
Salicin	+	+	+	+
D-celiobiose	+	+	+	+
D-maltose	+	+	+	+
D-lactose(bovine origine)	+	+	+	+
D-melibiose		+	-	+
D-sachharose(sucrose)	+	+	+	+
D-trehalose	+	+	+	+
Inuline	_	+	+	+
D-melizitose	+	+	+	+
D-raffinose	_	+	+	+
Amidon(starch)	_	_	_	_
Glycogen	_	_	_	_
Xylitol	_	_	_	_
Gentibiose	+	+	+	+
D-turranose	+	+	+	+
D-lyxose	_	_	_	_
D-tagatose	_	_	_	_
d-fucose	_	_	_	_
L-fucose	_	_	_	_
D-arabitol	_	_	_	_
L-arabitol	_	_	_	_
Potassium gluconate	_	_	_	_
Potassium 2-ketogluconate	_	_	_	_
Potassium 5-ketogluconate	_	_	_	_

TableI. Physiological and biochemical characteristics of *Lactobacillus* LB 36
Data are from Bergey's Manual (Vol.III, Second Edition)

Conclusion

An *in vitro* ability of *Lactobacillus* to quench free radicals has been reported in this study. From the results of this study, the highest level of antioxidant activity was observed in LB 36 *Lactobacillus* isolates obtained from goat milk, thus indicating presumptive protection against free radicals. These features could lead to the production of innovative functional foods.

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