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ISOLATION AND CHARACTERIZATION OF PROBIOTIC LACTIC ACID BACTERIA FROM DHOKLA DOUGH

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Abstract

Lactic Acid Bacteria (LAB) plays an essential role in the food fermentation and has potential due to their ability to enhance the gut health and inhibit the pathogens. This study was carried out to isolate and characterize the LAB from Dhokla Dough, a traditional Indian fermented food especially for Gujarat peoples, to evaluate Dhokla Dough probiotic properties. The isolate (DC-1) was obtained using the selective MRS media and characterized through morphological, various biochemical and physiological tests. The isolate was Gram-positive, Catalase negative, non-spore forming and non-capsulated. It shows tolerance to bile salts (up to 4%), NaCl (1-3%) and pH (6-7) which suggests adaptability of digestive tract conditions. The isolate evaluated by their antimicrobial activity against *E.coli*, *Salmonella typhimurium*, *Shigella spp.* and *Bacillus megaterium* in which the highest inhibition observed against *S.typhimurium*. MALDI-TOF analysis recognized the isolate as *Enterococcus faecium* with a score of 1.86. Protein profiling by SDS-PAGE indicated a molecular weight >97.4 kDa. These findings indicates the probiotic potential of LAB isolated from traditional fermented foods and their applicability in functional food development. Further investigation in vivo is required.

Keywords: Lactic Acid Bacteria (LAB), Probiotic, Fermented food, Dhokla, *Enterococcus faecium*, Antimicrobial activity.

Introduction

A diverse group of Gram-positive, non-spore forming, facultative anaerobic bacteria that produce lactic acid as the end-product during the sugar metabolism is Lactic Acid Bacteria (LAB). They are important in the fermentation and have been widely accepted for their physiological benefits (Hammes & Hertel, 2009: Fontana et.al. 2013). Probiotics defined by FAO/WHO as "live microorganisms which when administered in an adequate amounts, confer a health benefit on the host," have become an critical part of functional food research and human health strategies (FAO/WHO, 2001; Hill et al., 2014).

LAB impact mostly to the gut health by competing with the enteric pathogenic microbes, producing antimicrobial compounds such as bacteriocins, organic acids, and hydrogen peroxide, and modifying host immune responses (Gismondo et al., 1999; Ouwehand et al., 2002). They are widely used in fermented dairy products, including yoghurt, kefir and cheese but also present in cereal based fermentation such as sourdough and the traditional Indian foods (Steinkraus, 2002; Marco et al., 2017). Earlier research has illustrated that LAB strains isolated from the indigenous fermented foods exhibits the promising probiotic properties, such as resilience to stress conditions, the ability to adhere to intestinal epithelial cells and strong antimicrobial activity (Kumar et al.,2011; Rezac et al., 2018).

In Indian, fermented foods like Dhokla from Gujarat and Idli, Dosa from South India signify diverse collection of LAB diversity; however, their probiotic potential has not yet been fully evaluated (Sekar & Mariappan, 2007). Dhokla, a fermented chickpea and rice-based product, provides a favorable niche for LAB growth due to its carbohydrate rich matrix and mild acidic environment. Exploring LAB from Dhokla may reveals strains with the functional properties suitable for the development of probiotic formulations (Patel et al., 2020). The present study was undertaken to isolate and characterize LAB from Dhokla dough, evaluated their biochemical and probiotic properties and identify them using MALDI-TOF and protein profiling. The study emphasizes the potential of traditional fermented foods as valuable sources of probiotic bacteria with applications in functional food industries.

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Materials and Methods

Sample Collection and Isolation from fresh Dhokla Dough samples were collected under sterile conditions then inoculated in selective MRS media broth and incubated at 37 degree Celsius for 48 hours, followed by streaking onto MRS agar plates. Isolated colonies were purified for further analysis. Morphological and biochemical characterization. Isolates were identified by gram staining, capsule staining, spore staining, catalase test and coagulate test (Bergey's manual, 1994). Biochemical profiling included carbohydrate fermentation, MR-VP test, citrate utilization , hydrogen sulfide production, nitrate reduction, starch hydrolysis, oxidase test, casein hydrolysis, gelatin hydrolysis and motility test.

Physiological Characterization

Bile salt tolerance: Growth in MRS broth containing 1-4% bile salt at 37 degree Celsius for 48 hour.

NaCl tolerance: Growth in MRS broth containing 0-4% NaCl.

pH tolerance: Growth in MRS broth containing pH 2-7.

Antimicrobial Activity: Cell free culture supernatants (CFCS) of isolate was examined against *E.coli*, *Salmonella typhimurium*, *Shigella spp.* and *Bacillus megaterium* using agar well diffusion method on N-agar and MH-agar. Zones of inhibition were calculated after 24 hrs incubation at 37 degree Celsius.

Protein Analysis:

Protein precipitation was conducted using chilled acetone and concentration was estimated using the Folin-Lowry method (Lowry et al., 1951). Proteins were examined by SDS-PAGE to determine molecular weight profiles.

Identification by MALDI-TOF

MALDI-TOF mass spectrometry was used for species-level identification based on protein fingerprinting.

Result

Morphological and Biochemical Features:

The isolate was identified as Gram-positive, catalase-negative, coagulase-negative, non-capsulated and non-spore former. Sugar utilization tests unveiled positive fermentation for dextrose, lactose, galactose, and fructose, while sucrose, maltose, ribose, xylose, arabinose and mannitol were negative. MR and VP tests were positive; citrate utilization, H2S production, and nitrate reduction were negative.

Table 1:

Characteristics	Dc-1
Size	Medium
Shape	Circular
Margin	Entire
Elevation	Slightly raised
Texture	Smooth
Opacity	Opaque
Pigmentation	Off-white

Table 2: Carbohydrate fermentation test for DC-1

SUGAR	DC-1
Sucrose	Negative
Maltose	Negative
Dextrose	Positive
Xylose	Negative
Ribose	Negative
Arabinose	Negative
Lactose	Positive
Mannitol	Negative
Galactose	Positive
Fructose	Positive

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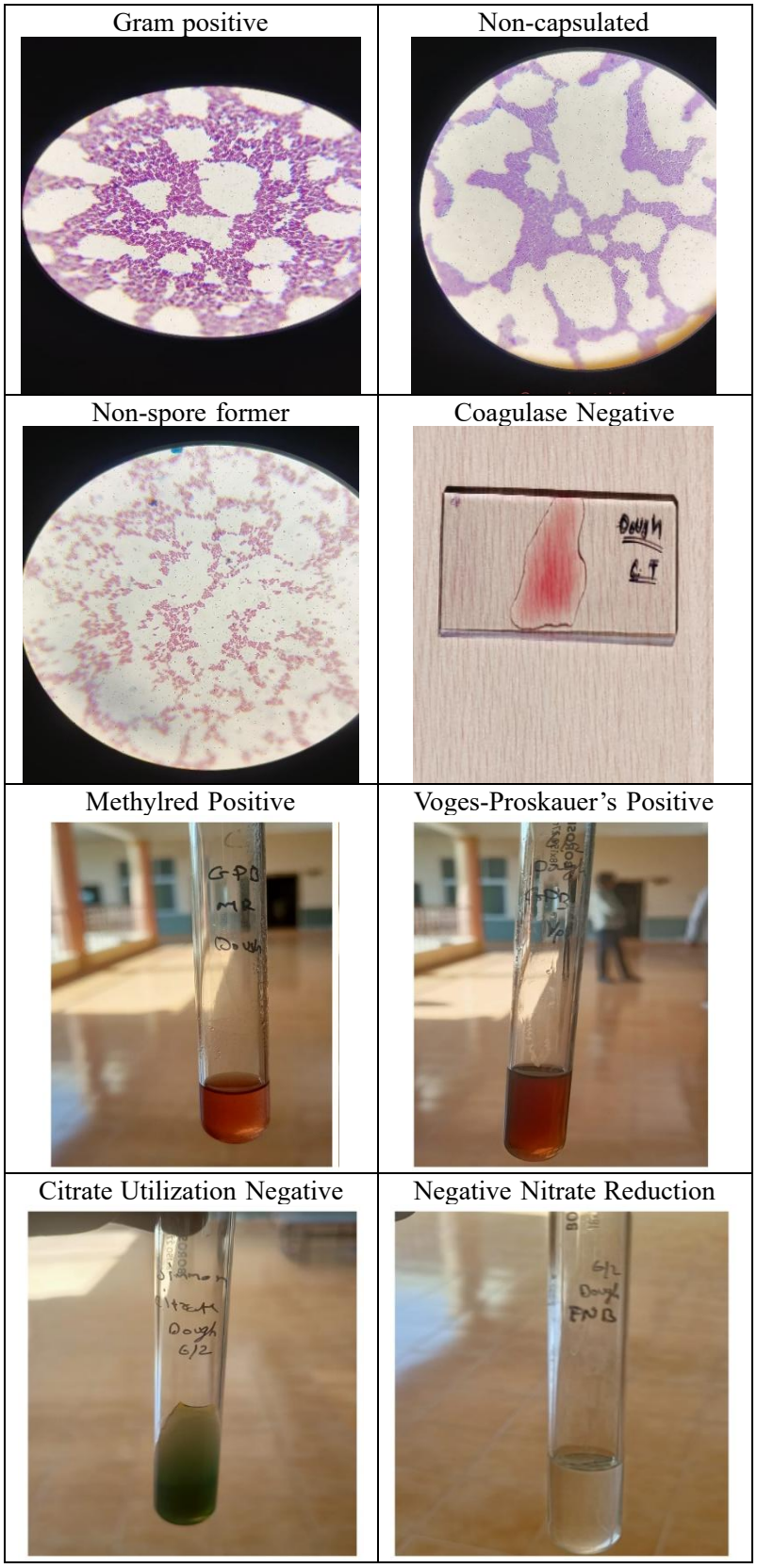


Figure 1:

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Physiological Properties

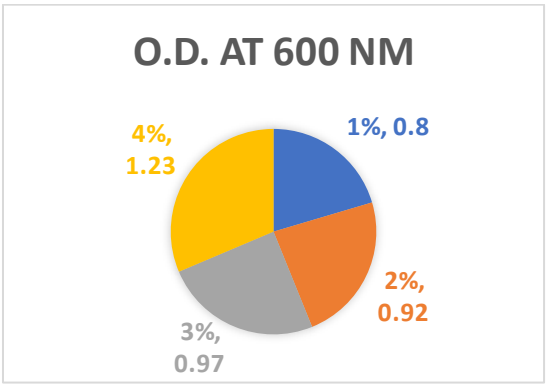


Figure 2: Bile salt tolerance: Growth sustained for bile salt up to 4% bile concentration

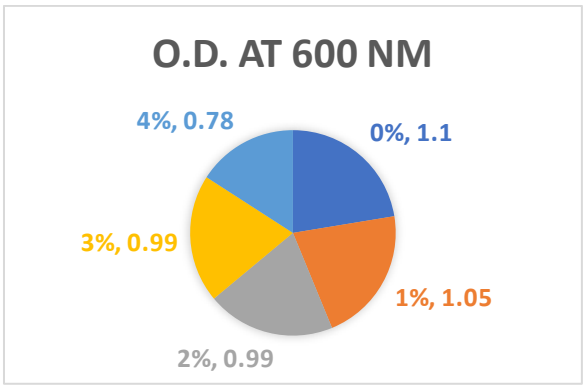


Figure 3: NaCl tolerance: Growth observed at 1–3% NaCl

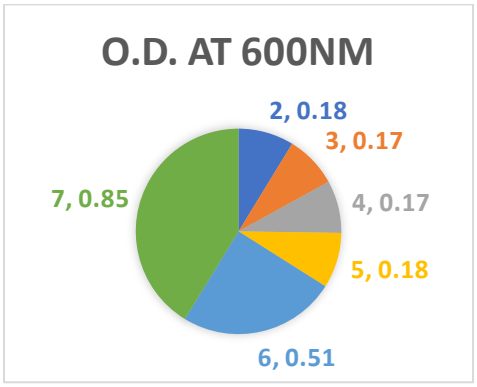


Figure 4: pH tolerance: Growth observed at pH 6–7 while limited growth observed at lowest pH

The isolate suppressed the common pathogens like *E.coli, S.typhimurium, Shigella and B.megaterium* upto certain extent.

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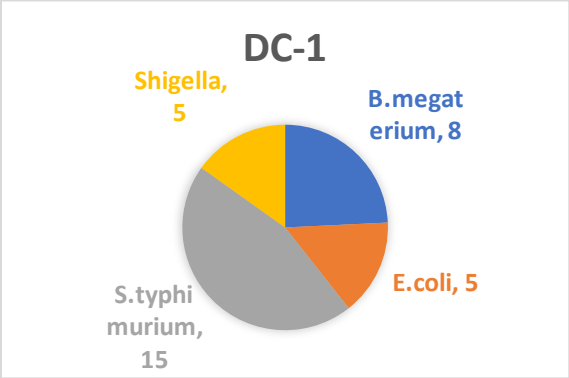


Figure 5: Zones of inhibition ranges from 5-15 mm, with maximum activity against *S.typhimurium*

Protein Estimation and SDS-PAGE

Protein concentration of the Dhokla dough sample was 0.56 mg/ml. SDS-PAGE unveiled a major protein band above 97.4 kDa.

Identification

MALDI-TOF evaluate the isolate as *Enterococcus faecium* with a score value of 1.86 indicating probable species level identification.

Discussion

The isolate from the Dhokla dough exhibited typical LAB features including Gram positive, catalase negative, coagulase negative and lactic acid fermentation capacity. These features are unvarying with previously reported LAB from fermented cereals, rice based food and dairy products (Hammes & Hertel, 2009; Fontana et al., 2013). The capability to sustain bile salts up to 4% and maintain growth at pH 6-7 indicates a strong capacity for gut environment survival, aligning with findings from Kumar et al. (2011) and Patel et al (2020), who reported alike tolerance in probiotic strains.

Antimicrobial activity against *E.coli*, *Salmonella typhimurium*, *Shigella spp.* and *Bacillus megaterium* advocate production of antibacterial peptides or bacteriocins, analogus work showed remarkable inhibitory effects on pathogens (Aween et al., 2012; Rezac et al., 2018). The strong inhibition against *S.typhimurium* is noteworthy, as alike results were documented by Gismondo et al (1999) emphasizing the role of LAB in pathogen control.

MALDI-TOF identification of isolate evaluate *Enterococcus faecium* conform with reports of its being successfully isolated from the fermented foods and considered for probiotic applications (Ouwehand et al., 2002; Marco et al., 2017). However, safety concerns related to antibiotic resistance and the opportunistic pathogenicity in the *Enterococcus* species require the careful evaluation. (Hill et al., 2014). Thus, genomic screening for the virulence and resistance gene is very essential before the commercial applications.

The SDS-PAGE analysis revealing proteins >97 kDa indicates potential functional proteins involved in stress adaptation, similar to findings of Hussey et al. (2012), where stress-response proteins contributed to probiotic resilience. Such proteins are likely to contribute in adhesion and survival in host environments, but further proteomic and genomic studies are needed.

Overall, this study confirms that indigenous Indian fermented Gujarat foods such as Dhokla are storehouse of novel probiotic strains. These results are reliable with Sekar & Mariappan (2007), who indicated the importance of indigenous fermented foods as sources of beneficial microorganisms. The probiotic potential of the isolate signify its possible role in functional food development, although in vivo validation and safety inspections remain crucial next steps.

Conclusion

This study was successfully carried out for the isolation and characterization of LAB from Dhokla dough. The isolate was diagnosed via MALDI-TOF was *Enterococcus faecium*, exhibiting probiotic attributes such as bile salt, NaCl and acid tolerance, and antimicrobial activity. These insights suggest its probiotic

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potential application in functional foods and its formulation. However, in vivo evaluation and genomic safety profiling are mandatory before commercial application.

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