RESEARCH ARTICLE

Investigation of Antifungal Activity of Purified Lectin from *Lactobacillus acidophilus* against Oral Candidiasis

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ABSTRACT

The lectins produced by *Lactobacillus acidophilus* and *Lactobacillus plantarum* have a wide range of abilities to hemagglutinate human erythrocytes, with more significant titers of hemagglutination blood group type O+ and lower titers of hemagglutination blood group type A+ and B|+. Also, *L. acidophilus* produced lectin with a higher titer than *L. plantarum.* It is extracted by glass beads and precipitated by acetone solvent. Six *Candida albicans* isolates were isolated from oral candidiasis. The highest antifungal activity of extracted lectin was against the tested *C. albicans* isolates in a dose-dependent manner so that the lectin has promised an alternative to overcome multiple antifungal resistance.

Keywords: *Candida albicans*, Lactobacilli, Lectin.

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INTRODUCTION

Lactobacilli are non-pathogenic commensals of human gut flora that are thought to be good for human health.[1] *Lactobacillus* species are commonly employed as probiotics due to their projected health-promoting effects.[2] A probiotic is a microbial feed supplement that improves the microbiological balance in the bowel and so benefits the host.[1] The concept of probiotic foods is based on the fact that the microflora in the gastrointestinal tract play a significant role in an individual's health status, which is influenced by a diet consisting of organisms,[3] which, when consumed in specific amounts, provide health benefits beyond their intrinsic nutritional value. A probiotic strain must meet all of the following requirements: human origin, generally recognized as safe (GRAS) status, production of antibacterial factors against invasive gram-negative pathogens, desirable metabolic activity, technological suitability, non-pathogenic, immunostimulatory, anti-carcinogenic, antimutagenic, and so on.[2,4] Probiotics have been increasingly popular as dietary supplements in recent years. In vitro approaches should be used to determine probiotic functional requirements, and the outcomes of these investigations should be reflected in controlled human studies. The following factors should be considered as major criteria for selecting an acceptable probiotic strain when choosing one.[4]

Lectins are non-immune divalent or multivalent carbohydrate-binding proteins that agglutinate cells and precipitate polysaccharides and glycoconjugates.[5] They could also be glycoproteins with at least one non-catalytic domain that has shown reversible binding to specific monosaccharides or oligosaccharides and can agglutinate human and/or animal erythrocytes by binding to the carbohydrate moieties on the surface of the erythrocytes and agglutinate the erythrocytes without changing the properties of the carbohydrates.[6,7]

Among the signal molecules that regulate the interactions mentioned above, lectins (the galectin family, among others) are thought to play an important role in various types of communications (bacteria, biofilm formation, bacterium cell, cell tissue organ whole organism, cell organelles, and so on).[7]

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The goal of this study is to find lectin-producing Lactobacilli in yogurt, extract lectin, and use it as an antifungal drug to treat oral candidiasis cases.

MATERIALS AND METHODS

Isolation of *Lactobacillus* from Yoghurt

Twenty yogurt samples were homogenized in 100 mL of sterilized phosphate buffer saline after being placed in sterile plastic bags. The bacteria were then serially diluted in phosphate buffer saline and inoculated on Lactobacillus MRS agar plates, where they were anaerobically incubated for 48 hours at 37ºC. Colonies that were morphologically distinct and well isolated were selected and transferred to new MRS agar plates for purity testing.

Identification of *Lactobacillus* Isolates

The morphological aspects of the selected bacterial isolates, such as gram staining and biochemical catalase and Oxidase assays, were used to characterize them. The results of the Vitek 2 system ANC ID card, which is developed for quick identification of Lactobacillus spp., are then used to determine the final identification of isolates.[8]

Quantitative Detection of Lectin

Serial two-fold dilutions of bacterial suspension in 0.02M phosphate buffer saline (PBS), pH 7.2, were combined with the same volume of a 2% solution of human erythrocytes type (A+, B+, AB+, and O+) in the same buffer and incubated at 37°C for 2 hours. Hemagglutination units were used to measure the activity (HU). The inverse of the maximum dilution capable of inducing agglutination was determined as one HU.[9]

Extraction and Preparation of Lectin

The cells of the chosen bacterial strain were cultured, then centrifuged, washed twice, and re-suspended in 0.02 M Phosphate Buffer Saline (PBS) pH 7.2. Glass beads were used to disrupt cells for 30 minutes at 4°C utilizing the vortex. Centrifugation at 8000 rpm for 20 minutes was used to remove any remaining complete cells or cell membrane fragments. The lectin's hemagglutination activity in crude cell extracts was determined using the supernatant as a starting point.[7] After several solvents such as ethanol, methanol, and acetone were used to precipitate the extract, the hemagglutination activity was measured.

Fungal Isolates

Six Candida albicans isolates were isolated from oral candidiasis on sabouraud dextrose agar, and chromogenic *Candida* agar then incubated aerobically for 48 hours at 37ºC. The diagnosis was confirmed by Vitek 2 system.

Antifungal Activity of Extracted Lectin

The antifungal activity of lectin at doses of 200, 250, and 300 µg/mL against isolated *Candida albicans* isolates was determined using the agar diffusion method. Using a sterile swab, the fungal isolates were dispersed on sabouraud dextrose agar plates at a concentration of 0.5 x 10^8 cell/ mL. A sterile cork borer with a diameter of 5 mm was used to create the wells, and 50 L of extracted lectin was placed in each well. The generated inhibitory zone was measured after a 48-hour incubation period at 37°C.[10]

RESULTS AND DISCUSSION

Quantitative Detection of Lectin

Lactobacillus spp. bacterial suspensions were tested against several types of human blood RBCs. As shown in Table 1, the lectins have a wide range of ability to hemagglutinate human erythrocytes, with larger titers of hemagglutination blood group type O+ and lower titers of hemagglutination blood group type A+ and B|+ (Table 1). Also, *L.acidophilus* produced lectin with a higher titer than *L.plantarum.* The lectin was more effective against the O+ blood group of humans than the other blood groups, and it's interesting to note that practically all of the major infectious diseases that plagued mankind before antibiotics have ABO blood group predilection for one or the other. Glycoconjugates, which are blood group antigens, are located on the surface of cells that line the digestive tract as well as on red blood cells (RBCs).[11]

Extraction and Preparation of Lectin

The extracted lectin from *L. acidophilus*[5] by glass beads led to an increase in the hemagglutination activity to 256 U/mL. The extract was partially purified by acetone, which increased the activity to 512 U/mL. At the same time, the other solvents don't lead to an increase in the hemagglutination activity as shown in Figure 1.

Antifungal Activity of Extracted Lectin

Using the agar well diffusion method, the antifungal activity of lectin was evaluated. The current investigation discovered that lectins with diameters ranging from 15 to 23 mm had the highest antifungal activity against the tested *Candida albicans* isolates, with diameters of 18, 19, and 23 mm at 200, 250, and 300 µg/mL, respectively (Figure 2). The hydrolytic enzymes can break down the *Candida* cell wall and biofilm, resulting in

Table 1: Screening leptin production by *Lactobacillus* spp.

	Human blood group			
Isolate	*A+*	*B+*	*AB+*	*O+*
L.acidophilus1	32	32	64	128
L.acidophilus2	16	16	32	64
L.acidophilus3	8	4	16	64
L.acidophilus4	8	8	8	16
L.acidophilus5	32	32	64	128
L.acidophilus6	4	2	8	16
L.acidophilus7	8	2	16	32
L.acidophilus8	16	16	32	128
L.plantarum1	4	16	32	64
L.plantarum2	4	8	16	32
L.plantarum3	16	16	32	128
L.plantarum4	8	8	16	64
L.plantarum5	2	8	16	16

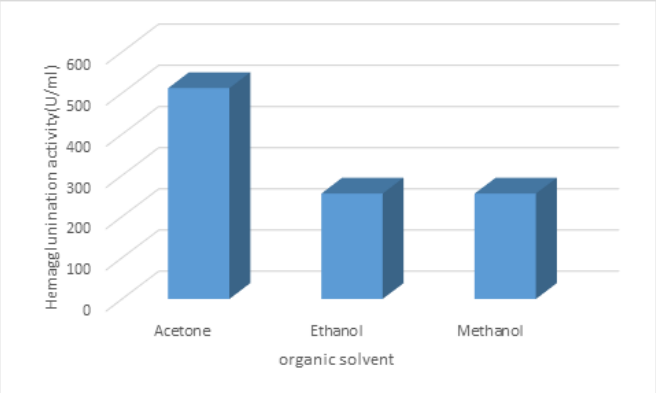

Figure 1: Precipitation of lectin from *Lactobacillus acidophilus*[5] by different solvents

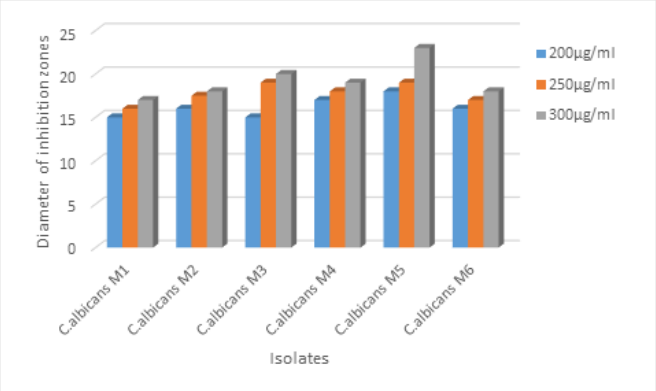

Figure 2: Antifungal activity of lectin evaluation

the formation of the – glucan layer, which protects the biofilm and boosts the immune response to *Candida* infections.[12] *Candida* pathogenesis is influenced by the production of biofilm and hypha, the secretion of certain enzymes, and adherence to the host's tissues.[13] As a result, the study concluded that dextranase could be utilized to prevent oral diseases such as dental caries. Against nystatin-resistant *Candida albicans* clinical strains, synergistic antipathogen actions between lectins and lectins and nystatin were identified.[14]

CONCLUSION

Lectin has promised an alternative to overcome multiple antifungal resistance.

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