

Isolation and Identification of Probiotic to Improve Nutritional Value of Diet Supplements

Urva Akmal¹, Muhammad Muneeb Subhani⁴, Shahid Usman², Salman Shabbir Abbasi³, Sonyia⁵, Ifra Ghor⁶, Shakira Ghazanfar^{7*} and Irum Arif⁷

¹Department of Environmental Sciences, Fatima Jinnah Women University Rawalpindi, Pakistan

²Department of Microbiology, Hazara University Mansehra, Pakistan

³Department of Biological Sciences, International Islamic University, Islamabad, Pakistan

⁴Center of Agri Biochemistry and Biotechnology (CABB), University of Agriculture Faisalabad, Pakistan

⁵Department of Biotechnology, Fatima Jinnah women University Rawalpindi, Pakistan

⁶Department of Microbiology, Govt College University Faisalabad, Pakistan

⁷National Institute of Genomics and Agriculture Biotechnology (NIGAB), National Agriculture Research Centre, Islamabad, Pakistan

***Corresponding Author:** Shakira Ghazanfar, National Institute of Genomics and Agriculture Biotechnology (NIGAB), National Agricultural Research Centre, Islamabad, Pakistan.

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Abstract

Probiotics are the live microbial feed supplement when administered in adequate amounts confers health benefits in the host. The objective of present research was to isolate the Lactic acid bacteria as potential probiotic. 10 bacterial strains were isolated from pickles and identified based on their morphological and biochemical characteristics. The non-sporulating, non-motile, gram positive, catalase and oxidase negative bacterial strains were selected for further probiotic characterization. All strains showed good probiotic potential. The *Pediococcus acidilactici* strain showed the best probiotic potential from all other strains. Our results demonstrated that the *Pediococcus acidilactici* is a unique bacteria for diet supplements. But, future research is required to produce nutritional diet supplements after assessing their advanced probiotic characteristics.

Keywords: Probiotics; *Pediococcus acidilactici*

Introduction

Assessment of current scenario related to food quality and safety determine that pathogenic microflora have gained resistance towards microbial agents through DNA vertical and horizontal transmission as a result of continual utilization of beneficial microflora [1]. As a result, these pathogenic microflora causes spoilage of food or make them unable for consumption. To resolve this reckless issue, scientists from all over the world are engaged to discover microorganisms (probiotics) proved to be beneficial to feed living organisms.

Probiotics are the live microbial feed additives, which improve the host health by improving gut microbiota. The immunomodulatory

properties of probiotics, however, are not displayed consistently and depend on the strain used, age of animals, and a number of other factors (Ohashi and Ushida 2009) It is advocated, therefore, to search for high-potential health beneficial bacteria from the human or animal microbiota that can be applied in a probiotic-like manner for this purpose. (van Baarlen, Wells, *et al.* 2013) As mentioned above, similarly to the mechanisms of action of probiotics in humans, the production of large amounts of lactic acid is thought to create an unfavorable environment for certain bacteria, including pathogenic strains of *E. coli*, *Salmonella*, or *Shigella*. Since the adoption of the term DFM (direct-fed microbial; a formulation of viable naturally occurring bacteria, fungi, or yeast) by FDA about two decades ago, there have been gigantic advances and progress

that have established the beneficial outcomes of exploiting micro-organisms and other feed additives in animal diets for improving animal's healthiness and productivity. The use of beneficial microbes in animal diets is appearing to be a promising mean for improved nutrient utilization and increased overall productivity of ruminants. A wide array of bacteria and yeasts have been screened out from the pools of naturally occurring microbes which are capable of positively manipulating and improving the production enhancements in ruminants such as nutrient digestion, nutrition, overall health, and productivity. Numerous DFMs are accessible today that have been rigorously documented for their positive effects on animal's weight gain, rumen development and digestion, restoration of intestinal microflora, producing bacteriocins, competitively reducing opportunistic pathogens, etc. In addition, several DFMs could also produce nutrients and stimulatory growth factors which in turn promote other beneficial microorganisms to flourish within the animal's digestive tract and boost the release of digestive enzymes thereby aiding in the feed digestion by the animal. Moreover, some selected DFM formulations have been proposed to perk up the immune responses as well as metabolism of the animals. Majority of such DFM mainly include lactic acid bacteria, probiotics, and yeasts, since these microbes confer several benefits when added to the animal's diet. These benefits may include stimulation of beneficial microbial growth in the rumen, decrease in ruminal acidosis, stabilization of the rumen pH, positive alteration of ruminal fermentation and end-product production, increase in ruminal propionate concentrations, increased plasma hormones, increase in nutrient flow, efficient nutrient digestibility, enhanced immune responses, and increased milk production. Generally, the population densities of rumen bacteria are dominating with the magnitude of 10^8 – 10^{11} , with rumen protozoa prevailing in the range of 10^5 – 10^7 and anaerobic fungi and some facultative anaerobic bacteria falling in the range of 10^4 (McAllister and Cheng 1996). However, these proportions of microbial population may vary significantly or slightly in response to animal diet types such as hay to concentrate rations, concentrate diets, complexity of fibers, pH fluctuations, etc. (Mackie, Gilchrist, et al. 1984).

Therefore, the present study was designed to determine whether probiotics (*Lactobacillus*) have probiotic potential or not. In this experimental based research, probiotics were isolated from traditional pickles, collected from the Rawalpindi region of Pakistan.

The pickle samples were of carrots (*Daucus carota* subsp. *Sativus*), radish (*Raphanus raphanistrum* subsp. *Sativus*), garlic (*Allium sativum*) and green chilli (*Capsicum annuum*).

Material and Methods

Isolation and biochemical characterization of bacterial strains

Isolation of probiotics was done by the procedure given by [2]. Serial dilution was done by transferring 1ml of brine from sample into 9ml of autoclaved saline. After that, serial dilution was further done by using this diluent. 0.02ml from each diluent was spread on MRS media plates and left for 24 hours at 37°C (Table 1). 10 Lactic Acid bacterial strains (PCR99, PCR100, PCR118, PCR119, PCR121, PCR125, PCR130, PCR137, PCR140 AND PCR141) were identified based on their morphological and biochemical characteristics by using the procedure given by [2-4]. Therefore, non-sporulating, non-motile, gram positive, catalase and oxidase negative LAB strains were selected for further probiotic characterization.

MRS Agar recipe X amount	
Ingredients	Amount (%)
Peptone	1.0
Beef extract	1.0
Yeast extract	0.4
Glucose	2.0
Sodium acetate	0.5
Polysorbate 80	0.1
Dipotassium hydrogen phosphate	0.2
Tri ammonium citrate	0.2
Magnesium sulfate	0.02
Manganese sulfate	0.005
Agar	1.0

Table 1: MRS Agar recipe X amount.

Identification of bacterial strains by 16S rRNA gene sequence

The bacterial strains were identified through 16S rRNA gene sequence by using the commercial service Macrogen Korea. The sequence was identified through EXBio Cloud service. The identified sequence was submitted to NCBI for accession numbers. The phylogenetic tree of the isolated strain was made through MEGA 7.

Probiotic characterization of the selective bacterial strains

Antibiotic resistance

For the determination of the media was prepared for the preparation of Mueller-Hinton plate, we used the standard method. The inoculum was prepared and the MH plates was inoculated in that plates. The antibiotic disc placement was done on these plates and then set them for overnight incubation. The zone of the size was measured through the standard CLSI guidelines.

Results

These 10 strains were molecularly identified as *Lactobacillus plantarum* (PCR99, PCR100, PCR125), *Lactobacillus. paracasei* (PCR118, PCR119, PCR121, PCR140, PCR141), *L. brevis* (PCR137) and *P. acidilactici* (PCR130). To utilize these isolated *Lactobacillus* and *Pediococcus strains* as probiotics, tolerance to low acidic and salt conditions is crucial. This tolerance makes them able to tolerate gastrointestinal tract severe conditions and function in digestive system. Results of present study shows that *Lactobacillus plantarum* (PCR100), *Lactobacillus paracasei* (PCR118) and *Pediococcus acidilactici* (PCR130) shows best response towards low acidic (pH 3 and pH 4) and bile salt conditions (0.3% and 0.5%) and show highest cell surface hydrophobicity values 84.62%, 82.39 and 86.12%. *Pediococcus acidilactici* showed the best results from all strains. All the strains show different enzymatic potential responses indicating that this property is strain-dependent. Furthermore, study relating to their antibiotic susceptibility done by using the procedure given by [5] resulted that *Lactobacillus plantarum* (PCR100) is resistant to Ampicillin, tetracycline, Kanamycin, polymyxin B, Metronidazole, Amoxycillin, Nalidixic Acid, Cefuroxime, Vancomycin. While, *Lactobacillus paracasei* (PCR118) showed resistance towards Cefuroxime, Amoxycillin, Metronidazole, Penicillin G, Ampicillin, Polymyxin B, Bacitracin. Besides, *Pediococcus acidilactici* (PCR130) showed resistance towards Metronidazole, Amoxycillin and Penicillin G. Antibiotic resistance among probiotic bacterial strains is desirable or not is a debated since some scientists claimed that they can pass their resistance to pathogenic microflora and make them more detrimental for health. On contrary to this, other scientists have different point of view that probiotics are healthy as they feed beneficial digestive microflora which are killed and destroyed by using antibiotics.

Figure 1: Colony morphologies of bacterial isolates on MRS agar media.

Figure 2: Microscopic observation of gram staining isolated bacterial species.

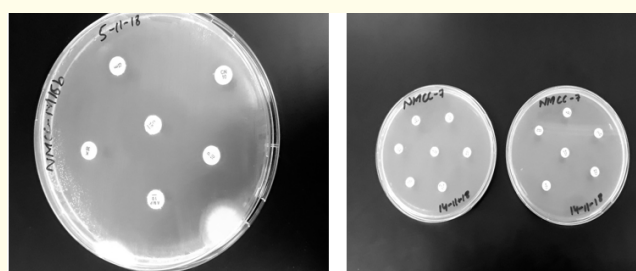


Figure 3: Antibiotic susceptibility of the *Pediococcus acidilactici*.

Lab ID	Color	Form	Opacity	Elevation	Margins	Texture	Pigment	Gram staining	Catalase
1	Off white	Small round	Translucent	Flat	Entire	Smooth	No	Positive Rods	Negative
2	Creamy white	Small round		Slightly Raised	Entire	Smooth	No	Positive Rods	Negative
3	White	Punctiform	Transparent	Flat	Entire	Smooth	No	Positive Rods	Negative
4	Creamy white	Round	Translucent	Slightly Raised	Entire	Smooth	No	Positive Rods	Negative
5	Off- white	Small Round	Translucent	Slightly Raised	Entire	Smooth	No	Positive cocci	Negative
6	Creamy off white	Small Round	Translucent	Slightly Raised	Entire	Smooth	No	Positive Rods	Negative
7	Creamy white	Small Round	Opaque	Slightly Raised	Entire	Smooth	No	Positive Rods	Negative
8	Off white	Small Round	Opaque	Elevated	Entire	Smooth	No	Positive cocci	Negative
9	Creamy white	Small circular	Translucent	Slightly Raised	Wavy	Smooth	No	Positive Rods	Negative
10	Creamy white	Round	Translucent	Slightly Raised	Entire	Smooth	No	Positive Rods	Negative

Table 2: Morphological Characterization Samples.

Figure 4: Phylogenetic tree of NMCC-10 bacterial strain.

Conclusion

Results of the present study concluded that *Lactobacillus Para-casei* (PCR118), *Lactobacillus plantarum* (PCR100) and *Pediococcus acidilactici* (PCR130) are best for their utilization to produce nutritional supplements for human beings and animal as they showed beneficial and desirable responses to low acidic and bile salt conditions, possess highest hydrophobicity values and displayed sensitivity towards antibiotics. Therefore, future research is required to produce nutritional diet supplements after assessing their advanced probiotic characteristics.

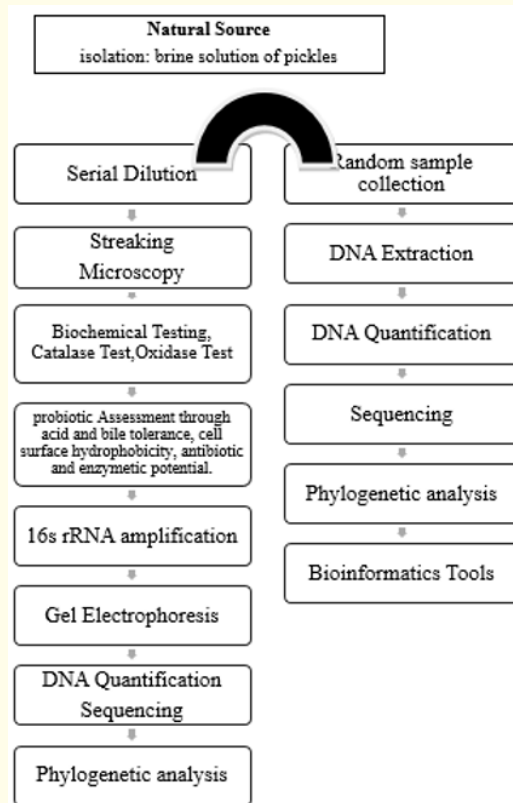


Figure 5: Isolation and Characterization of Probiotic Lactic Acid Bacterial Strains From Traditionally Fermented Pickles.

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