VOLUME - VIII, ISSUE - I - JANUARY - MARCH - 2019
AJANTA - ISSN 2277 - 5730 - IMPACT FACTOR - 5.5 (www.sjifactor.com)

12. Acid and Bile Tolerance of Lactic Acid Bacteria Isolated from Chicken GIT

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Abstract

The aim of this research was to obtain the lactic acid bacteria (LAB) with good probiotic potential including resistance to bile salt and acid conditions. In this study, 5 backyard poultries were located and samples were collected. The collected samples were inoculated on MRS Agar and incubated at 37°C in anaerobic jars. A total of 11 different isolates identified based on morphological and some biochemical characters. 04 out of 11 isolates were further used for the in vitro study of probiotic potential based on antagonistic nature. All strains showed good to moderate tolerance for highly acidic pH. Except LAB 4, all other LAB isolates were able to survive higher concentrations of bile salt (4000 ppm).

Key-words: Acid Tolerance, Bile Tolerance, Chicken, Lactic Acid Bacteria, Probiotic

Introduction

The gastrointestinal tract (GIT) is the major digestive and absorbing organ which plays an important role in animal growth and health. In bird, the GIT microflora has been regarded as fulfilling crucial roles in nutrition, growth performance and protection against pathogenic bacteria (Mead et al 2000; Jun Wang et al 2012). Antibiotics have been frequently used in intensively reared poultry production in order to improve growth performance (Butaye et al., 2003). Recently, increasing concern on the antibiotic resistance in bacteria has led to greater interest in the use of probiotics in poultry production to control bacterial infection and reduce the use of antibiotics.

Probiotics are defined as live microbial food supplements that beneficially affect the host by improving intestinal microbial balance. Among the potential probiotics, lactic acid bacteria (LAB) are reported to have important effects on animal performance. The gram positive lactic

acid bacteria (LAB) have been well known for thousands of years for their important role in the food industry due to their fermentative capacities (McKay and Boldvin, 1990).

In recent years, the role of these bacteria in health and functionality of human and animal intestine has been well emphasized, mainly because of their ability to lower the pH and to produce antimicrobial agents (Delves-Broughton, 1990; Ten Brink et al., 1994).

It is well known that lactic acid bacteria have adapted to grow under widely different environmental conditions, and they are widespread in nature. Apart from food sources, lactic acid bacteria are commonly found in the gastrointestinal tract of various endothermic animals, mice, rats, pigs, fowl and humans (Weese et al., 2004).

The present experiment aimed to isolate and identify lactic acid bacteria as a suitable probiotic candidate from GI tract of backyard chicken. A few Probiotic properties were also investigated to find out high potential probiotic of selected strain to use in poultry feed preparations.

Materials and Methods

Lactobacillus Enumeration and Isolation

Lactic Acid Bacteria (LAB) were isolated from the GIT of backyard chicks aged between 20 to 30 d after birth. Samples were collected from five different backyard poultries and inoculated in 100ml MRS broth and incubated at 37°C for 24hrs in anaerobic jars. Later the bacterial consortium was streak on de Man Rogosa Sharp (MRS) agar supplemented with bromo-cresol purple (Jimenez et al., 2008). After incubation, colonies producing a clear zone and with different morphologies were collected and purified on the same medium. Only Gram positive, non-spore forming and catalase negative bacterial isolates were selected for further investigation.

Probiotic characteristics

pH tolerance

Acid tolerance of the selected bacterium was investigated at different pH. First, MRS broths with different pH including 2, 4 and 6 were prepared using HCl 1N (Sigma) and NaOH 1N (Sigma) (Samelis et al., 1994). The broths media along with control bottles were autoclaved at 121°C for 15 min and then inoculated with overnight culture of the selected strain in MRS broth followed by incubation at 30°C. Optical density (OD) as growth rate of bacteria was measured by spectrophotometer (Shimadzu, UV-1601, Japan) at 600 nm after 24 h incubation.

Bile Tolerance

The modified method of Arihara et al. (1998) was used to determine bile tolerance of selected LAB. Before testing for bile tolerance, LAB strains were grown at 37°C for 24 hour in MRS broth without bile. 1ml of the culture broth was poured onto MRS agar with bile salt concentrations of 2000, 3000 and 4000 ppm. Bacterial growth was determined after incubation at 37°C for 48 hour.

Results

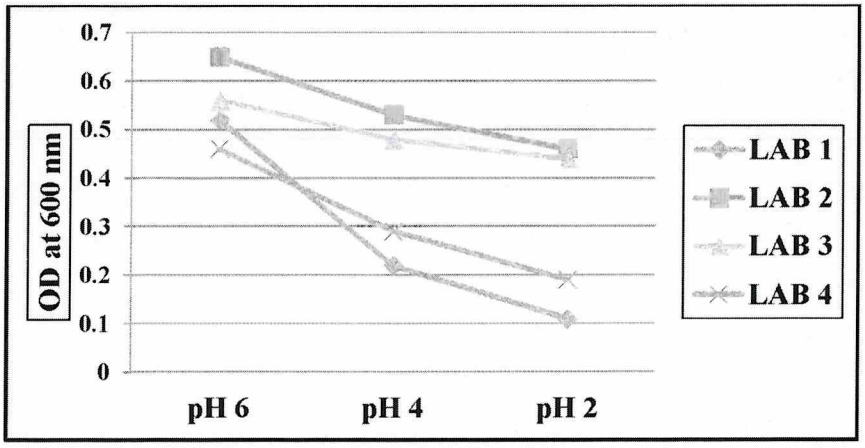
Lactobacillus enumeration and isolation

The bromo-cresol purple in MRS agar as an indicator causes yellow colonies in color for lactic acid bacteria (Badis et al., 2004; Rengpipat et al., 2008). A total of eleven colonies of lactic acid bacteria were isolated from collected samples. The isolates were gram-positive, catalase-negative and short rod or coccobacilli in shape.

Based on antimicrobial potential of LAB isolates against common poultry pathogens, 04 strains were further selected for the study of pH and Bile tolerance.

pH tolerance

In this study, all the four isolates were able to survive in from pH 6 to pH 2. LAB 2 and LAB 3 were found to actively multiplying even at extreme acidic conditions (pH 2). On other hand, LAB 1 and LAB 4 showed poor growth at pH 2. The survival of all isolates at low pH confirm that these isolates can transit through stomach and may be able to multiply in extreme conditions.



Graph 1: pH Tolerance of Lactic Acid Bacteria isolated from Chicken GIT

Bile Tolerance

Except LAB 4, all remaining isolates showed satisfactory growth at higher bile salt concentrations. LAB 1 and LAB 3 were not only able to survive in higher bile concentrations but

found to be effectively multiplied under highest concentration (4000 ppm). Whereas, LAB 4 did not survived at 4000 ppm concentration of Bile salt.

	Bile salt concentration		
	2000 ppm	**3000 ppm**	**4000 ppm**
LAB 1	+++	++	++
LAB 2	+++	++	+
LAB 3	+++	+++	++
LAB 4	++	+	-

Table 1: Bile salt Tolerance of Lactic Acid Bacteria isolated from Chicken GI

Discussion

As said earlier, the major aim of this study was to isolate potential probiotic candidate from chicken GIT. Here, we had collected GIT sample from five different backyard poultries. From these sources we got eleven different Lactic Acid Bacteria colonies on MRS medium. Out of which four isolates were further selected to study their probiotic potential based on their antagonistic effect against enteric pathogens similar type of screening was carried out by Ten Brink et al., 1994 for separation of effective probiotic strains from other less effective strains.

The isolates were checked with some of the important probiotic properties including pH tolerance and Bile salt tolerance.

pH is crucial factor which significantly affect bacterial growth. There is an always extreme acidic condition in gastric environment and potential probiotic candidate must tolerate it. In present work, all LAB isolates were able to survive up to extreme acidic pH (pH 2) similar results were demonstrated by Ram Kumar Pundir et al., 2013.

In present work 2000ppm to 4000 ppm of bile concentrations were used, as it corresponds to that found in the chicken intestinal tract. 2500 ppm is the maximum bile concentration in healthy bird. LAB 1, LAB 2 and LAB 3 were able to survive at 4000 ppm.

In conclusion, the experimental results revealed that, isolated Lactic Acid Bacteria are able to show satisfactory probiotic potential including tolerance to pH and Bile salt.

Reference

1. Arihara, K., Ota, H., Itoh, M., Kondo, Y., Sameshima, T., Yamanaka, H., Akimoto, M., Kanai, S. and Miki, T. 1998. Lactobacillus acidophilus group lactic acid bacteria applied to meat fermentation. J. Food Sci. 63(3): 544-547.
2. Badis, A., Guetarni, D., Moussa-Boudjemaa, B., Henni, D. E., Tornadijo, M. E., & Kihal, M. 2004. Identification of cultivable lactic acid bacteria isolated from Algerian

raw goat's milk and evaluation of their technological properties. Food Microbiology, 21: 343-349.

3. Delves-Broughton, J. 1990. Nisin and its uses as a food preservative. Food. Technol. 44:100-112.
5. Jimenez, E., L. Fernandez, A. Maldonado, R. Martin, M. Olivares, J. Xaus and J.M. Rodriguez. 2008. Oral administration of Lactobacillus strains isolated from breast milk as an alternative for the treatment of infectious mastitis during lactation. Appl. Environ. Microbiol. 4(15): 4650–4655.
6. McKay, L. L. and K. A. Boldwin. 1990. Applications for biotechnology: present and future improvements in lactic acid bacteria. FEMS Microbiol. Rev. 87:3-14.
7. Mead G. 2000. Prospects for competitive exclusion treatment to control salmonellas and other foodborne pathogens in poultry. Vet. J., 159(2): 111-123.
8. Nguyen TDT, Kang J H, Lee MS. 2007. Characterization of Lactobacillus plantarum PH04, a potential probiotic bacterium with cholesterol-lowering effects. Int. J. Food Microbiol. 113: 358-361.
9. Ram Kumar Pundir, Satish Rana, Neha Kashyap and Amandeep Kaur. 2013. Probiotic potential of lactic acid bacteria isolated from food samples: an in vitro study. Journal of Applied Pharmaceutical Science Vol. 3 (03): 085-093.
10. Rengpipat, S., Phianphak, W., Piyatiratitivorakul, S., & Menasveta, P. 1998. Effect of probiotics on Black tiger shrimp Penaeus monodon survival and growth. Aquaculture, 167: 301-313.
11. Samelis J, Maurogenakis F, Metaxopoulos J. 1994. Characterisation of lactic acid bacteria isolated from naturally fermented Greek dry salami. Int. J. Food Microbiol. 23: 179-196.
12. Ten Brink, B., M. Minekus, J. M. B. M. van der Vossen, R. J. Leer and J. H. J. Huis in't Veld. 1994. Antimicrobial activity of lactobacilli: preliminary characterization and optimization of production of acidocin B, a novel bacteriocin produced by Lactobacillus acidophilus M46. J. Appl. Bacteriol. 77:140-148.
13. Weese JS, Anderson ME, Lowe A, Penno R, da Costa TM, Button L, Goth KC. 2004. Screening of the equine intestinal microflora for potential probiotic organisms. Equine Vet J. 36(4):351-355.