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EFFECT OF VARYING CULTURE CONDITIONS ON ANTIMICROBIAL ACTIVITY OF POTENT PROBIOTIC *Lactobacillus plantarum* DM 69

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Abstract: Effect of various factors like temperature (15 ^{0}C, 25 ^{0}C, 30 ^{0}C and 37 ^{0}C), pH (3.0, 4.0, 5.0, 6.0 and 7.0), salt concentrations (2.0, 4.0, 6.0 and 8.0%) and incubation period (18, 24, 30, 36, 42 and 48 h) on antimicrobial activity of potent probiotic *Lactobacillus Lactobacillus plantarum* DM 69 was performed. The optimum antimicrobial compound production was judged by their different zones of inhibition of crude supernatant.
Keywords: Culture conditions, probiotic Lactobacillus species, antimicrobial activity.

Introduction

Lactobacilli are characterized as gram-positive, rods or coccobacilli, non-motile and catalase-negative, non-spore-forming, aerotolerant and strictly fermentative. It constitutes an important part of the indigenous microflora of man and widely distributed throughout the gastrointestinal tract. They ferment lactic acid from glucose in both homofermentative and heterofermentative way (Gomes and Malcata, 1999). *Lactobacilli* stimulate the growth of indigenous beneficial gut microbata by inhibiting the growth of opportunistic pathogenic microbes by producing antimicrobial substances, such as organic acids, fatty free acids, ammonia, hydrogen peroxide, biosurfactant and Bacteriocins (Gomes et al 2012). Different antimicrobial compound exhibits different inhibition profile on pathogenic microorganisms. In order to increase the productivity of such compounds, a better understanding of factors affecting their production is essential. Bacteriocin production has been reported to be affected by several factors including carbon and nitrogen sources; and fermentation conditions, such as pH, temperature and agitation.

Materials and methods

Effect of temperature on antimicrobial activity of potent probiotic *Lactobacillus* DM 69

To find the effect of temperature on antimicrobial activity of potent probiotic *Lactobacillus* isolate, the isolate was inoculated (1% v/v) in 10 mL of sterile MRS broth and incubated at different temperatures such as 15 ^{0}C, 25 ^{0}C, 30 ^{0}C and 37 ^{0}C for 24 h. At the end of

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incubation period, the cell-free supernatant was collected by centrifugation (10000 rpm for 10 min at 4 ^{0}C) and filtered through a sterilized 0.22 µm MILLEX-GP filter. They were checked for their antimicrobial activity against test pathogens such as *E. coli* ATCC 25922, *Bacillus cereus* ATCC 10702, *Salmonella typhi* MTCC3216, *Salmonella enterica* subsp. *enteric* ATCC 35640, *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* MTCC 902 by the agar well diffusion method.

Effect of pH on antimicrobial activity of potent probiotic *Lactobacillus* DM 69

To find the effect of pH on antimicrobial activity of potent probiotic *Lactobacillus* isolate, the isolate was inoculated (1% v/v) in 10 mL of sterile MRS broth adjusted in different pH (3.0, 4.0, 5.0, 6.0 and 7.0). At the end of incubation period, the cell-free supernatant was collected by centrifugation (10000 rpm for 10 min at 4 ^{0}C) and filtered through a sterilized 0.22 µm MILLEX-GP filter. They were checked for their antimicrobial activity against same pathogens by the agar well diffusion method.

Effect of salt (NaCl) concentration on antimicrobial activity of potent probiotic *Lactobacillus* DM 69

To find the effect of salt concentration on antimicrobial activity of potent probiotic *Lactobacillus* isolate, the isolate was inoculated (1% v/v) in 10 mL of sterile MRS broth containing different concentrations of NaCl (2.0, 4.0, 6.0 and 8.0%) and incubated at 37 ^{0}C. At the end of incubation period, the cell-free supernatant was collected by centrifugation (10000 rpmfor 10 min at 4 ^{0}C) and filtered through a sterilized 0.22 µm MILLEX-GP filter. They were checked for their antimicrobial activity against same pathogens by the agar well diffusion method.

Effect of incubation period on antimicrobial activity of potent probiotic *Lactobacillus* DM 69

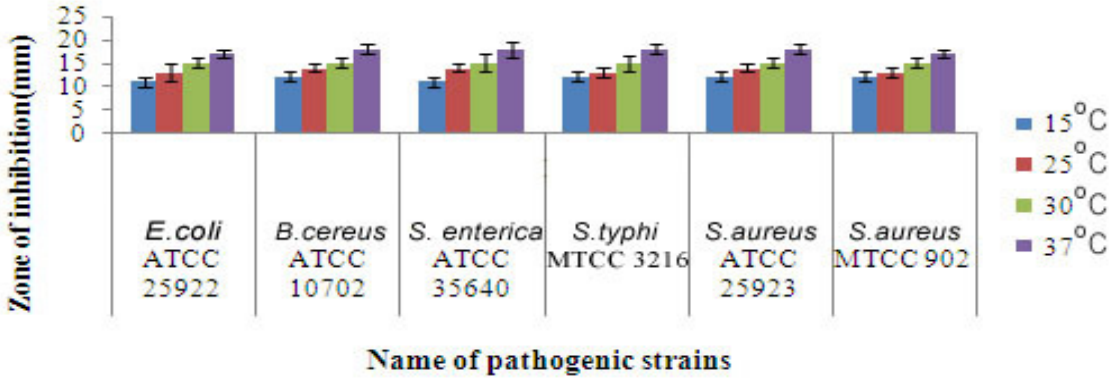
To find the effect of incubation period on antimicrobial activity of potent probiotic *Lactobacillus* isolate, the isolate was inoculated (1% v/v) in 100 mL of sterile MRS broth. Inoculated flask was incubated at 37 ^{0}C for 18, 24, 30, 36, 42 and 48 h. At the end of each incubation period, the cell-free supernatant was collected by centrifugation (10000 rpm for 10 min at 4 ^{0}C). They were filtered through a sterilized 0.22 µm MILLEX-GP filter and checked for their antimicrobial activity against same pathogens by the agar well diffusion method.

Result

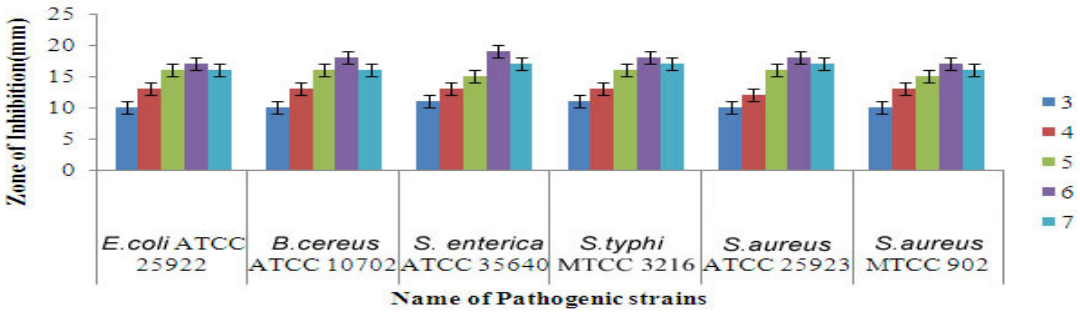
The effect of varied incubating temperatures on crude supernatant activity of the isolate DM 69 in de Man Rogosa Sharpe broth was determined by its antibacterial activity against the

indicator strains. The result showed that antibacterial activity was highest at 37 ^{0}C followed by 30 ^{0}C. It was moderate at 15^{0}C and 25 ^{0}C as seen in Graph 1 A. The results obtained in this study regarding crude supernatant activity in media incubated at varied pH values (Graph 1 B) showed optimum activity at pH 6.0 followed closely by pH 5.0 and 7.0 for indicator organisms. Moderate activity was found at pH 3.0 and 4.0. This pH tolerance is an extremely important feature since the isolates have the ability to survive, grow and produce antimicrobial compound under acidic and alkaline conditions. Furthermore, the effect of varied sodium chloride (NaCl) concentration in medium on crude supernatant activity was also evaluated. Highest zone of inhibition of crude supernatant was observed at NaCl concentration of 2.0%, but started reducing as salt concentration increased (Graph 1 C). The effect of varied incubating duration on crude supernatant activity was affected as different zones of inhibition were observed against the indicator organisms (Graph 1 D). Optimum crude supernatant activity was observed after 18 h judged by the zones of inhibition against the indicators. There were observable reduction in crude supernatant activities as incubation time dropped.

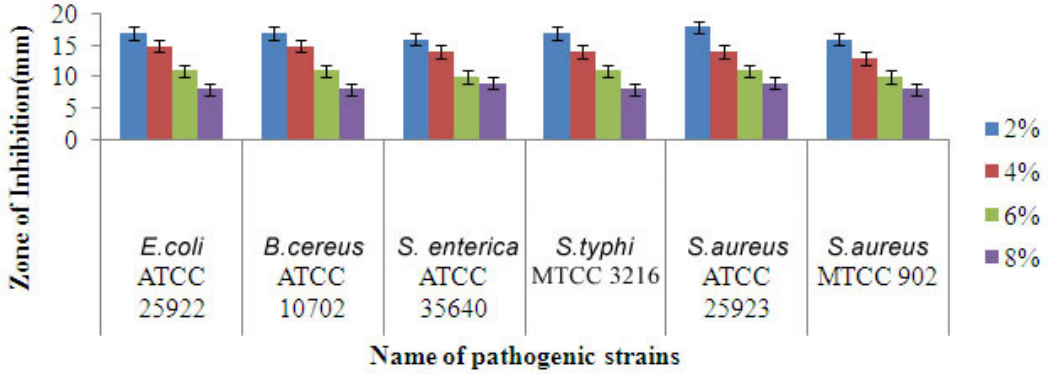
Graph 1: Effect of different factors on antimicrobial activity of *L. plantarum* DM 69
(A) Effect of temperature on antimicrobial activity of *L. plantarum* DM 69



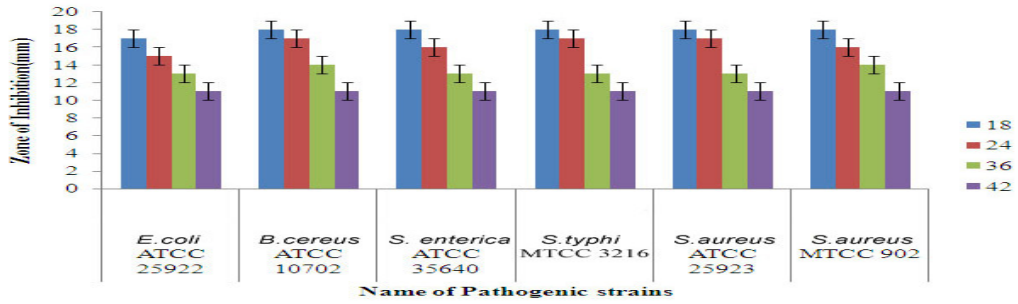
(B) Effect of pH on antimicrobial activity of *L. plantarum* DM 69



(C) Effect of salt (NaCl) concentration on antimicrobial activity of *L. plantarum* DM 69



(D) Effect of incubation period on antimicrobial activity of *L. plantarum* DM 69



Discussion and conclusion

It has been reported that the antimicrobial activity can be influenced by physical factors such as temperature, pH, salt concentrations and incubation period of *Lactobacillus* isolates (Onwuakor et al., 2014). In the present study, effect of such factors on antimicrobial activity of potent probiotic *Lactobacillus* strainwas performed as per the protocol reported in Onwuakor et al., (2014). The result showed that antimicrobial activity was highest at 37 ^{0}C followed by 30 ^{0}C where moderate activity was found in 15 ^{0}C and 25 ^{0}C. In addition, antimicrobial activity was found maximum at pH 6.0 closely followed by pH 5.0 and 7.0 for

indicator organisms. Moderate activity was found at pH 3.0 and 4.0. On other hand, highest zones of inhibition of crude supernatant was observed at NaCl concentration of 2.0%, but started reducing as salt concentration increased. In case of incubation period, optimum crude supernatant activity was observed after 18 h which was judged by the zones of inhibition against the indicators. There was observable reduction in crude supernatant activities as incubation time dropped. These findings were matched with the previous reports on effect of temperature, pH, NaCl concentration and incubation period on antibiosis activity of *Lactobacillus* species (Djadouni and Kihal, 2012; Onwuakor et al., 2014). As per Djadouni and Kihal (2012), the effect of incubation temperature on the antimicrobial activity of crude supernatant of *Lactobacillus* strain showed that the highest inhibitory effect was obtained in MRS agar after 24 h of incubation at 30°C; increasing the incubation temperature had a significant adverse effect on active compounds concentration. Different pH values ranging from 3.5-9.5 were used to test the production of antimicrobial protein (bacteriocin) by *Lactobacillus* strainunder investigation. Low levels of antimicrobial protein (bacteriocin) activity was recorded when both the test strains LBbb0141 and LBdc0103 were cultured in MRS agar with an initial pH of 3.5. Similarly, as per the report of Onwuakor et al., (2014), the effect of varied incubating temperatures on crude supernatant production by the isolates in de Man Rogosa Sharpe broth was determined by its antibacterial activity against the indicator strains. The result showed that antibacterial activity was highest at 30 ^{0}C followed by 35 ^{0}C. The results obtained in this study regarding crude supernatant activity from media incubated at varied pH values showed optimum activity at pH 6.0 followed closely by pH 5.0 for both indicator organisms. Furthermore, the effect of varied medium percentage sodium chloride (NaCl) concentration on crude supernatant production and activity was also evaluated. Highest zones of inhibition and consequent optimum crude supernatant production was observed at NaCl concentration of 4.0%, but started reducing as salt concentration increased further. Ogunbanwo et al., (2003) found that bacteriocin was produced during the pre-and early exponential growth phases and reached a maximum level at late stationary phase. Verellen et al., (1998) found that maximum production of plantaricin by a strain of *Lactobacillus plantarum* coincided with the onset of logarithmic phase and early stages of stationary phase when nutrients were available for metabolic activity.

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