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Optimization of cultural parameters for the production of antimicrobial compound from *Lactobacillus fermentum* (MTCC No. 1745)

Abstract

To improve the productivity of antibacterial compounds of *Lactobacillus fermentum* by optimizing its nutrient and physical factors and screened for its antimicrobial activity by agar well diffusion method. In order to improve its efficiency, the effects of medium components carbon and nitrogen sources, temperature, pH, agitation, incubation time, were optimized and its productivity was determined by agar well diffusion method against four bacterial strains obtained from MCC, Pune, India namely *E.coli, Bacillus subtilis, Staphylococcus aureus*, *Psudomonas aeruginosa*. The bacterial inhibition rate was more in the optimized medium composition (g/100mL), containing dextrose 3.0, tryptone 1.5 and incubation time for 72hrs, temperature 36±2°C and pH 7. Compared to basal medium the optimized medium shown about 1.2 fold increased in the zone of inhibition by *Lactobacillus fermentum*. The results from this study confirmed that the antibacterial substances produced by *Lactobacillus fermentum* were found to be more effective after its optimization.

Keywords: *lactobacillus fermentum*, zone of inhibition, optimization, antimicrobial compounds, lactic acid bacteria

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Introduction

Lactic acid bacteria (LAB) are economically important since they used in food and feed fermentation. They produce different types of substrates with the antimicrobial properties which can be used as bio preservatives[1] *Lactobacillus* are gram positive bacteria fitting within the general definition of LAB. LAB has been used in the production of a variety of dairy, vegetables and meat fermented foods for many centuries. *Lactobacillus* are used in probiotics improving the microbial balance of intestine or assay beneficial agent in the treatment of gastro enteritis in humans and animals.[2] In addition to the contribution to the typical sensory characteristics of these foods,[3] LAB exert a strong antimicrobial activity against many microorganisms, as a result of the production of hydrogen peroxide, organic acids, inhibitory enzymes, antimicrobial compounds and bacteriocins.[4] Production of antimicrobial compounds by an *Lactobacillus fermentum* depends on many parameters like nutrients, salt concentration, pH and temperature. In fact, the composition of culture medium closely associated with the metabolic capacities of the producing strain and significantly influences the biosynthesis of secondary metabolites.[5] The concept of medium optimization for secondary metabolites production involves the exploitation of medium components and cultural condition to obtain the desired product in a cost effective manner.

In the present investigation an attempt has been made to investigate the effect of different nutrients and cultural conditions for the maximum production of Zone of inhibition by *Lactobacillus fermentum*.

Materials and methods

Materials

MRS broth, yeast extract, Tryptone, lactose, nutrient broth, malt extract, dextrose and galactose were procured from Himedia, Mumbai, India. Maltose, beef extract, Sucrose, nutrient agar, Xylose, fructose, from Merck, India. All chemicals used were of analytical grade.

Methods

The isolate *Lactobacillus fermentum* (MTCC No. 1745) used in this study was procured from MTCC (Microbial type culture collection), Chandigarh, India.

Test organisms used in the study

E.coli, Bacillus subtilis, Psudomonas aeruginosa, Staphylococcus aureus, were obtained from MCC, Pune. The cultures obtained were in the form of lyophilized powders in sealed vials. The cultures were revived in Nutrient broth and stored in agar slants for further study.

Effect of different carbon sources

Lactobacillus fermentum was inoculated in the basal media and kept in incubator shaker at optimized speed and temperature for 36hours. Various carbon sources used in the medium were arabinose, fructose, dextrose, galactose, glucose, lactose, maltose, mannose and sucrose at a final concentration of 1%. A flask without any carbon source was kept as a control.

Determination of optimum concentration of best carbon source

Among different carbon sources used, the carbohydrate which supported the maximum growth of *Lactobacillus fermentum* and production of Zone of inhibition was further optimized by changing its concentration from 1 % to 6% and determined the optimum concentration of the best carbon source.

Effect of different nitrogen sources

The growth and production of Zone of inhibition was controlled

by using different nitrogen sources like L-asparagine, tyrosine, casein, beef extract, peptone, soybean meal, tryptone and yeast extract at a final concentration of 1%.

Determination of optimum concentration of best nitrogen source

The maximum production of Zone of inhibition shown by nitrogen source was further optimized by altering its concentration from 0.5% to 3.0%, to determine the optimum concentration.

Effect of pH

To evaluate the effect of pH on growth and zone of inhibition was determined by changing the pH (5.0 to 9.0) by adjusting to required value by addition of 1N HCl or 1N NaOH of the optimized media containing best carbon and nitrogen source.

Effect of temperature

The optimized media containing best nitrogen, carbon sources at optimum pH was incubated at various temperatures ranging from 20°C to 50°C, to determine the optimum temperature required for maximum growth and production of Secondary metabolite.

Effect of incubation period

The optimum incubation period required for the growth and production of Zone of inhibition was determined by incubating the optimized media with best carbon, nitrogen and amino acid sources at optimum pH and temperature at different incubation periods (12h to 96h).

Statistical analysis

The results analyzed in this study were the mean or SD (Standard Deviation) of three independent experiments. The data was statistically analyzed by one way ANOVA and the means were assessed by DMRT (Dunken Multiple Range Test) at 0.5% level of significance.

Results and discussion

Effect of different carbon sources

Maximum zone of inhibition shown by *Lactobacillus fermentum* was observed with dextrose as a carbon source (Figure 1). Whereas minimum zone of inhibition was observed with lactose.

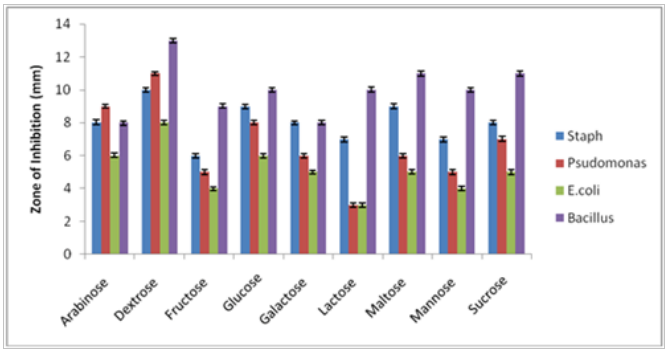

Figure 1 Effect of carbon source on the growth of *Lactobacillus fermentum* and zone of inhibition.

Determination of optimum concentration of best carbon source

As shown in the Figure 2, there is an increase in the zone of inhibition production at 3g of dextrose.

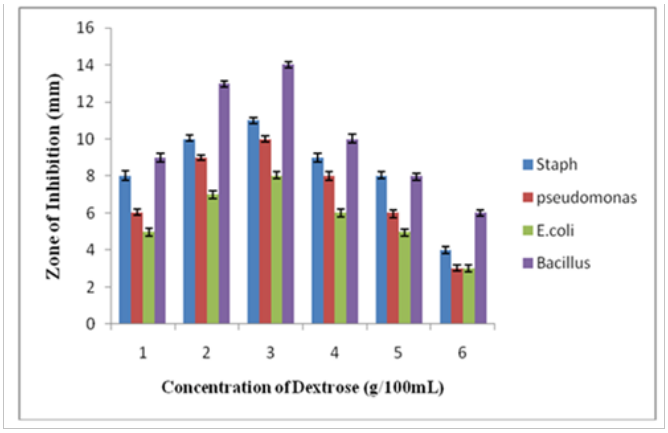

Figure 2 Determination of optimum concentration of best carbon source.

Effect of different nitrogen sources on of zone of inhibition

Among eight different nitrogen sources used, maximum zone of inhibition was observed with tryptone followed by beef extract and soybean meal (Figure 3). Low Zone of inhibition production was observed in the medium containing casein.

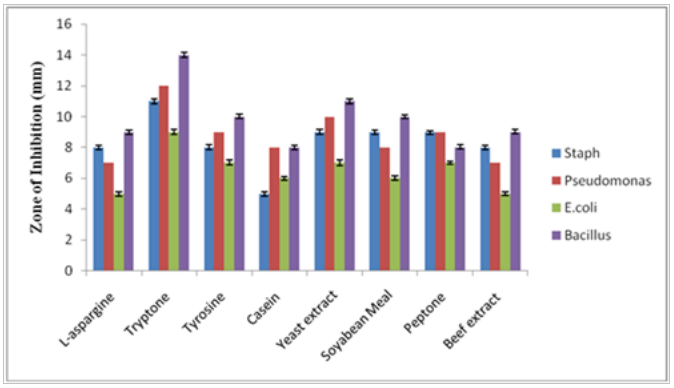

Figure 3 Effect of Nitrogen source on *Lactobacillus fermentum*.

Determination of optimum concentration of best nitrogen source

As shown in Figure 4, there is a continuous increase in the growth of *Lactobacillus fermentum* and zone of inhibition production from 0.5 to 3g of trptone. However, further increase in the tryptone concentration showed a gradual decrease in Zone of inhibition.

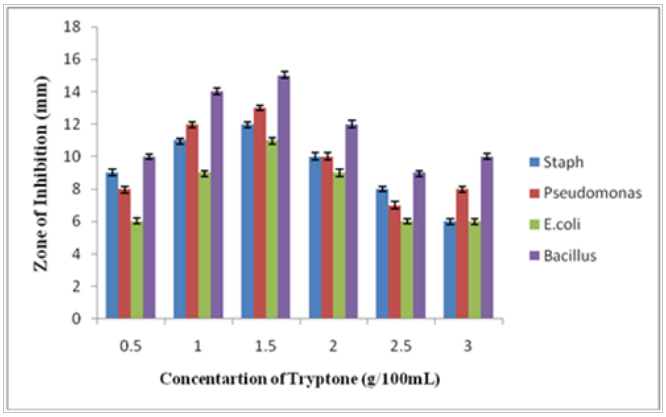

Figure 4 Effect of tryptone on growth and zone of inhibition.

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Effect of pH on growth of *Lactobacillus fermentum* and of zone of inhibition

The zone of inhibition was observed at pH 6.0 and beyond this there is a sudden decrease in zone of inhibition at pH 8.0 (Figure 5).

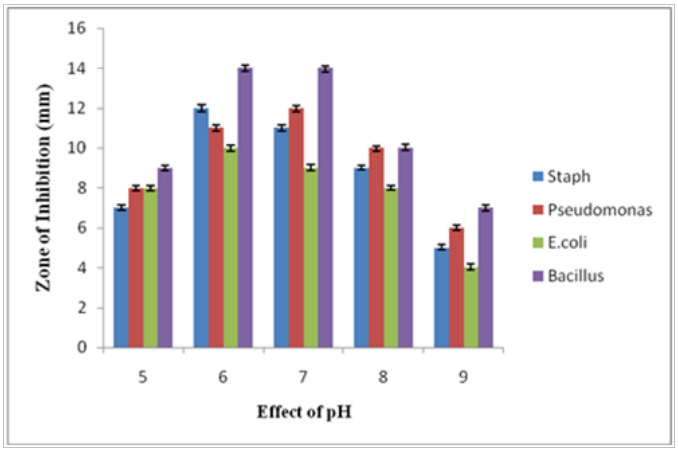

Figure 5 Effect of pH on growth and Zone of inhibition production.

Effect of on temperature *Lactobacillus fermentum* and zone of inhibition

Figure 5, shows the optimum zone of inhibition at 35°C and beyond optimal temperature, zone of inhibition was less.

Effect of incubation time on *Lactobacillus fermentum* and zone of inhibition

There was a sharp increase in the growth of *Lactobacillus fermentum* and Zone of inhibition from 48hours of incubation and gradually increased up to 72h of incubation (Figure 6).

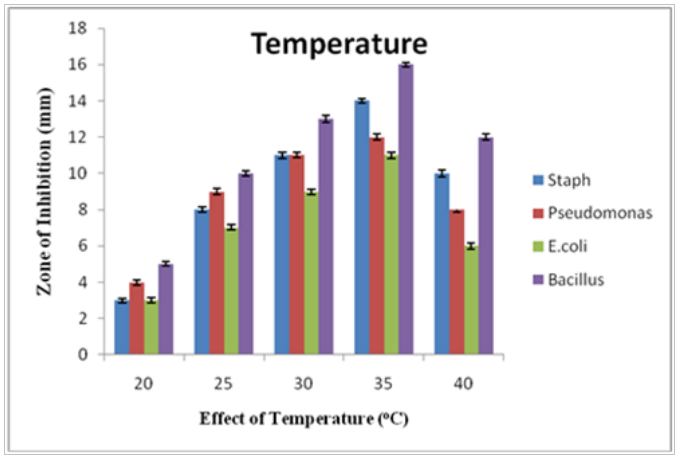

Figure 6 Effect of temperature on growth and Zone of inhibition production.

Mushood et al.,[6] reported optimization of temperature and pH conditions for the production of secondary metabolite using *Lactobacillus faecium* B3L3 at pH 8 and 37°C. Ouardy et al.,[7] has reported 2% tryptone and pH 6.5 has increases the antimicrobial activity compared to control in *Lactobacillus durans* E204.

Conclusion

Based on the above optimized studies, the composition of the nutrient medium and physical parameters required for the optimum growth and zone of inhibition by *Lactobacillus fermentum* were presented in Table 1. When compared to basal medium the optimized medium showed about 1.2 fold increased in the production of Zone of inhibition by *Lactobacillus fermentum* (Figure 7 & 8). Similar reports were observed by Marine bacteria *Lactobacillus* with a 1.6 fold increase.[8,9]

Table 1 Optimized production medium and culture conditions for *Lactobacillus fermentum*

Composition of optimized production medium and cultural conditions	(g/100mL)
Dextrose	3
Tryotone	1.5
Yeast extract	0.5
Sodium acetate	0.5
Di-potassium phosphate	0.3
pH	6.5
Temperature	36°C
Aeration	160rpm
Incubation time period	76h

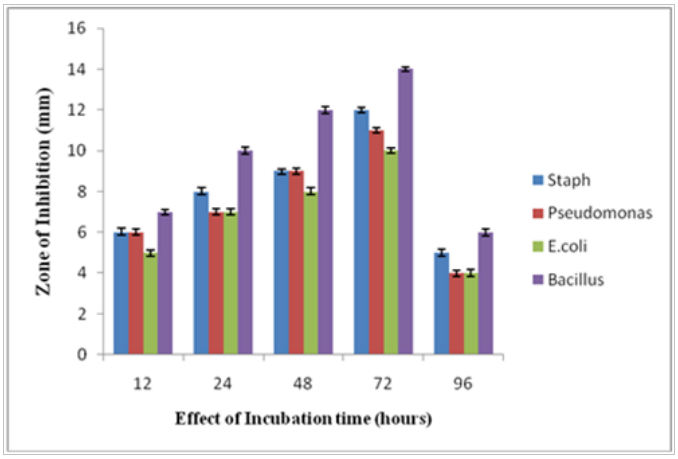

Figure 7 Effect of Incubation time on growth and zone of inhibition production.

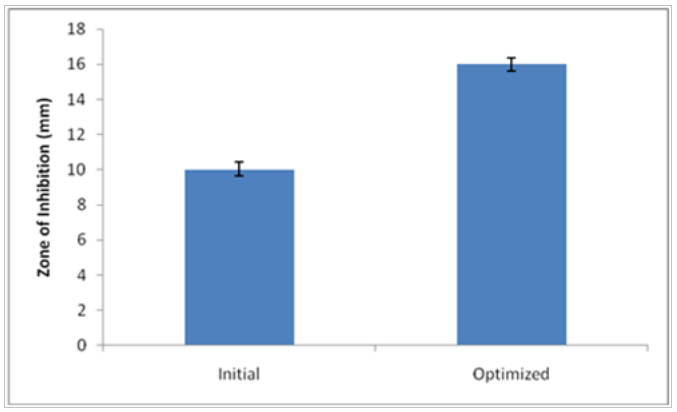

Figure 8 Zone of inhibition production by basal and optimized media.

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Conflict of interest

The author declares no conflict of interest.

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