Academic Sciences

International Journal of Current Pharmaceutical Research

ISSN- 0975-7066 Vol 7, Issue 3, 2015

Original Article

OPTIMIZATION OF CULTURAL PARAMETERS FOR THE PRODUCTION OF ANTIMICROBIAL COMPOUND FROM *ENTEROCOCCUS FAECIUM* CST-1 (MCC-2729)

CHANDRA MOULI LALAM*[1,2], T. SRINIVASAN[1], VSSL PRASAD TALLURI[1], NAIDU P. Y.[2]

[1]Department of Biotechnology, GITAM Institute of Science, GITAM University, Visakhapatnam 530045, India, [2]Analytical research and development, Hospira, Chennai, India
Email: chandramoulilalam@gmail.com

Received: 21 May 2015, Revised and Accepted: 30 Jun 2015

ABSTRACT

Objective: To improve the productivity of antibacterial compounds of *Eenterococcus feacium* CST-1 by optimizing its nutrient and physical factors and screened for its antimicrobial activity by agar well diffusion method.

Methods: 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 MTCC, Chandigarh, India namely *Bacillus subtilis, E. coli, Psudomonas aeruginosa, Staphylococcus aureus*,.

Results: The bacterial inhibition rate was more in the optimized medium composition (g/100 ml), containing tryptone 1.5, dextrose 3.0 and incubation time for 76 hrs, temperature 35±2 °C and pH 6.5. Compared to basal medium the optimized medium shown about 1.2 fold increased in the zone of inhibition by *Enterococcus feacium* CST-1.

Conclusion: The results from this study confirmed that the antibacterial substances produced by *Eenterococcus feacium* CST-1 were found to be more effective after its optimization.

Keywords: *Enterococcus feacium,* Zone of inhibition, Optimization, Antimicrobial compounds.

INTRODUCTION

Lactic acid bacteria (LAB) have been used in the production of a variety of dairy products, vegetables and meat fermented foods for many centuries and also economically important because since they were used in food fermentation. They produce different types of substrates with the antimicrobial properties which can be used as bio preservatives [1]. In addition to the contribution to the typical sensory characteristics of these foods 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 [2, 3]. *Enterococcus faecium* are gram positive bacteria fitting within the general definition of LAB. Enterococcus is used in probiotics because of its importance in enhancing the microbial balance of intestine or assay beneficial agent in the treatment of gastro enteritis in humans and animals [4]. Production of antimicrobial compounds by an *Enterococcus feacium* 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 *Enterococcus feacium*.

MATERIALS AND METHODS

Materials

MRS broth, NaCl, peptone, yeast extract, nutrient broth, starch, lactose, nutrient agar, dextrose and galactose were procured from Himedia, Mumbai, India. Maltose, beef extract, Sucrose, Xylose, malt extract, fructose, from Merck, India. Dextrose, trisodium citrate, urea, citric acid were procured from Qualigens, mumbai, India. All chemicals used were of analytical grade.

Methods

The isolate *Enterococcus feacium* used in this study was isolated from visakha dairy soil sample [6]. The secondary metabolite production was done by growing the *Enterococcus feacium* in the MRS broth medium using a 500 ml Erlenmeyer at pH 6.5±2 and 37 °C for 72 hrs. To estimate the zone of inhibition activity, the fermentation broth was centrifuged at 10,000 rpm for 10 min at 4 °C and the supernatant was used to perform the antimicrobial acitivity [7]. Agar well diffusion method was used for determination of antimicrobial activity [8].

Test organisms used in the study

Bacillus subtilis (MTCC10403), E. coli (MTCC1652), Pseudomonas aeruginosa (MTCC4676), Staphylococcus aureus (MTCC3160), were obtained from MTCC, Chandigarh. 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

Enterococcus feacium was inoculated in the basal media and kept in incubator shaker at optimized speed and temperature for 36 hours. Various carbon sources used in the medium were arabinose, fructose, dextrose, glucose, galactose, 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 *Enterococcus feacium* 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 were 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 the 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) adjusted to required value by addition of 1 N HCl or 1 N 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 were 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 the best carbon, nitrogen sources at optimum pH and temperature at different incubation periods (12 h to 96 h).

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 DISCUSSIONS

Effect of different carbon sources

Maximum zone of inhibition shown by *Enterococcus feacium* was observed with dextrose as a carbon source (fig. 1). Whereas, minimum zone of inhibition was observed with lactose.

Determination of optimum concentration of best carbon source

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

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 (fig. 3). Low Zone of inhibition production was observed in the medium containing casein.

Determination of optimum concentration of best nitrogen source

As shown in fig. 4, there is a increase in the growth of *Enterococcus feacium* and zone of inhibition production at 1.5g of tryptone. However, further increase in the tryptone concentration showed a gradual decrease in Zone of inhibition.

Effect of pH on growth of *Enterococcus feacium* and of zone of inhibition

The zone of inhibition was observed at pH 6.0 and pH 7.0, beyond there is a sudden decrease in the zone of inhibition at pH 8.0 (fig. 5).

Effect of on temperature *Enterococcus feacium* and zone of inhibition

Fig. 6, shows the optimum zone of inhibition at 35 °C and beyond optimal temperature, zone of inhibition was less.

Effect of incubation time on *Enterococcus feacium* and zone of inhibition

There was a sharp increase in the growth of *Enterococcus feacium* and Zone of inhibition from 48 hours of incubation and gradually increased up to 72 h of incubation (fig. 7) [9].

Reported optimization of temperature and pH conditions for the production of the secondary metabolite using *Enterococcus faecium* B3L3 at pH 8 and 37 °C [10] has reported 2% tryptone and pH 6.5 has increases the antimicrobial activity compared to control in *Enterococcus durans* E204.

Table 1: **Optimized production medium and culture conditions for *Enterococcus feacium***

Composition of optimized production medium and cultural conditions	(g/100 ml)
Dextrose	3.0
Tryptone	1.5
Yeast extract	0.5
Sodium acetate	0.5
Di-potassium phosphate	0.2
pH	6.0
Temperature	35°C
Aeration	160rpm
Incubation time period	76 h

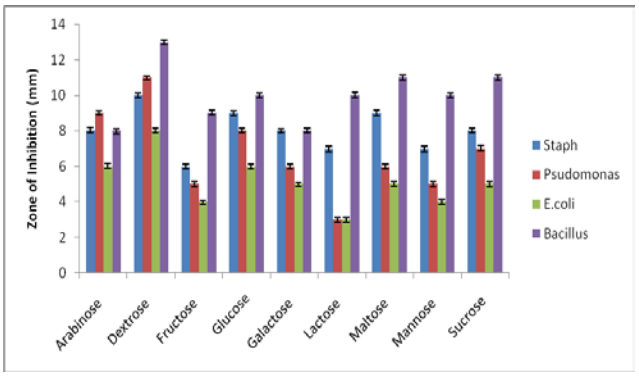

Fig. 1: Effect of carbon source on the growth of *Enterococcus feacium* and zone of inhibition

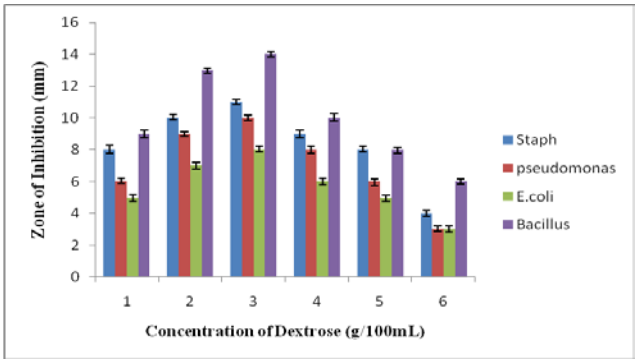

Fig. 2: Determination of optimum concentration of best carbon source

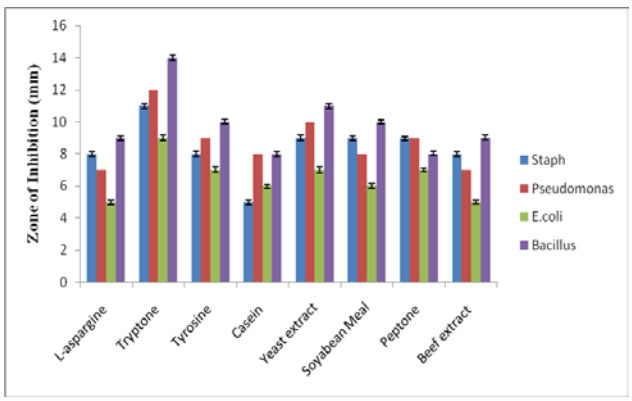

Fig. 3: Effect of Nitrogen source on *Enterococcus feacium*

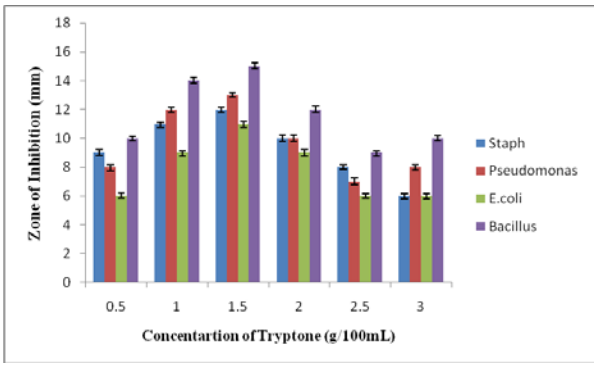

Fig. 4: Effect of tryptone on growth and zone of inhibition

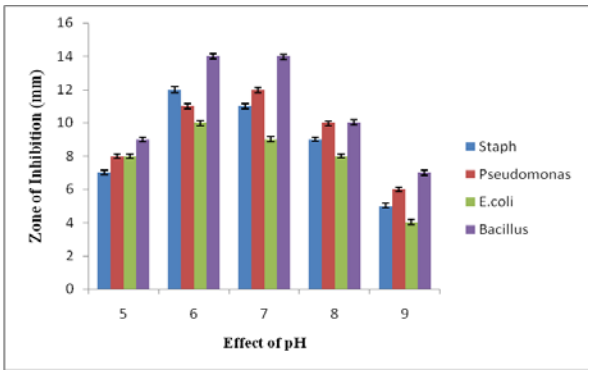

Fig. 5: Effect of pH on growth and Zone of inhibition production

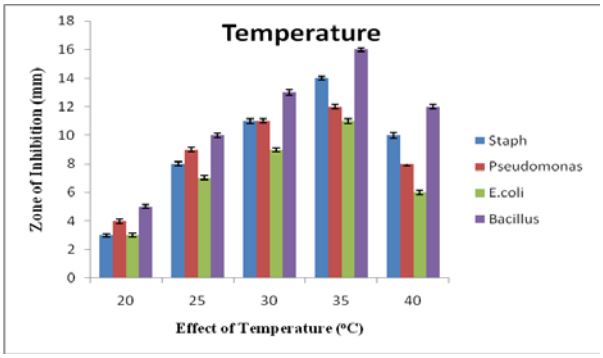

Fig. 6: Effect of temperature on growth and Zone of inhibition production

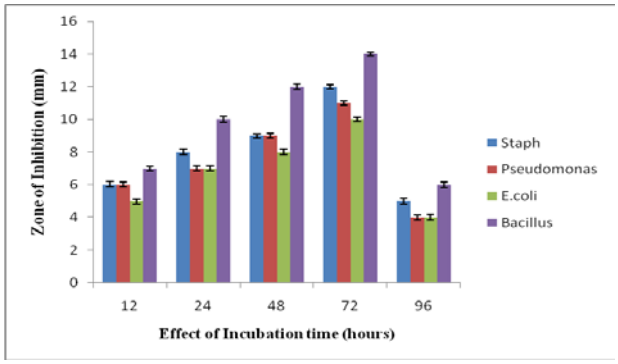

Fig. 7: Effect of Incubation time on growth and zone of inhibition production

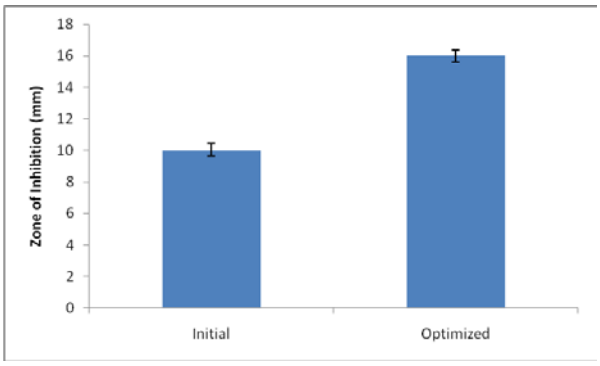

Fig. 8: Zone of inhibition production by basal (initial) and optimized media

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 *Enterococcus feacium* was 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 *Enterococcus feacium* CST-1 (fig. 8). Similar reports were observed by [11] Marine bacteria *Enterococcus feacium* with a 1.6 fold increase.

CONFLICT OF INTERESTS

Declared None.

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