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IMPACT OF CULTURE CONDITIONS ON THE PRODUCTION OF CURVACIN A BY *LACTOBACILLUS CURVATUS* LC05

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ABSTRACT

Curvacin A, a type of bacteriocin produced by *Lactobacillus curvatus* LC05 has large spectrum of inhibition against human pathogenic, food spoilage microorganisms and many strains of *Lactobacillus* were isolated as a test organisms. The Curvacin A inhibited *Enterococcus faecalis* EF1 *Escherichia coli* NCTC 10418 but did not inhibit *Candida albicans* ATCC 10321. The antibacterial activity of the strains were observed between mid lograthmic and stationary phase of growth. Larger quantity of Curvacin A was found produced when the medium is supplemented with yeast extract (3.0%) sodium chloride (1.0-2.0%), glucose (1%) and Tween 80 (0.5%). Supplementation of sodium acetate, magnesium sulphate, tri ammonium citrate and dipotassium phosphate had no effect on production of Curvacin A.

KEY WORDS: Bacteriocin, Curvacin A, growth media, *Lactobacillus curvatus* LC05, antagonistic activity

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INTRODUCTION

Lactic acid bacteria, a physiologically related group of Gram positive bacteria are widely used in the production of various fermented products and even cosmetics ingredients [1] . The presence of these bacteria proved to produce desirable and unique flavour in the food stuffs. They produce various compounds such as organic acids, diacetyl, hydrogen peroxide and bacteriocin during lactic acid fermentations[2] .Lactobacilli show strong antagonistic activity against many microorganisms, including food spoilage organisms and human pathogens.production of secondary metabolites, decrease in pH and Lactic acid synthesis are the main preserving factor in food fermentation. In addition, some lactobacilli produce other inhibitory substances such as bacteriocins. Bacteriocins are antimicrobial proteinaceous substances that are inhibitory to sensitive strains and are produced both by Gram positive and Gram negative bacteria [3].

Usage of bacteriocins as a natural food preservative is increasing during last decades. But the in depth research on antibacterial properties of lactic acid bacteria have not been fully carried out. [4] Maximal production of Curvacin A, a type of bacteriocin of *Lactobacillus curvatus* could be obtained when culture medium is supplemented with growth regulating factors such as carbon and nitrogen sources, vitamins and also regulating pH of the medium and growth temperature.

MATERIALS AND METHODS

Sample collection

The dairy products like raw milk, curd, cheese and fermented rice products like dosa mix were procured from retail markets in Tiruchengode, Namakkal district, Tamilnadu. For each variety, 20 samples were collected in a sterile container adopting aseptic procedure. They were packed in ice boxes and transferred to the laboratory within 2 h.

Isolation of Lactic Acid Bacteria

Man Rogosa Sharpe (MRS) broth and agar (Hi Media, Mumbai) were used for enumeration and culture of lactic acid bacteria. The samples were homogenized in a stomacher blender using saline, serially diluted and pour plated on MRS plated. The plates were overlaid with MRS agar and incubated at 37 C for 48 – 72 h[5].

Identification of the bacterial strains

The cultures were identified according to their morphological, cultural, physiological and biochemical characteristics.

Bacteriocin assay

Lactobacillus curvatus LC05 was cultivated in MRS broth for 48 h at 35 C. Extraction of bacteriocin was carried out.. The culture supernatant was purified to ensure that only bacteriocin (Curvacin A) was assayed. Bacteriocin was detected at every stages of the purification. Antagonistic activity of Curvacin A against indicator human pathogenic bacteria was performed using well diffusion assay. [6] The unitage of antimicrobial activity of Curvacin A is defined as the reciprocal of the highest dilution showing inhibition of the indicator lawn. It is expressed as activity units per ml (AU/ml)[7]

Influence of medium component on the production of curvacin A

The effect of medium ingredients on curvacin A production was evaluated using composed media. The supplements studied were tryptone (0.0 – 3.0%), yeast extract (0.0 – 3.0%), beef extract (0.0 – 3.0%), triammonium citrate (0.0-2.0%), sodium acetate (0.0-0.5%), magnesium sulphate (0.0-0.1%), di potassium phosphate (0.0-0.2%), sodium chloride (0.0-3.0%) and tween 80 (0.0-1.0%) [8].

Influence of growth conditions on the production of curvacin A

Effect of temperature

The effect of incubation temperature and time on production of curvacin A was carried out. Three portions of composed media were inoculated (1% v/v) with an overnight culture of curvacin A producing organism; incubated at 25,30,37,45 and 55 C for 48 h and the absorbance values (580 nm), pH and curvacin A activities of cultures were determined [9].

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Effect of initial pH

To determine the effect of initial pH on production of curvacin A, 100 ml of composed media were adjusted to initial pH values of 4.5,5.0, 5.5, 6.0, 6.5, 7.0 and 7.5, using 5 mMol hydrochloric acid or 5 mMol NaoH. Each medium was inoculated (1% v/v) with an overnight culture of curvacin A producing organism and incubated at 30 C for 48 h. Absorbance values (580 nm) pH and bacteriocin activities were determined [10].

Effect of incubation time

The effect of incubation period was studied in the same manner as described for pH. Active cultures of producer organism (1% v/v) were inoculated into 100ml aliquots of sterile composed in Erlenmeyer conical flasks. Inoculated at 37 C for periods of 12, 24,36,48,60 and 72 h. Individual flasks were kept for each incubation period. At the end of each incubation period, bacteriocin activity, pH and absorbance values (580 nm) were determined[11].

RESULTS AND DISCUSSION

The present investigation was aimed primarily at optimizing the cultural conditions for obtaining stable and viable production of curvacin A by *Lactobacillus curvatus* which had wide range of antagonistic effect over many Gram positive and Gram negative pathogenic bacterial species (Table 1)

Table 1
Antagonistic activity of Curvacin A produced by Lactobacillus curvatus LC05 against human bacterial pathogens

Organism	Strain No	Zone of inhibition
Bacillus cereus	ATCC 9634	9 mm
Bacillus subtilis	NCBI 8222	7mm
Staphylococcus aureus	ATCC 14458	8mm
Staphylococcus faecalis	ATCC 19433	12mm
Listeria monocytogenes	CHRL 587	8mm
Candida albicans	ATCC 10231	-
Escherichia coli	NCTC 10418	11mm
Vibrio cholerea	AP 15534	11mm
Shigella dysentry	AP 23498	10mm
Salmonella typhimurium	ATCC 13311	10mm
Serratia marcescens	Soil sample	10mm
Klebsiella pneumoniae	Clinical sample	09 mm
Micrococcus futeus	NCIB 196	10mm

Note : '_' indicates , no zone of inhibition.

The influence of culture medium components on the production of curvacin A was analysed using *Escherichia coli* as indicator organism[13]. The results showed that curvacin A production was observed when main nutrients were present in the medium. When the medium was supplemented with tween 80(0.5%), yeast extract (2-3.0%), glucose (1.0%) and NaCl (1-2.0%), larger quantity of bacteriocin production was found synthesized. Addition of other chemical supplements like tri ammonium citrate, manganese sulphate, sodium acetate magnesium sulphate and potassium phosphate had no effect on curvacin a production (table 2). This proves the role of constituents in medium might have an influence on the production of Curvacin A by *Lactobacillus curvatus LC05*[14].

Table 2
Effect of Nutrient components on Curvacin A production by Lactobacillus curvatus LC05

Medium component	%	Growth(580nm)	Final pH	Activity of CurvacinA
MRS + Beef extract	0.0	0.6±0.01	3.90±0.12	1500±0.00
	1.0	0.7±0.16	3.95±0.23	3100±0.00
	2.0	1.5±0.12	3.96±0.34	700±0.00
	3.0	1.7±0.12	4.01± 0.12	300±0.00
MRS + glucose	0.0	0.6±0.03	4.11±0.12	1500±0.00
	1.0	0.7±0.03	4.03±0.12	6500±0.00
	2.0	1.1±0.02	3.98±0.22	3300±0.00
	3.0	1.3±0.01	3.89±o.11	700±0.00
MRS+ Sodium acetate	0.0	0.4±0.00	4.04±0.01	3100±0.00
	0.1	0.6±0.11	4.06±0.12	3100±0.00
	0.2	0.9±0.11	4.10±0.13	3100±0.00
	0.3	1.1±0.12	4.12±0.12	3100±0.00
MRS+ Sodium chloride	0.0	0.9±0.02	3.80±0.15	3100±0.00
	1.0	0.8±0.01	3.83±0.22	6500±0.00
	2.0	0.5±0.22	3.85±0.11	3100±0.00
	3.0	0.3±0.12	3.89±0.12	700±0.00
MRS+ Tri ammonium citrate	0.0	0.6±0.03	4.05 ±0.11	3100±0.00
	1.0	0.7±0.02	4.06 ±0.11	3100±0.00
	2.0	1.1±0.01	4.07±0.12	3100±0.00
	3.0	1.2±0.02	4.08±0.11	3100±0.00
MRS+ Tryptone	0.0	0.3±0.11	3.50±0.11	3100±0.00
	1.0	0.8±0.12	3.81±0.11	3100±0.00
	2.0	1.3±0.11	4.00±0.21	700±0.00
	3.0	1.6±0.11	4.05±0.11	700±0.00
MRS+ Tween 80	0.00	0.7±0.11	4.31±0.11	1500±0.00
	0.1	0.9±0.11	3.80±0.21	3100±0.00
	0.5	0.7±0.22	3.85±0.11	6300±0.00
	1.0	0.2±0.11	3.90±0.22	1500±0.00
MRS+ Yeast extract	0.0	0.7±0.11	4.01±0.11	700±0.00
	1.0	0.8±0.11	4.02±0.22	3100±0.00
	2.0	1.2±0.11	4.02±0.13	6500±0.00
	3.0	1.5±0.11	4.05±0.11	6500±0.00
MRS+ Magnesium sulphate	0.0	0.6±0.11	3.89±0.11	3100±0.00
	0.1	0.9±0.22	3.99±0.12	3100±0.00

	0.2	1.0±0.22	4.01±0.11	3100±0.00
	0.3	1.1±0.11	4.03±0.22	3100±0.00
	0.4	1.2±0.11	4.06±0.21	3100±0.00
MRS+ Dipotassium	0.0	0.7±0.11	3.90±0.11	3100±0.00
hydrogen	0.1	0.8±0.12	3.80±0.21	3100±0.00
phosphate	0.2	1.2±0.11	3.45±0.11	3100±0.00

Similar observations have been made previously. Biswas[15] compared the production of pediocin ACH by *Peidcococcus acidilactici* H grown in TGE broth, MRS broth and several modifications of it. TGE broth containing various concentration (0-2.0 %), of trypticase glucose and yeast extract found produced maximum quantity of pediocin at the 1% level. When cultivated in normal MRS broth, 15% less pediocin production was observed compared to the yield in TGE broth. Sanni *et al*[16]. also reported that highest activity of curvacin A was observed when the concentration of peptone and glucose were varied from 0.25% and 0.5% in MRS broth. Alteration of nutrients in growth media should also to be considered for maximum production of curvacin A that has potential use as food preservative From this observation we note that inexpensive production medium could be formulated for better production of bacteriocin like Curvacin A[17]

During analysis of growth conditions of the test isolate, minimum yield of curvacin A was observed at early and mid lograthmic growth phase and increase in yield was observed at stationary phase [18] Beyond stationary phase a decline in bacteriocin production was observed. This may be due to the synthesis of proteinase enzyme by the test organism, which got accumulated in culture flasks. But some reports indicate that the production of bacteriocin was found throughout the growth phase and not particularly during exponential and stationary phases[19,20,21].

CONCLUSION

The influence of growth temperature, incubation period and pH of the medium on production of Curvacin A was also investigated. The use of constituted medium at 37 C of growth temperature, initial pH 6.0 and for 36 to 48 h enhanced the best production of Curvacin A by *Lactobacillus curvatus LC05.*

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