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ORIGINAL RESEARCH ARTICLE

Effect of Storage Time and Temperature for Maximum Bacteriocin Production by Lactic Acid Bacteria

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ABSTRACT

Lactic acid bacteria (LAB) occur naturally in several raw materials like milk, meat and flour used to produce foods. LAB are used as natural or selected starters in food fermentations in which they perform acidification due to production of lactic and acetic acids flavour, protection of food from spoilage and pathogenic microorganisms by LAB is through producing organic acids, hydrogen peroxide, diacetyl, antifungal compounds such as fatty acids or phenullactic acid and bacteriocins. In the present study, effect of storage time and temperature for maximum bacteriocin production by lactic acid bacterial isolates was studied. Among the bacteriocins produced, the bacteriocin of *Streptococcus thermophilus* could be inacted only after a storage period of five weeks though there was a gradual decrease in the activity

Key words: Lactic acid bacteria, Bacteriocin, Storage time and Temperature.

1. INTRODUCTION

Lactic acid bacteria (LAB) are those that produce lactic acid as the sole product or major acid from the energy yielding fermentation of sugars. They can be broadly defined as Gram positive, anaerobic, microaerophilic or aerotolerent bacteria, either rod or coccus, catalase negative and fastidious in their growth. The most important contribution of these bacteria to fermented products is to preserve the nutritive qualities of the raw material and inhibit the growth of spoilage and pathogenic bacteria [1]. This inhibition may be due to the production of many metabolites such as organic acids (lactic and acetic acid), hydrogen peroxide, diacetyl and bacteriocins [2]. Some bacteriocins kill only bacteria belonging to the same species whereas other bacteriocins kill a broad range of Gram positive bacteria [3, 4].

Bacteriocin of LAB is considered as safe natural preservatives or biopreservatives, as it is assumed that they are degraded by the proteases in gastrointestinal tract. Bacteriocins are extracellularly released peptides or protein molecules, with a bactericidal or bacteriostatic mode of action against closely related species. The inhibitory spectrum of some bacteriocins also includes food spoilage and/or food borne pathogenic microorganisms. The discovery of nisin, the first bacteriocin used on a commercial scale as a food preservative dates back to the first half of last century but research on bacteriocin of LAB has expanded in the last two decades, searching for novel bacteriocin producing strains from dairy, meat and plant products, as well as traditional fermented products.

Bacteriocins are of special interest due to their potential uses as natural preservatives. Bacteriocins are antimicrobial proteinaceous compounds that are generally inhibitory towards sensitive strains and are produced by both Gram-positive and Gram-negative bacteria. Among the Gram-positive bacteria, bacteriocins produced by many lactic acid bacteria used in food fermentation and dairy products, including strains in the genera *Lactococcus, Lactobacillus, Pediococcus* and *Leuconostoc*. In USA, only nisin produced by *Lactobacillus lactis* is permitted as a food preservative. Bacteriocins are protein molecules synthesized for various lineages of Gram positive and Gram negative bacteria when exposed it stressful conditions. Bacteriocins have been characterized as molecules of high antimicrobial property even at low concentrations by antibiosis [5]. Bacteriocins are generally defined as extracellularly released peptide or protein that shows a bactericidal activity against more

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distantly related species like *Listeria monocytogenes*.

2. MATERIALS AND METHODS

2.1. LAB used

The lactic acid bacterial isolates used in this present study were,

a) *Lactobacillus acidophilus*
b) *Lactococcus lactis* sub sp. *lactis*
c) *Pediococcus acidilactici*
d) *Streptococcus thermophilus*

2.2. Effect of storage time and temperature

Bacteriocin was stored at 4°C (refrigerator), -20°C (deep freezer, blue star) and 37°C for a period of six weeks. At different time intervals *viz.*, 0, 1, 2, 3, 4, 5 and 6th weeks, samples were taken from the stored materials for the determination of the remaining activity of bacteriocin by Agar spot test.

3. RESULTS AND DISCUSSION

The influence of storage period and storage temperature on the inhibitory activity of bacteriocin of all lactic acid bacteria *viz. Lactobacillus acidophilus, Lactococcus lactis, Pediococcus acidilactici* and *Streptococcus thermophilus* were studied and the results are presented in the tables 1-4. The bacteriocins of LAB were stored for a period of six week at three different temperatures *viz.*, -20, 4 and 37°C. The results revealed that, the inhibitory activity of *Lactobacillus acidophilus* was affected only by 20% at a storage temperature of -20°C and affected by 40% at a temperature of 4°C for the storage period up to six months and five months respectively. It was also found that there was a gradual 20% reduction of activity at the storage temperature of 37°C and after 4 week of storage, the activity was completely lost (**Table 1**). The same trend of activity was observed for the bacteriocin of *Lactococcus lactis, Pediococcus acidilactici* and *Streptococcus thermophilus* at -20 and 4°C storage temperatures; whereas the inactivation of bacteriocin of *Lactococcus lactis* and *Pediococcus acidilactici* was noted after three weeks of storage period (**Table 2 & 3**). Among the bacteriocins produced, the bacteriocin of *Streptococcus thermophilus* could be inacted only after a storage period of five weeks though there was a gradual decrease in the activity (**Table 4**).

Bacteriocin production by the test isolate displayed secondary metabolite kinetics. For example, bacteriocin was produced during the pre- and early exponential growth phases and reached a maximum level at late stationary phase resulted in a decrease in bacteriocin activity. The effect of incubation temperature incubation period and initial pH of medium on production of bacteriocin was also investigated [6]. The use of constituted medium at 30°C incubation temperature, initial pH 5.5 and for 48 to 60 hours fostered the best production of bacteriocin by *Lactobacillus acidophilus* [7,8].

Despite the high number of bacteriocin producing LAB isolated and characterized so far, further search for new strains belonging to all genera of LAB having different spectra of action and isolated from different environments is worthwhile. According to Klaenhammer [9], 99% of all bacteria may make at least one bacteriocin. Bacteriocins are antimicrobial agents produced by bacteria which are active against closely related bacteria as claimed by Klaenhammer [10]. They have been proved active against many other bacteria also including pathogens described by Flythe and Russell [11]. Hence, they may be used as probiotic or as biopreservatives especially in the acid fermentation of food.

Anne Vaughan *et al*. [12] investigated the antimicrobial efficacy of a bacteriocin for the development of microbiologically stable beer. *Lactococcus lactis* was shown to produce the antimicrobial activity during growth under specific conditions. The capacity of the bacteriocin to prevent microbial spoilage of bacteriocin containing beer at 30°C or room temperature resulted in antimicrobial activity. Aly Savadogo *et al*. [13] isolated eighty strains of lactic acid bacteria producing bacteriocin were isolated from Burkina Faso fermented milk samples. These strains were identified to species *Lactobacillus fermentum, Pediococcus* sp., *Leuconostoc mesenteroides* and *Lactococcus.* Isolated bacteriocin exhibited antibacterial activity against *Enterococcus faecalis, Bacillus cereus, Staphylococcus aureus,* and *Escherichia coli* using the agar drop diffusion test. The inhibition diameters obtained with bacteriocin are between 8 mm and 12 mm. Gram positive indicator bacteria were most inhibited.

Ogunshe *et al*. [14] isolated 50 bacteriocin producing *Lactobacillus* strains from some Nigerian indigenous fermented foods and beverages and characterized as *Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus fermentum, Lactobacillus lactis* and *Lactobacillus plantarum* were screened for these inhibitory potentials against food borne pathogenic from the same or similar to fermented food sources and against clinical indicator bacterial isolates. The

survival rates of the pathogenic indicator bacteria in the fermented food sources were between 8 and 14 days while the clinical isolates survived in simulated fermented food samples between 5 and 9 days.

Lue De Vuyst and Frederic Leroy [15] described the production, purification and food applications of bacteriocins from lactic acid bacteria. In fermented foods, lactic acid bacteria display numerous antimicrobial activities due to the production of organic acids and other compounds such as bacteriocins. Michael Baker Diop *et al*. [16] evaluated that bacteriocin produced by *Lactobacillus lactis* and *Enterococcus faecium* show antimicrobial activity against *Listeria monocytogenes* and *Bacillus coagulans* whereas only that produced by *Lactococcus lactis* has an activity against *Bacillus cereus*. Bacteriocin producing *Lactococcus lactis* strains were found in variety of traditional foods indicating a high potential of growth of this strain in variable ecological complex environment and has been selected for application in food preservation. Adetunji and Adegoke [17] demonstrated bacteriocin and cellulose production by lactic acid bacteria isolated from West African soft cheese. The correlation between cellulose productions and bacterial growth was highly significant after 72 hours of incubation. The bacteriocin produced by the strains could be good for biopreservation.

Table 1: Effect of storage time and temperature on the inhibitory activity of bacteriocin produced from *L. acidophilus*

Organisms	Storage time (weeks)	Inhibitory activity (%)		
		-20°C	4°C	37°C
L. acidophilus	0	100	100	100
	1	100	80	80
	2	100	80	60
	3	80	80	40
	4	80	80	40
	5	80	60	-
	6	80	60	-

Table 2: Effect of storage time and temperature on the inhibitory activity of bacteriocin produced from *L. lactis*

Organisms	Storage time (weeks)	Inhibitory activity (%)		
		-20°C	4°C	37°C
L. lactis	0	100	100	100
	1	100	80	80
	2	100	80	60
	3	80	80	20
	4	80	80	-
	5	80	60	-
	6	80	60	-

Table 3: Effect of storage time and temperature on the sensitivity of bacteriocin produced by *Pediococcus acidilactici*

Organisms	Storage time (weeks)	Inhibitory activity (%)		
		-20°C	4°C	37°C
P. acidilactici	0	100	100	100
	1	100	80	80
	2	100	80	80
	3	80	80	40
	4	80	80	–
	5	80	60	–
	6	80	60	–

Table 4: Effect of storage time and temperature on the sensitivity of bacteriocin produced by *Streptococcus thermophilus*

Organisms	Storage time (weeks)	Inhibitory activity (%)		
		-20°C	4°C	37°C
Strp. thermophilus	0	100	100	100
	1	100	80	80
	2	100	80	80
	3	80	80	60
	4	80	80	40
	5	80	60	40
	6	80	60	–

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