Partial Purification and Optimization of Bacteriocin like Inhibitory Substances from Indigenous Bacteria

P. Muthukumaran*, T.Paramasivan, R. Janani, K. Soundharya and S. Thanishka

Department of Biotechnology, Kumaraguru College of Technology, Coimbatore,
Tamil Nadu - 641 049
* Corresponding author muthukumaran.p.bt@kct.ac.in

Abstract

In this study, the bacterial isolate from oats fermented broth was used to optimize the production parameter. Initially, bacteriocin like inhibitory substance (BLIS) was partially purified by ammonium sulphate (40%) precipitation followed by dialysis. The antimicrobial activity of partially purified BLIS was studied by agar well diffusion method by using various human pathogens. The culture was optimized under different physio-chemical conditions like pH, temperature, NaCl, surfactants, organic acids, proteolytic enzyme and metal ions. The respective antimicrobial activity was tested. The bioactive showed considerable stability in activity and was not inhibited by the external physical and chemical conditions. The zone of inhibition for the antimicrobial activity was found to be 4-5 mm. The temperature and pH did affect the activity as the zone of inhibition was 2-3 mm. The yield of protein (BLIS) in the culture was about 25 mg/ml on an average under most of the physio-chemical conditions. The scope of application of bacteriocin / BLIS in food preservation is included in progression of research.

Keywords: antimicrobial, bacteriocin, optimization, stability

I. INTRODUCTION

Bacteriocins are generally recognized as “natural” compounds able to influence the safety and quality of foods. In the past years, a lot of works have been aimed to the detection, purification and characterisation of bacteriocins, as well as to their use in food preservation strategies. These bio preservatives can be used in a number of ways in food systems and application of bacteriocins from lactic acid bacteria (LAB) to promote the microbial stability of both fermented and non-fermented vegetable food products using bacteriocinogenic strains as starter cultures, protective cultures or co-cultures and the employment of pure bacteriocins as food additives. Bacteriocins can be incorporated into foods as a concentrated, though not purified, preparation made with food-grade techniques [1]. The isolation of bacteriocin and its characterization from fermented broth cultures are done in order to make the bioactive material more available for future preservation applications. Bacteriocin and bacteriocin-producing strains of lactic acidbacteria (LAB) have been the focus of extensive research inrecent years due to their potential as bio preservatives. Initial studies on bacteriocin production by LAB were focused on isolates associated with dairy products. More recently, bacteriocinogenic activity has been discovered in bacterial strains from meat silage and fermented vegetables [2].Biochemical characterization of the proteins and peptides produced by the bacteria are done by conducting several tests in order to characterize the protein’s reaction towards a particular reagent, chemical or a different medium. Fermentation with different sugars will enable the characterization of the bacterial strain hydrolysis with the carbohydrate. The turbidity of the broth will indicate the growth of the organism in the particular sugar [3]. Bacteriocins can be used as food additives. For instance, nisin is commercially made in a partially purified form and a marketed preparation with the pediocin PA-1 (AcH) producer is available. As an alternative to the addition of Bacteriocins to foods, bacteriocins may be produced directly in the food as a result of starter culture or co-culture activity. Several studies have indeed indicated that LAB starter cultures or co-cultures are able to produce their bacteriocins in food matrices, and consequently display inhibitory activity towards sensitive food spoilage or pathogenic bacteria. The latter trait has mainly been documented forfermented sausage, fermented vegetables and olives, and dairy products[4].Food fermentation are typically carried out by mixed cultures consisting of multiple strains or species [5]. Different strains of bacteria are more common in fermentation of food. *Lactobacillus*/lactic acid bacteria are the most prevalent bacteria that are identified in most of the foods. The species include: *Lactobacillus fermentum*; *Lactobacillus planatarum*; *Lactobacillus brevis*; *Lactobacillus cellobiosus*; *Leuconostoc mesenteroids*;

Lactobacillus delbrueki;*Lactobacillus corniformis* and *Lactobacillus casei*[6].Bacteriocin produced by Lactobacillus sp. MSU3IR was partially purified by dialysis[7] and determined by SDS-PAGE analysis contained two distinct bands weighing 39.26 kDa and 6.38 kDa. Molecular mass of polyfermenticin SCD, a newly isolated bacteriocin by [8] had a molecular mass of 14.3kDa after SDS-PAGE. Adsorption of bacteriocins onto Micro-Cell was done[9] that involved both electrostatic and hydrophobic interactions. The biological activity of purified bacteriocin produced [10]was rapidly destroyed by several proteases that affect the activity of proteins. Study by [11] Ammonium sulfate precipitation of *L.plantarum*LPCO10 produced the total bacteriocin activity about 15 times higher than that present in the initial supernatant collected.

II.EXPERIMENTAL MATERIALS AND METHODS

A. Partial purification of bacteriocin producing bacterial culture

As a beginning 1litre nutrient broth was prepared and inoculated with the bacterial strain producing bacteriocin obtained from fermented oats. The broth was incubated for 24hrs at 37°C. The sample was centrifuged at 10000rpm for 10min and the supernatant was collected. To the supernatant30%, 40%, 50%, 60%, 70% and 80% ammonium sulphate salt was added and precipitation was done. The fractionated sample as centrifuged at 10000rpm for 15min and the pellet was suspended in 0.1M phosphate buffer. The pellet was now subjected to dialysis (10k Da pore size) overnight.

B. Optimization of bacteriocin producing bacterial culture

Different factors like pH, temperature, NaCl at various concentration, surfactants, culture media, carbon and nitrogen sources were considered for the optimization of bacteriocin producing culture. Each of the optimization was done with 25ml of nutrient broth in boiling test tubes. The pH levels were adjusted from 4-10, temperature 10°C to 60°C, NaCl concentration varying from 1-5% surfactants like Tween 80, SDS, Trition X-100, carbon sources like fructose, xylose, lactose, maltose and mannose were added in 5% concentration(w/v) and nitrogen sources such as ammonium chloride, sodium nitrate and sodium sulphate in 5%(w/v) concentration. Luria broth, Muller Hinton and nutrient broth were used as culture media optimizers. All the samples were incubated for 48hrs at RT but the temperature optimization was done only for 24hours. The study [12] wasused for well diffusion assay procedure & for optimizing Lactic Acid Bacteria inhibitor-producersthis was measured at OD600 for 18 h at 30°C.

C. Test organisms

Pseudomonas aeruginosa MTCC - 424, *Aspergillus nidulans* MTCC –1344 and *Candida albicans* MTCC - 227 were used as test organisms, which were obtained from Institute of Microbial Technology (IMTECH), Chandigarh.

D. Antimicrobial activity by agar well diffusion assay

The incubated samples were now subjected to antimicrobial activity by agar well diffusion method. Nutrient agar plates, Yeast and fungal media plates were prepared for respective test organisms. The plates were streaked with the test organisms and well were filled with the respective optimization factors and incubated for 24hrs at 37°C. The antimicrobial activity was obtained as zone of inhibition in mm.

III. RESULTS AND DISCUSSION

A. Partial purification of bacteriocin producing bacterial culture

The culture that was fractionated using different saturation levels of ammoniumsulphate was tested for antimicrobial activity and 40% concentration was found to be optimum and showed maximum activity. Table 1 indicates antimicrobial activity of different ammonium sulphate precipitation (%) from screened bacterial isolates. Zone of inhibition of E. Coli (12.06 mm) *P. aeruginosa* (13.00 mm) *S.typhi* (13.10 mm) *K.pneumonia* (11.10 mm) & *S.aureus* (10.40 mm) was reported [13].*A.wentii* ATCC 1778 (12 mm), *Candida utilis* (16 mm), *S.fibuligera (*15 mm) &*T.reesi* (16mm) were the zone of inhibition reported by [14].

TABLE 1. PARTIAL PURIFICATION OF BACTERIOCIN PRODUCING BACTERIAL CULTURE

Test Organisms	Zone of inhibition (mm)
Bacillus subtilis MTCC – 121	-
Pseudomonas aeroginosa MTCC - 424	20
Klebsiella pneumonia MTCC – 109	40
Staphylococcus aereus MTCC – 737	30
Aspergillus niger MTCC - 343	40
Aspergillus fumigans MTCC – 1244	45
Candida albicans MTCC - 227	-

B. Antimicrobial activity of optimized samples

The bacterial cultures that were optimized with different factors showed positive results when tested for antimicrobial

activity. The factors differed in the extent of their activity but still they were observed to show good antimicrobial property against the tested organisms. The organisms are infectious but were inhibited in growth and development by the strains isolated from the fermented oats. Figure 1, 2, 3 indicates antimicrobial activity of protein from screened bacterial isolates.Effect of pH on activity of bacteriocin by *L. brevis* OG1 was found stable at pH 2 to 8, while for *L. plantarum* F1, it was found to be stable at pH 2 to 6[15].

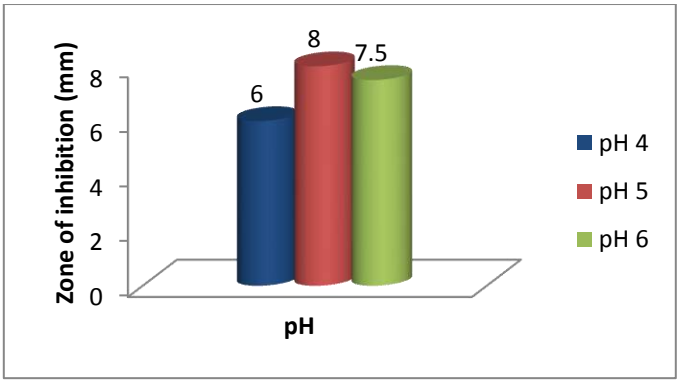

Fig. 1. Antimicrobial activity of protein against *Pseudomonas aeruginosa* MTCC – 424 at different pH

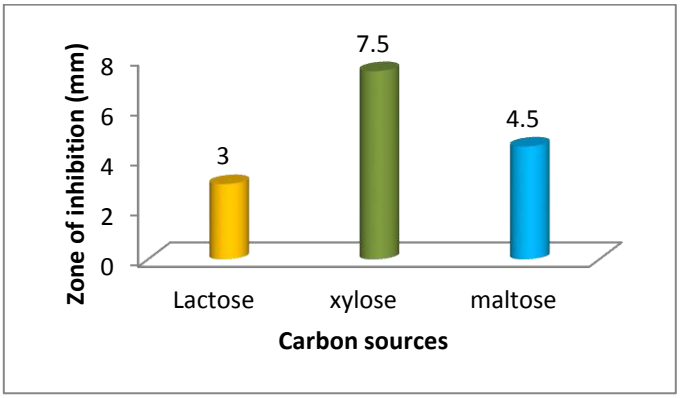

Fig. 2. Antimicrobial activity of protein against *Pseudomonas aeruginosa* MTCC – 424 at 3 different carbon sources

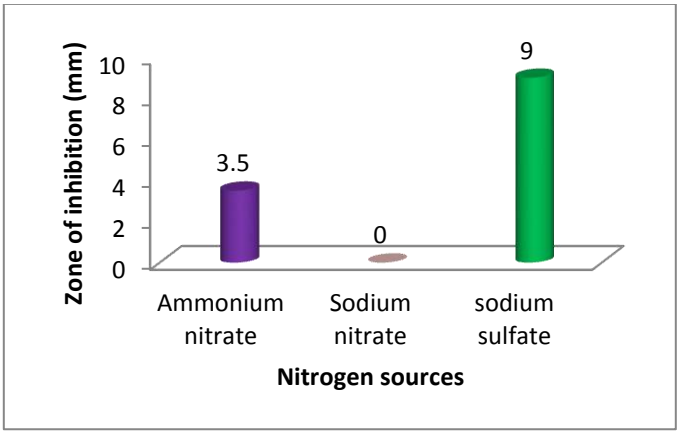

Fig. 3. Antimicrobial activity of protein against *Pseudomonas aeruginosa* MTCC – 424 at 3 different Nitrogen sources

IV. Conclusion

The partial purification and optimization Bacteriocin producing bacterial culture was isolated from fermented broth of oats. This bacterial culture containing bioactive compound was found to be efficient and showed up positive results in order to carry out application procedures. The study revealed that Bacteriocin producing bacterial isolates were isolated from oats possess a wide spectrum of inhibitory activity against the major test organisms such as *Pseudomonas aeruginosa* MTCC - 424, *Staphylococcus, Aspergillus nidulans* MTCC - 1344, *Candida albicans* MTCC - 227. As a note the bacterial culture are very effective against the bacterial pathogens and to an extent the fungal pathogens. Therefore it has a potential for application as a biopreservative in different food products as such or in combination with other preservation methods. Since lactic acid bacteria are found to be more predominant as a preservative the same method of lactic acid fermentation can be combined with the screened bacterial isolates and tested for efficiency. Thus to conclude the isolated strains are effective against disease causing microbes and also bacteriocin culture is stable under optimization with physio-chemical factors, thereby can surely be applied on the preservation and packaging criteria with respect to food technology. The probiotic activity of the strains is expected not to affect the contents of the food leading to any alterations or degradation.

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