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THERMAL STABILITY AND PH TOLERANCE OF AN ANTIMICROBIAL PRODUCING BACTERIA ISOLATED FROM SPOILED FOOD

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ABSTRACT: Lactic acid bacteria (LAB) have a long history of application in fermented foods because of their capacity of production of antimicrobial compounds. They cause rapid acidification of the raw material through the production of lactic acid and other organic acids. In addition, their production of aroma compounds, bacteriocins, acetic acid, exo-polysaccharides, ethanol, and several enzymes is of importance**.** The most important contribution of these microorganisms is the preservation of the nutritional qualities of the raw material through extended shelf life and the inhibition of spoilage and pathogenic bacteria. This contribution is due to competition for nutrients and the presence of inhibitor agents produced, including bacteriocins, organic acids, hydrogen peroxide etc. LAB produces antagonistic compounds that increase their competitive value. This century has been a major effect in describing, cataloging, and characterizing the wide variety of antagonistic compounds produced by lactic acid bacteria. Apart from lactic acid, LAB may produce other inhibitory substances such as diacetyl, hydrogen peroxide, reuterin (ß-hydroxy propionaldehyde) and bacteriocins. Several food-grade lactic acid bacteria, used in food fermentation, are known to have these antimicrobial properties. The main object of this work is to find out the temperature optima, pH optima, antimicrobial spectrum study and to study the morphological characteristic of the isolated bacteria IPM-1 from spoiled palm food (fermented Taal bara). It was found that the isolate, IPM-1 is producing some antimicrobial substance and that effective at neutral pH range and temperature near about 70˚C at which the activity persists.

KEYWORDS: Antimicrobial substances, Lactic Acid Bacteria (LAB), pathogenic organisms, thermal stability, antimicrobial spectrum.

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1.INTRODUCTION

Lactobacillus is widely used as probiotics in fermented foods. Lactic acid bacteria (LAB) have antioxidant effects that can chelate ferrous ions and degrade nitrite and cholesterol as observed in in-vitro analysis [1-2]. LAB are natural microbes and therefore its metabolic products are generally regarded as safe [3]. For example, nisin, which is an antimicrobial bio-preservative, is allowed as food preservative obtained from the *Lactococcus lactis* to prevent the growth of spoilage causing organisms. LAB metabolic products, such as acid, hydrogen peroxide, and bacteriocin, can inhibit some bacteria and fungi [4]. All strain unable to perform antimicrobial activities because their metabolic production is insufficient or minimal [5]. Therefore, LAB with high antibacterial activities should be screened, and their antibacterial components should be analyzed. Considering that LAB have these effects in vitro and that their metabolites may target and play a role in the competitive exclusion of pathogens, we should screen bacteria that produce numerous antimicrobial peptides and acids that are good biopreservatives [6]. Therefore, our study aimed to extend the screening of bacteria from fermented food products to obtain novel and good strains. This century has been a major effect in describing, cataloging, and characterizing the wide variety of antagonistic compounds produced by LAB. LAB produces lactic acid, lactic & acetic acids and may also produce other inhibitory substances such as diacetyl, hydrogen peroxide, reuterin (ß-hydroxy propionaldehyde) and bacteriocins. The most important contribution of these microorganisms is the preservation of the nutritional qualities of the raw material through extended shelf life and the inhibition of spoilage and pathogenic bacteria. A group of antimicrobial peptides, so-called bacteriocins, have a wide range of antimicrobial activity and are produced by many Gram-positive bacteria. Bacteriocins are ribosomally synthesized peptides and most of them are of small size, cationic and heat-stable [7- 10]. They kill sensitive bacteria by forming pores on the cytoplasmic membrane or by inhibiting synthesis of the cell wall. This trait is believed to be an important part of the antimicrobial action that prevents the growth of pathogens during food fermentation and preservation [11-14]. However, strains belonging to species of other LAB have been used in the past as probiotics as well, such as *Enterococcus. faecium*, *Enterococcus. faecalis*, *Streptococcus. thermophilus*, *Lactococcus. lactis* subsp, *Leuoconostoc mesenteroides*, and *Pediococcsu. Acidilactici.* Most antimicrobial components remained stable at high temperatures. The molecular weight of antimicrobial peptides, which are secondary protein structures (α-helix, β-folding, β-rotation angle, and random crimp), is between 3 and 10 kDa. Their low molecular weight and secondary structure may lead to the high-temperature resistance of most antimicrobial peptides [15]. This finding indicated that the antibacterial components of LAB could be applied as biological preservatives for high-temperature treatments of food. Earlier there are some reports regarding the biocontrolling of pathogens. There are beneficial microorganisms in soil that may increase the resistance of the plant against the pathogens in the root region of the plant [16]. The most important group of these rhizobacteria—which have been well known for many years as plant biocontrol agents for different crops—are bacteria from the genera *Pseudomonas* or *Bacillus* [17–20]. *Pseudomonas fluorescens, Agrobacterium radiobacter, Bacillus subtilis, B. cereus, B. amyloliquefaciens, Trichoderma virens, Burkholderia cepacia, Saccharomyces sp., Gliocladium sp. and Streptomyces sp*. have been demonstrated in previous studies to be used as antimicrobial agents against several phytopathogenic fungi [21]. In recent years, LAB have attracted much attention as antimicrobial agents. There are various researches on LAB applications in traditional food and feed fermentation and preservation [22-23]. Resistance of chemical control agents by pathogens poses a threat to agricultural products and ultimately economic loss [24]. For controlling mycotoxin-contaminated food and feed several physical and chemical methods have been developed but only few are practically considered [25]. Therefore, a potent method has to be employed to reduce the presence of mycotoxins. Meanwhile, natural preservatives could be an alternative against chemical control [26]. Lactic acid bacteria is gaining much attention, due to consumers' demand for reducing the use of chemical preservatives [27]. As a result of this, several researchers have exploited antagonistic and antimicrobial compounds such as bacteriocins produced by LAB which are able to control pathogenic microorganisms and undesirable moulds in food and feed [28-31]. Main objectives of this investigation are to study the effect of Temperature and pH on the activity of the antimicrobial substance of isolated bacteria IPM-1 from spoiled palm food (fermented Taal bara), study the its antimicrobial spectrum and the morphological characteristic features.

2. MATERIALS AND METHODS

Microorganism: IPM -1 (isolated from palm product), **Indicator strain:** The indicator organisms used to observe the inhibition activity of the anti microbial substance(s) produced by the isolated organism(s) were *LP* sp.2083. This was procured from MTCC, Chandigarh. *Aspergillus sp.* Isolated from rotten Mango.

Screening and isolation of potent antimicrobial producing bacteria from various sources

Twenty five samples were collected altogether from various sources like rotten fruits, putrid meat, salted fish, water samples etc. First of all, MRS broth medium was prepared. Colonies emerging on MRS agar media were purified and are used to inoculate in broth (MRS broth) and kept in incubator for overnight (37^o C). Indicator culture (LP 2083) was mixed with previously prepared molten MRS soft agar media and inoculated on MRS hard agar plates and kept for solidification for approximately 15 mins. In the mean time, 1.5 ml of each broth culture of isolated organisms taken in sterilized eppendorf tube and centrifuged at 10,000 rpm for 10—15 mins. Supernatant of 20 µl was taken and prick on indicator culture lawn and incubated for overnight at 37^o C. After 24 hours the inhibition zone on lawn of indicator microorganisms was observed. From total samples one isolate IPM-1 from rotten palm (fermented Taal Bara) product was found to be most potent inhibitor against the indicator used in this experiment.

Determination of temperature optima of potent antimicrobial produced by the isolated organism

This experiment was performed by heating the antimicrobial compound, isolated from the cell free soup of IPM-1, at different temperature in hot water bath. The temperature levels were selected arbitrarily as 50ºC, 60^0C, 70ºC and 80ºC. The isolated antimicrobial compound was kept for 10 minutes at respective temperatures. After centrifugation, 20 µl of supernatant were prick from each treatment on indicator culture lawn and incubated for overnight at 37º C. After 24 hours the inhibition zone on lawn of indicator microorganisms was observed. Results showed that antimicrobial activity persist upto 70^0 C.

3. RESULTS AND DISCUSSION

Antimicrobial spectrum study of the secreted antimicrobial compound from the isolated microorganism was shown in table 1. The anti microbial spectrum study shows that the anti microbial substance can inhibit a number of organisms tested here the most sensitive one was LP 2083 (16 mm) followed by *Aspergillus sp*. (15 mm) *E. coli* (12 mm) and *Salmonella typhi* (11 mm).

Table 1. The anti microbial spectrum of IPM -1against different microorganisms

	organisms	Inhibition zone(mm)
1.	*Bacillus subtilis*	0
2.	*Salmonella typhae*	11
3.	*Bacillus megaterium*	0
4.	*Escherichia coli*	12
5.	*Alcaligenes viscosus*	0
6.	*LP2083*	16
7	*Aspergillus sp.(isolated from rotten Mango*	15

Temperature effects shows that antimicrobial compound secreted by the organism IPM-1 has the capabity to retain its antimicrobial activity at temperature upto 70^0C temperature (Table -2, Fig. 1) The figures showing inhibition zone after treating of the supernatants at 60ºC(A) and 70^0 (B) for 10 min.(Fig. 1). The test organism was *Aspergillus sp.* Isolated from rotten mango.

Table 2. Effect of different temperature on antimicrobial activity.

Temperature	Activity of the amp
50^0 C	+
60^0 C	+
70^0 C	+
80^0 C	-

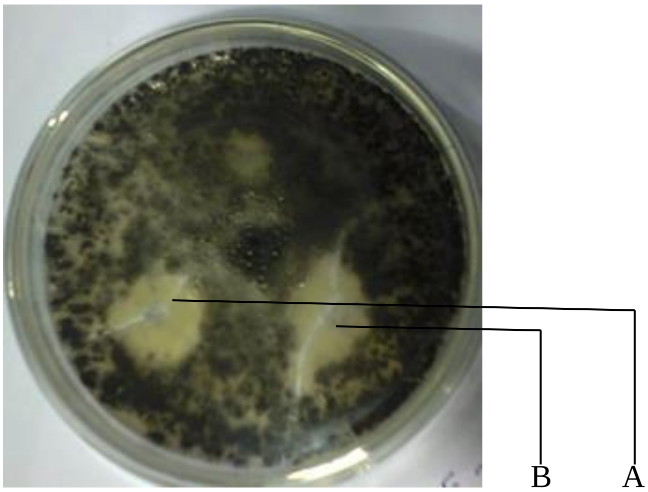

Fig 1: Activity of the Antimicrobial compound obtained from IPM-1 in different temperature

Above figures showing inhibition zone after treating of the supernatants at 60^{o}C(A) and 70^{0} (B) for 10 min.(Fig. 1).

Determining the pH optima of the production of antimicrobial compound by the organism

This experiment is a very important way to know the range of pH in which the organism can grow to its highest growth rate and produce the best antimicrobial compound in respect to the quantity and quality. To determine this we have prepared media of different pH. MRS broth at different pH (pH 6.0, 7.0 and 8.0) were inoculated with IPM-1. After 24 hrs. 20 µl Supernatants were taken pricked into the MRS containing indicator organism (LP 2083). The result of the two sets of experiment showed that the organism is growing better and also producing better antimicrobial compound within the range of pH 6.0 to 8.0 (Fig. 2).

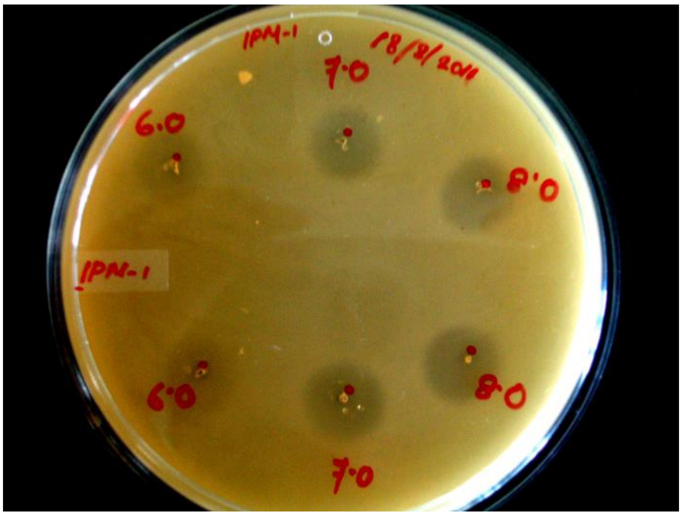

Fig 2: Inhibition Zones of indicator organism in presence of IPM-1 cell free extract soup

Morphological study of the isolated species

The organism was streaked on MRS agar and incubated for 24 hours to obtain single colonies (Fig.3A). The Gram staining technique was applied to observe the morphological features and Gram character. It shows that the organism is a rod shaped bacterium that is Gram positive. (Fig. 3

B)

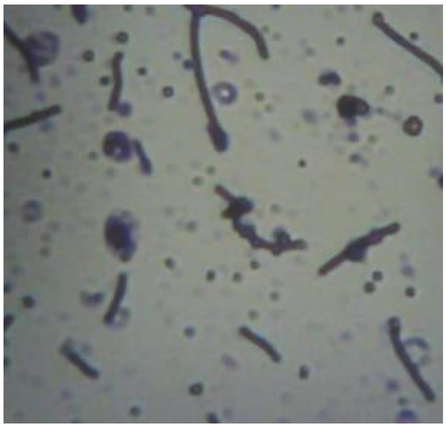

Fig. 3 A: Single colonies of IPM-1 **Fig. 3B: Rod shaped Gram + IPM-1**

Growth curve study of the isolated bacteria.

This is an important tool to know that whether the bacterium is rapidly dividing or very slowly or it may be at a moderate rate. That growth curve of the isolated IPM – 1 cell is given below along with the data. The OD value at different time had been measured. The result shows that IPM-1 is fast grower and enters at stationary phase within 13 hours of growth (Fig. 4)

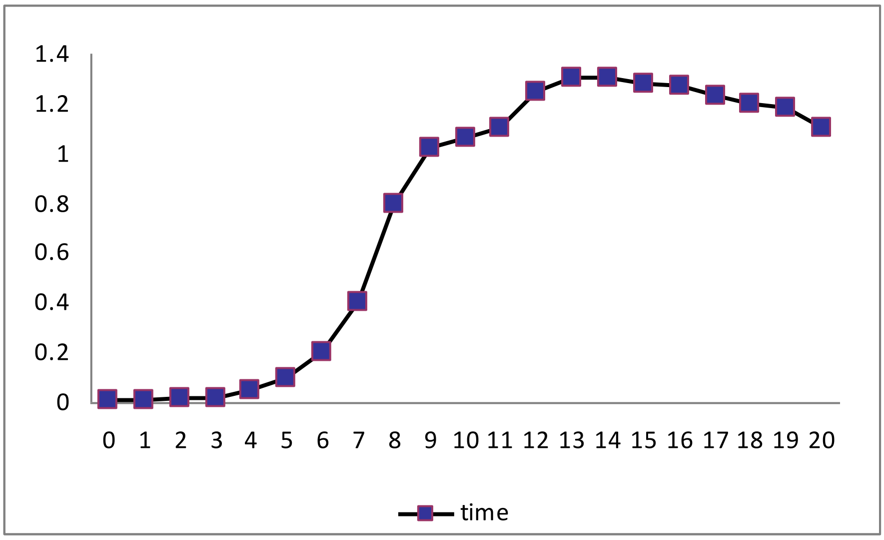

Fig. 4. Growth curve of IPM-1. The horizontal axis denotes the time and the vertical axis denotes the O.D values

4. CONCLUSION

The production of this antimicrobial substance will be at its best between the pH range 6.0 to 8.0, that is quite neutral environment can promote the growth and also the production of the antimicrobial substance. Heat treatment of the antimicrobial substance at different temperature was done and the treated cell free extracts were spotted on the agar plate spreaded with the indicator organism. It shows that the antimicrobial produced by the isolated organism can tolerate high temperature up to 70˚C. The growth curve shows that the bacterium enters at stationary phase after 13 hrs. of incubation. The antimicrobial production is also high at this stage of growth. The isolation and partial characterisation of the organism, IPM-1, was done and the results of the experiments were discussed above. The above discussion reveals that the bacterium/ Bacterial products may be used for different purposes like food preservation, biological eradication of many harmful organisms etc.

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CONFLICT OF INTEREST

Authors have no any conflict of interest.

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