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Antibacterial activity lactobacillus plantarum

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ABSTRACT

Lactic acid bacteria can synthesize antibacterial substances which are used in fermentation and food biopreservation. Six strains of lactic bacteria Due mainly to the genera Lactobacillus, were selected from brine artichokes, olives and carrots. The strain of the genus Lactobacillus SMBL1 was kept to its high bactericidal activity primarily vis- à-vis of Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 25921, Salmonella enteritidis and Streptococcus agalactiae. This antibacterial activity comes from an extracellular substance, protein and heat resistant in nature.

KEYWORDS

Lactic acid bacteria;
Bactericidal;
Brine fermentation
bioconservation.

INTRODUCTION

Lactic acid bacteria combine a group of irregular species whose common point is the synthesis of lactic acid. They belong to different genres as Bifidobacterium, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, Streptococcus, Aerococcus, Carnobacterium and Alloicoccus.

They work in the dairy industry and in plenty of other food fermentation similarly bakery products, hard-smoking fish, meat, meats, pickling vegetables and wine making, etc.. They work with the texture, the taste of food and in the synthesis of aromatic compounds.

They ferment carbohydrates into lactic acid, thus reducing the pH suitable for food storage. Their antagonistic capacity drift identically a competition for substrates[1], and when the conditions are favorable for development, production of bacteriocins[2] as nisin[3].

Bacteriocins are antibacterial peptides[4]. They have a narrow spectrum of activity behind pathogenic species[5].

They have a maximum solubility, stability and activity at acid pH. They are heat stable and are neutralized by proteases.

In the following work, strains of lactic acid bacteria were isolated to test their bactericidal effect, their acidifying power and sought the type of inhibitory substances.

MATERIALS AND METHODS

Isolation and identification of lactic strains

Six strains of lactic acid bacteria were isolated from three different habitats : brine carrots (SMBL1 and SMBL2), brine artichoke (SMBL3 and SMBL4)

and olive brine (SMBL5 and SMBL6) Only Gram-positive bacteria and catalase negative were selected. The isolation is carried out on MRS medium (Man Rogosa Sharp, Difco, Detroit, USA) [6] solid medium adjusted to the typical search lactobacilli. The cultures were incubated 24 hours at 30°C in Petri dishes in the dark. The purification is performed by five consecutive subcultures distribution solid MRS medium. Conservation is inclined on MRS medium at +4°C in test tubes in the dark.

Identification is established based on various biochemical and morphological characteristics : catalase, growth temperature, carbon dioxide production, fermentation of various sugars[7]. For the identification of the strains, were placed 0.1 ml of a 24 hour culture at 30° C in the wells of a API 20E (Bio Mérieux) / B. After 24 hours of incubation, the identification strains is carried out according to the indication of the manufacturer who showed that this strain is lactobacillus plantarum.

pH lactic acid bacteria in liquid MRS medium

The six bacterial strains were grown 48 h on MRS liquid medium in 100 ml flasks at 30° C ± 2 °C in the dark.

The initial and final pH were made using a pH meter Orion Research Type in combination electrode.

The titration acidity is carried out on 10 ml culture with a solution of 0.1 N sodium hydroxide using a burette to Mohr tap, there is a drop of a methanol solution of 1% phenolphthalein used as a color indicator.

Percent acidity is expressed as mg of lactic acid (MW = 90.08 g) per 100 ml culture. To evaluate this, the six bacterial strains were grown two days in MRS liquid medium in 100 ml flasks at 30 ° C in the dark and with a fixed starting pH 6.46 with 1N HCl and 1N NaOH.

Power antibacterial strains

Numerous methods set for the Exploration of synthesising lactic strains of bacteriocins are based on the principle that these protein substances can spread in a solid culture medium or semi solid which previously inoculated with a target strain. Bacteriocin production is distinguished by the inhibitory potential of the filtrate

Microorganism tested on the development of the target[8] germ.

Six strains of lactic acid bacteria after culture on MRS medium two days at 30°C were tested for antibacterial activity by the method of diffusion[9] TSA agar (Tryptic Soy Agar, Difco, Detroit, USA).

Six small wells are dug per Petri dish of 5 mm in diameter, with four boxes per condition and with three replicates at different times.

Plates are sheltered by the pathogenic strain Staphylococcus aureus ATCC 25923, then the wells are filled by 60-80 mu.l of filtered supernatant obtained after centrifugation at 10 000 rev / min in 20 ml MRS medium cultured with lactic strain. The supernatant was neutralized with 0.1 N NaOH to obtain a pH of 6.50, and then a few drops of catalase to avoid the effect of oxygen peroxide.

After 24h of incubation at 30±2°C, the diameters of inhibition zones around the wells appear were measured (average of two perpendicular diameters).

The inhibition is registered positive when it exceeds 1 mm[10].

Antibacterial spectrum of strains with high acidifying power

For testing antibacterial activity, a serie of seven pathogenic microorganisms has been renamed. They were isolated and identified from infected Biologics Research Laboratory and medical analyzes Idrissi hospital Kenitra. All these strains were tested for resistance using ATB galleries (Bio- Mérieux SA, France).

These seven strains were purified and stored on nutrient agar slants (TSA Tryptic Soy Agar) at 4°C in the dark. Prior to their use in the inhibition tests, they were activated by transfer of nutrient broth and incubated 16-18 hours at 37° C.

The two most powerful for their acidifying power lactic strains, which were stored at 4° C, were activated before use in the inhibition of transfer tests on MRS broth supplemented with 2% yeast extract to optimize the culture medium, and then incubated 24 hours at 30 ° C to obtain young cells with a maximum yield of inhibitory substances. A volume of 20 ml was centrifuged ten minutes 10 000 revolutions / min. The filtrate was kept at + 4°C in

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the dark after neutralization.

As before, the diffusion method is applied agar TSA to highlight the possibility bactericide.

Location of the inhibitory substance SMBL1 strain

From a culture of strain SMBL1 MRS liquid medium, incubated for 24 hours at 30 ± 2°C, a volume of 50 ml of culture was centrifuged ten minutes at 10,000 revolutions / min. The inhibition tests are subsequently applied as both the supernatant, indicating the extracellular portion, and on the cap, coincides with the cellular portion.

The influence of the neutralization and the addition of a few drops of catalase was tested on these two fractions. For each of the seven pathogens, four Petri dishes with two wells per box (with and without neutralization and addition of catalase) were used with three replications.

Evidence of the presence of bacteriocins

Lactic acid bacteria can synthesize separate inhibitory substances bacteriocins especially lactic acid, acetic acid and oxygen peroxides. To remove the effect of organic acids, especially lactic and acetic acid, the supernatant was neutralized to pH 6.5 by NaOH 0.1N order to remove the hydrogen peroxide in the medium heaped culture, the supernatant was also treated with a catalase two hours at 30 ° C prior to test inhibition.

TABLE 1 : Pathogenic bacteria used in the test

	Pathogenic strains	Collection
Gram négatif	Escherichia coli ATCC 25921	Urinary
	Klebsiella pneumoniae	Urinary
	Proteus mirabilis	Urinary
	Pseudomonas aeruginosa ATCC 27853	Pus
	Salmonella enteritidis	Stool
Gram positif	Staphylococcus aureus ATCC 25923	Urinary
	Streptococcus agalactiae	Vaginal

Study of the thermal stability of the bacteriocin

The bacteriocins are known to be resistant to elevated temperatures proteins, The thermostability of inhibitory substances was tested by heating to 100° C for 0, 15, 20 and 30 minutes from the supernatant SMBL1 strain. As before, the diffusion method is used agar TSA to highlight the bactericidal power of Staphylococcus aureus ATCC 25923 with three replicates for each test.

RESULTS

Antibacterial activity and acidifying power of six isolates

Six strains of lactic acid bacteria belong to the genera Lactobacillus, Only SMBL1 and SMBL2 show strong antibacterial activity against Staphylococcus aureus ATCC 25923 (TABLE 2).

SMBL1 the strain was identified as Lactobacillus most competitive not only for its antibacterial activity, but also by its high percentage of acidity (TABLE 2).

Antibacterial spectrum of the two selected strains

The SMBL1 strains and SMBL2, maintained for the important acidifying power, have a generally high vis- à-vis bactericidal effect of pathogens tested (TABLE 3, Figure 1).

The SMBL1 strain exhibits strong bactericidal activity (TABLE 3) In both Gram positive bacteria Staphylococcus aureus ATCC 25923 (27 mm inhibition zone) and Streptococcus agalactiae (zone of inhibition of 18 mm).

TABLE 2 : Features six strains grown in MRS liquid medium for 48 hours at 30°C in the dark, with bactericidal effect vis-à-vis Staphylococcus aureus ATCC 25923

strains	Détermination	Biotope	pH		Acidity (%)	catalase	Gram	Effect Bactéricidal
			initial	final				
SMBL1	Lactobacillus Plantarum	brine carrot	6,55	3,72	1,22	-	+	++++
SMBL2	Lactobacillus sp	brine carrot	6,37	3,84	1,21	-	+	+++
SMBL3	Lactobacillus sp	Brine Artichoke	6,38	4,67	0,88	-	+	++
SMBL4	Lactobacillus sp	Brine Artichoke	6,56	4,75	0,84	-	+	++
SMBL5	Lactobacillus sp	Brine olive	6,44	4,82	0,68	-	+	++
SMBL6	Lactobacillus sp	Brine olive	6,27	4,96	0,64	-	+	++

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TABLE 3 : Diameter of the inhibition of two strains of bacteria lactic vis- à-vis seven pathogenic strains by the method of diffusion on TSA area after 24 hours at 30°C in the dark

Pathogenic strains	Diameter of inhibition (mm)	
	SMBL1	SMBL2
Escherichia coli ATCC 25921	25	9
Klebsiella pneumoniae	14	7
Proteus mirabilis	15	5
Pseudomonas aeruginosa ATCC 27853	14	12
Salmonella enteritidis	17	6
Staphylococcus aureus ATCC 25923	27	22
Streptococcus agalactiae	18	10

Among the Gram-negative bacteria, is particularly active in Escherichia coli ATCC 25921 (25 mm inhibition zone) and Salmonella enteritidis (17 mm inhibition zone).

SMLBL2 The strain also has a bactericidal activity vis-à- vis the strains pathogenic gram positive Staphylococcus aureus ATCC 25923 (22 mm inhibition zone) and Streptococcus agalactiae (10 mm inhibition zone).

Among the Gram-negative bacteria, is particularly active against Pseudomonas aeruginosa ATCC 27853 (12 mm inhibition zone) and Escherichia coli ATCC 25921 (9 mm inhibition zone).

Localization of the inhibitory effect of the strain SMBL1

Cellular part (base) no result on the growth of pathogenic strains used (TABLE 4). For cons, the

TABLE 4 : Antibacterial activity of extracellular and cellular parts of the strain SMBL1 by the diffusion method on TSA after 24 hours of incubation at 30°C.

Pathogenic strains	Diameter of inhibition (mm)	
	Extracellular fraction	Cellular fraction
Escherichia coli ATCC 25921	22	-
Klebsiella pneumoniae	14	-
Proteus mirabilis	15	-
Pseudomonas aeruginosa ATCC 27853	14	-
Salmonella enteritidis	17	-
Staphylococcus aureus ATCC 25923	25	-
Streptococcus agalactiae	18	-

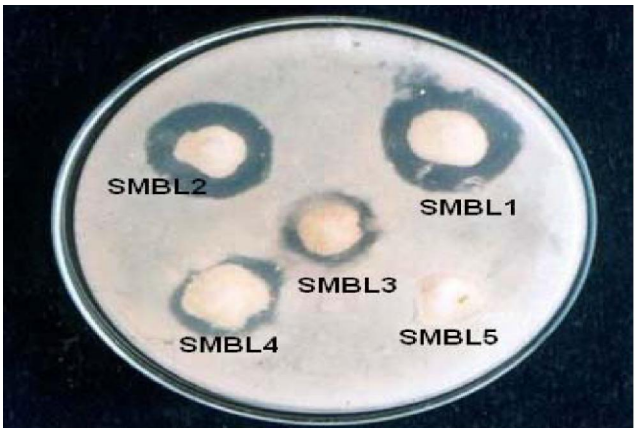

Figure 1 : Antibacterial activity of lactic strains and SMBL1 SMBL2 vis-à -vis Staphylococcus aureus ATCC 25923 (1) by diffusion method Tryptic Soy Agar medium after 24 hours of incubation at 30 ° C in the dark

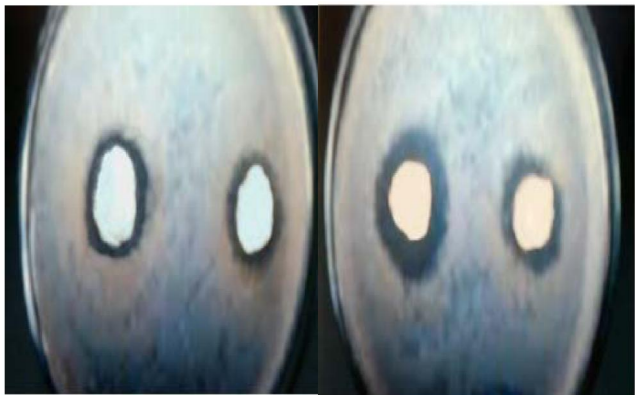

Figure 2 : confirmation of the antibacterial activity of the extracellular portion of the lactic strain smbl1 vis- à-vis Proteus mirabilis (left) and Staphylococcus aureus ATCC 25923 (right) by diffusion method tryptic soy agar medium after 24 hours of incubation at 30 ° ca darkness

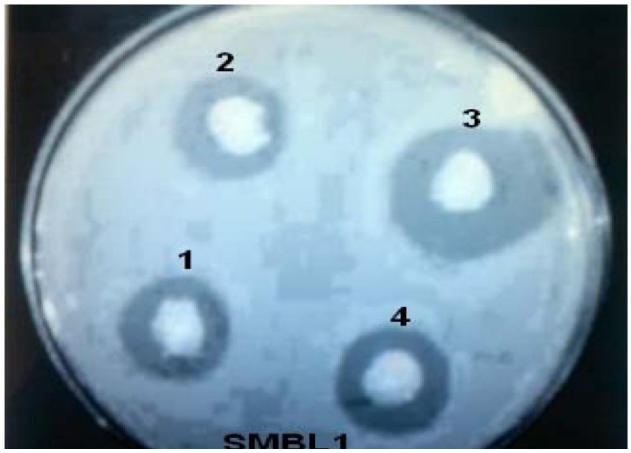

Figure 3 : Antibacterial effect vis-à -vis Staphylococcus aureus ATCC 25923 extracellular part of the SMBL1 strain after thermal treatment at 100°C by the method of diffusion on Tryptic Soy Agar medium after 24 hours of incubation at 30°C in the dark; (1) Witness ; (2) : 15 min ; (3) : 20 min ; (4) : 30 min

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extracellular portion corresponding to the supernatant shows a strong antibacterial ability (Figure 2). This result is consistent with the work of[11] and[12].

As before the inhibitory effect is dominant for the two gram-positive strains Staphylococcus aureus ATCC 25923 (25 mm inhibition zone) and Streptococcus agalactiae (18 mm inhibition zone).

Among the Gram-negative bacteria, is particularly active in Escherichia coli ATCC 25921 (22 mm inhibition zone) and Salmonella enteritidis (17 mm inhibition zone).

Thermal treatment of substance inhibiting the strain SMBL1

Whatever the time of heat treatment at 100 °C, the supernatant retains its antibacterial activity (Figure 3).

DISCUSSION AND CONCLUSION

In the six strains of lactic acid bacteria isolated, bactericidal activity and the percent acidity and without changing see very likely related to the environment. The two most operating stem, SMBL1 SMBL2 and do not show the same spectrum of action with respect to pathogenic bacteria. They are all excited about the Gram+, but only SMBL1 strain is operative against Proteus mirabilis, Gram -. By choosing from a variety of environments, it is therefore feasible to select strains of interest.

Pathogenic bacteria are Gram + regularly more sensitive to the bactericidal effect of lactic acid bacteria[13]. Bacteriocins act by forming pores in the cytoplasmic membrane which cause disturbances of cell functions[14]. Song and Richard[15]. Presented in Listeria, Gram + bacteria, cells that strong bacteriocins have a membrane composition different from that in sensitive cells.

The bactericidal activity of the strain is recovered SMBL1 strictly in the culture medium. We therefore constitution of extracellular substances.

These different organic acids and oxygen peroxide. Indeed, neutralization of the supernatant and the addition of catalase did not result in reduction of inhibition diameter (Figure 2).

The cancellation of the effect of lactic acid and oxygen peroxide acid rather promote the activity of antibacterial substances.

For treatment at 100°C for 20 min and 30, significant inhibition of the area that the conduit or inhibitory molecules are thermostable. These specifications suggest that dealing with bacteriocins[16].

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