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Selection of Bacteria Strains Antibacterial Lactic

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Abstract:- **The oranges wastes have a big impact on pollution and health, to overcome these impacts, the work has allowed biological transformations.**
At this point twenty strains of lactic bacteria belonging particularly to Streptococcus, Lactobacillus, Enterococcus were selected from orange pulp. The strains have been selected for its Strong bactericidal activities, especially towards Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 25921, and Klebsiella pneumonia, Morganella morganii.
Lactic bacteria are used in the fermentation and organic food preservation, thanks to the production of organic acids and other antibacterial substances such as bacteriocins by inhibiting certain pathogenic strains. Their interactions led to the occurrence of important zones of inhibition [1].
The results obtained showed that the production of lactic acid lowers the pH and creates an
environment that is unfavorable to pathogens.

Keywords: Lactic bacteria, antibacterial substances, valorization of waste, fermentation.

INTRODUCTION

The orange tree is a small tree of the citrus group, grown in Mediterranean countries and in warmer regions. Its fruit is round with orange skin, sometimes streaked with red; the juicy and tart pulp is divided into quarters, with or without glitches.
These citrus undergo transformation process to obtain other products such as juice, jam, compote etc. The large amounts of residues from these process are easily degraded and may contribute to the increase rate of Air pollution [2].
Hence the biological treatment of waste is obligatory.
So our study involves the use of lactic bacteria capable of processing this waste, these lactic bacteria inhibit the development of unwanted bacterial flora that is pathogenic or alteration, which secrete bacteriocins possess inhibitory activity against pathogenic bacteria , They ferment carbohydrates into lactic acid consequently, PH decrease which is favorable to the bio food preservation. [3]
In this task, we isolated strains of lactic, acidifying tested their power, their bactericidal and search the nature of inhibitory substances.

Materials and Methods

Isolation and identification of lactic strains :

During the different stages of the transformation process, twenty strains of bacteria lactic was isolated from orange juice (BL1, BL2 ....BL20).
The isolation is performed on solid environment, MRS (Man Rogosa Sharp, Difco, Detroit, USA) [5], an adapted and suitable environment for specific research about lactobacilli. The cultures were incubated for 24 hours at 30 ° C in Petri dishes in the dark. The purification is performed by four successive subcultures spreading in MRS solid medium. The conservation is inclined on MRS medium at + 4 ° C in test tubes in the dark.
For strain identification, 0.1 ml of a culture in 24 hours at 30 ° C into the wells of API 20E (bioMérieux) / B. After 24 hours of incubation[6].
the identification was based on the determination of morphological and biochemical characteristics : catalase, growth temperature, carbon dioxide production, fermentation of various sugars.

The pH of lactic acid bacteria in MRS liquid :

Twenty bacterial strains were grown in three days on MRS liquid in vials at 30 ± 2 ° C in the dark. The initial and final pH are measured using a pH meter Orion Research as a combination electrode. The titration acidity is carried out on 10 ml culture with a sodium hydroxide solution 0.1 N using a burette Mohr valve, in the presence of a drop of a methanol solution of 1% phenolphthalein that is used as a color indicator. The acidity is expressed as mg of lactic acid (MW = 90.08 g) per 100 ml of culture.

The antibacterial power of strains:

Many methods were described for the detection of bacteriocin-producing lactic strains that are based on the principle that these proteinaceous substances can diffuse into a solid culture medium or semi-solid, which was previously inoculated with a target strain. The production of bacteriocins is detected by the inhibitory power of the tested microorganism filtrate on the growth of the target organism[4]. The 20 lactic acid bacteria strains are tested for their antibacterial power of the broadcast method [7] agar TSA (Tryptic Soy Agar, Difco, Detroit, USA). Three to five wells per Petri dish of 5 mm in diameter were carried out, with four cans per condition and with three replications at different times. Plates are flooded by the pathogenic strain Staphylococcus aureus ATCC 25923, and then the wells are filled with 60-80 .mu.l of filtered supernatant. The supernatant was neutralized with 0.1 N NaOH to obtain a pH of 6.50, and then a few drops of

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catalase to eliminate the effect of oxygen peroxide. After incubating of 24 hours at 30 ± 2 ° C, the diameters of inhibition zones appeared around the wells and they were measured (average of two perpendicular diameters).

The antibacterial spectrum of strain with higher acidifying power:

For the tests of antibacterial activity, some pathogenic microorganisms were chosen. They were isolated, and identified from contaminated biological products research laboratory, and medical analysis of Kenitra (Maâmora). These strains were tested for their resistance.

TABLE1: The removal of pathogenic bacteria used in the test

pathogenic strains	**Sample**
Negative gram	
Escherichia coli ATCC 25921	urinary
Klebsiella pneumoniae	broncho- pulmonary
Morganella morganii	urinary
Positive gram	
Staphylococcus aureus ATCC 25923	urinary

These strains were purified and stored in a Nourishing oblique gélose TSA (Tryptic Soy Agar) at 4 ° C in the dark. Before using them in the inhibition assays, they were activated by transfer of nutrient broth, and incubated for 16 to 18 hours at 37 ° C.

The three most efficient lactic acid strains for their acidifying power, which were stored at + 4 ° C, they were activated before their use in inhibition tests by transferring them on MRS broth. Supplemented with 2% yeast extract to optimize the culture medium, and then incubated for 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 stored at 4 ° C in the dark after neutralization. As before, we use the TSA agar diffusion method to demonstrate the bactericidal.

The location of the inhibitive substance of the BL1 strain:

From a culture of BL1 strain liquid MRS medium, incubated for 24 hours at 30 ± 2 ° C, a volume of 50 ml culture is centrifuged for ten minutes at 10,000 rev / min[8]. The inhibition tests are carried out as above in both the supernatant, representing the extracellular fraction, and the pellet corresponding to the cell fraction. The influence of the neutralization and the addition of a few drops of catalase [9] were tested on these two fractions. For each of these pathogens, four Petri dishes with two wells per box (with and without neutralization and addition of catalase) have been used with three replications.

Confirmation of the presence of bacteriocins:

Lactic acid bacteria can produce inhibitory substances. To ensure the presence of the latter, we took a young culture of 18 hours, which was cultured in 50 ml of modified MRS broth and incubated at 37 ° C.

After incubation, the tubes were centrifuged at 4000 rev / min to recover the supernatant. To eliminate the effect of the organic acids especially lactic and acetic acids, the supernatant was neutralized (pH = 7) by adding a solution of 0.1 N NaOH in the presence of a drop of a solution of 1% methanolic phenolphthalein used as a color indicator and devoid of catalase [9].

Results

PH and acidity:

Monitoring the pH and acidity shows a progressive decrease in pH for all strains isolated. BL1 and BL2 strains have a very strong acid value (Table 2). This table shows the results:

TABLE2: Measurement of pH and acidity lactic acid bacteria isolated from prepared samples, grown in MRS liquid / 24 at 30 ° C in the dark

lactic acid bacteria	pHi	pHf	acidity mg lactic acid / 100ml
BL1	5.77	3.96	0.98
BL2	5.95	3.98	0.98
BL3	5.85	3.97	0.94
BL4	6.22	5.01	0.73
BL5	6.21	5.01	0.93
BL6	6.02	5.98	0.72
BL7	6.32	5.97	0.79
BL8	5.93	4.12	0.79
BL9	6.05	5.98	0.83
BL10	6.23	5.01	0.69
BL11	6.56	5.04	0.93
BL12	6.12	5.14	0.70
BL13	6.08	5.21	0 .50
BL14	6.21	5.09	0.68
BL15	6.31	5.02	0.72
BL16	5.92	4.98	0.36
BL17	5.84	4.97	0.36
BL18	5.82	4.82	0.34
BL19	5.53	4.94	0.23
BL20	5.52	4.78	0.35

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The antibacterial spectrum of the three most acidifying strains:

All these lactic bacteria have a significant zone of inhibition but the first 3 are the most acidifying strains (Table 2).

The results of the study of the diameter of the inhibition zone of three strains of lactic acid bacteria, counterpart of four pathogenic strains by the diffusion method TSA after 24 hours at 30 ° C in the dark are shown in the following table 3.

TABLE 3: The diameter of inhibition zone of three strains of lactic acid bacteria.

pathogenic strains	**inhibition diameter (mm)**		
	BL1	**BL2**	**BL3**
Staphylococcus aureus	18	10	11
Morganella morganii	16	15	17
Escherichia coli ATCC 25921	9	11	8
Klebsiella pneumoniae	9	13	7

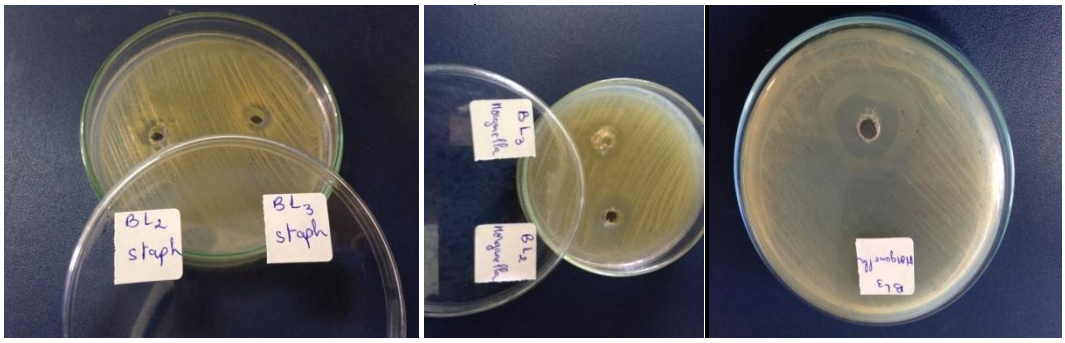

Photo 1 and 2 and 3: antibacterial activity of lactic strains BL2 and BL3. Counterpart, staphylocolus aureus and morganella by diffusion method tryptic soy agar medium TSA 24heurs after incubation at 30 ° C

The strains BL1, BL2 and BL3 are selected for their high acidifying power, have a bactericidal effect generally high vis-a-vis of the tested pathogens germs, BL2 strain has potent bactericidal activity on Escherichia coli ATCC 25921 (zone 11 mm inhibition), and Klebsiella pneumoniae, with an inhibition zone of 13mm (Table 3).
The other two strains are less active, BL1 strain exhibits bactericidal activity strong on Staphylococcus aureus (18 mm inhibition zone).
and the BL3 strain shows strong bactericidal activity on Morganella (17 mm inhibition zone).

The location of the inhibitory effect of the strain BL1:

To study the antibacterial activity of extracellular and cellular fractions of BL1 strain we came to carry the diffusion method TSA after 24 hours of incubation at 30 ° C.

TABLE 5 : Antibacterial activity of the extracellular and cellular fractions of the BL 1 strain by diffusion method TSA after 24 hours of incubation at 30°C

pathogenic strains	inhibition diameter **(mm** supernatant (extracellular fraction)	base (cellular fraction)
positive gram		
Staphylococcus aureus	17	0
Negative Gram		
Morganella morganii	16	0
Escherichia coli ATCC 25921	9	0
Klebsiella pneumoniae	9	0

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The cell fraction (base) has no effect on the growth of pathogenic strains used (table5). For cons, the extracellular fraction of the supernatant has strong antibacterial power

Photo 4: antibacterial effect of BL1 of staphylococcus aureus by the diffusion method tryptic soy agar medium TSA 24heurs after incubation at 30 ° C.

As before the inhibitory effect is dominant for the Gram positive bacterium Staphylococcus aureus (17 mm) among Gram-negative bacteria, it is mainly active on Morganella morganii (16 mm) .By cons the other two strains of Escherichia coli ATCC 25921 and Klebsiella pneumoniae have an inhibitory effect which remains low compared to other pathogenic strains.

DISCUSSION AND CONCLUSION

Waste fermentation citrus from pure lactic acid bacteria culture has given better results in relation to the stability and change the organoleptic characteristics of citrus waste.

Production inhibitors by the fermentation and lowering the pH of a level or most microorganisms are inhibited are important phenomena in the processing [10].

From the 20 strains of lactic acid bacteria isolated, the bactericidal activity and acidity appear highly variable and no apparent relationship with the habitat. [11]

The three most effective strains, BL1, BL2, BL3 do not have the same vis-à-vis spectrum of action of pathogenic bacteria.

The comparison between the results of the pH and acidity shows that the acidity increases with decreasing pH.

The bactericidal activity of the strain BL1 is found exclusively in the culture medium. It was therefore formation of extracellular substances.

From the results obtained, neutralization of supernatant and the addition of catalase did not cause decrease in the diameter of inhibition.

Gram-positive bacteria are generally more sensitive to the bactericidal effect of lactic bacteria [12].

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