

## Characterization of milk strains isolated from household waste

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### ABSTRACT

For over 4000 years, lactic acid bacteria are used to make many products, including fermented dairy products (cheese, yogurt...).

Fermentation gives food flavor and distinctive texture, can keep better and also provides some nutritional and health benefits. If this practice was the intuitive origin, its scientific basis are now better understood.

Waste collection will be available to households that are sorted by the following ground and subjected to spontaneous fermentation several physico chemical and microbiological analyzes are periodically established, we raise in this paper follow microbiological counts in order to see the effects lactic acid bacteria on the fate of other pathogenic bacteria and in order to achieve purification and characterization of isolates.

Analyzes to characterize bacterial strains are by physicochemical methods, biological tests for the specification of lactic strains for the selection of seeds, the most acidifying and more efficient, that would be fundamental to the preparation of a close which is subsequently used in a controlled fermentation.

As noted below strains Nos. 9 and 7 are the most acidifying this brings us to the preparation of a close under good conduct controlled fermentation.

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### KEYWORDS

Lactic acid bacteria;  
Ferment;  
Fermentation;  
Waste;  
Counts characterization.

### INTRODUCTION

With an ever larger and more diversified world-wide consumption, waste production is increasing in quantity and quality and generating enormous risks on the receiving environment<sup>[1,2]</sup> and therefore on the health of populations.

Our society is faced with a real problem so much more acute that ' formerly for many reasons including: ' s ' s population growth resulting increase in consumer

products, the decrease in the lifetime of goods and products, increase ' non- naturally destructible packaging ' s rising printed information (newspapers, concentration of populations and habitat type ' ...). This could cause parasitic diseases including helminths causing morbidity and mortality worldwide<sup>[3]</sup>.

This situation is much more serious in developing countries (DCs) in particular because of the considerable delay in the field due to their lack of resources and difficulty of addressing the issue with a suitable context approach.

The lack of data on waste characterization - which is a prerequisite for any management strategy and the difficulty to update the data possibly because of the exorbitant costs of the methodologies used, often for the settings of the northern countries, are the main constraints for the implementation of effective policy and sustainable waste management in developing countries. And landfills<sup>[4]</sup>.

Chemical and microbiological composition of household waste creates a special importance by researchers in the field of recycling of by-products and organic waste. Indeed, these wastes can not be used directly or in the rough because of their dangerous microflora from the point of view that alteration of hygiene.

Biological treatment of waste based on the ability of microorganisms to transform some compounds considered waste products re-recoverable. Following the use of anaerobic fermentation, our study involves the use of bacterial microorganisms capable of converting household waste into a stable by-product for two reasons:

- Lead accumulation areas, confine the reappearance of pollution are increasingly perceived in recent years and defend the neighboring people landfills méfaits deal with this kind of waste<sup>[5,6]</sup>.
- Regain a rich fountain of organic matter and other elements whose interest may be true at feeding cattle or rabbits or agriculture<sup>[7]</sup>.

Lactic acid bacteria are a diverse group of microorganisms that produce lactic acid as the main product of the metabolism. They are non-pigmented, anaerobic facultative aerobic, Gram - positive, catalase negative with the exception of certain types pseudo catalase and tolerant to acidic pH. Their shape may be coccoid, coccobacillary or bacillary.

They are generally mesophilic. Based on the characteristics of fermentation, the lactic acid bacteria are homofermentative and heterofermentative. In the first case, only the product is lactic acid. In the second, in addition to the lactic products are acetic acid, ethanol, carbon dioxide and formic acid.

Currently, lactic acid bacteria include twelve different bacterial genera :

Lactobacillus, Leuconostoc, Lactococcus, Enterococcus, Streptococcus, Pediococcus, Carnobacterium,

Oenococcus, Weissella, Aerococcus, and Tetragenococcus Vagococcus.

### Waste collection

Periodic samples of waste were made from the landfill. They are collected in crates of 30 kg capacity. Sampling was carried out at random. The funds are returned to the laboratory to sort. The nature and percentage of each compound were determined immediately after the release of the contents of each box.

### Preparation of study material

Organic waste is isolated after the sorting operation. They are then placed in a grinder at 1500 rpm. The ground material obtained is placed in pots of 5 kg for analysis. Periodic samples are prepared to perform Physico- chemical and microbiological analyzes.

### Analysis microbiologique

Microbiological analyzes were focused on the flora of Hygienic and biotechnological interest

### Enumeration of lactic strains

Several authors isolated lactic acid bacteria from different habitats belonging including the genera Streptococcus, Lactobacillus, Enterococcus and Lactococcus. Other species referenced and unidentified gave better results in the field of biotechnology<sup>[8]</sup>. The assessment of their abundance in a biotope is by culture in solid MRS (Man Rogosa Sharp, Difco, Detroit, United states). And that after incubation for 24 hours of incubation at a temperature of 30°C.

### Strains of lactic acid isolation

Ten strains of bacteria were isolated lactaiques ; The isolation is carried out on MRS medium (Man Rogosa Sharp, Difco, Detroit, USA) solid medium suitable for specific research lactobacilli. The cultures are incubated at 30 ° C for 24 hours in Petri dishes in the dark. Purification is achieved by four successive subcultures in spreading solid MRS medium. Conservation is inclined on MRS medium at +4 ° C in test tubes in the dark.

Ten bacterial strains were grown two days (48h) on MRS liquid medium in 100 ml flasks, at 30 ° C in

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the dark. with an initial pH of 6.46, 1N NaOH and 1N HCl.

### Physicochemical analyzes

#### Determination of pH

The pH was measured using a pH meter Type Orien Research. The measurement values are collected after calibrating the apparatus. The standards used are pH 4 and 7.

#### Determination of acidity

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

Percent acidity is expressed as mg of lactic acid (MW = 90.08 g) per 100 ml of culture :

$$\text{Acidity (\%)} = \frac{N \text{ NaOH Vol NaOH} \times 90.08 \times 100}{1000 \times \text{sample weight}}$$

#### Catalase test

The catalase test emulsion is used by a bacterial culture them in a drop of hydrogen to 30 g/l placed on a slide. The positive reaction resulted in the presence of a gas bubble disengagement.

#### Test gram

The cultures were examined by conventional staining gram, colored in purple bacteria are positive grams those colored pink are negative grams.

#### Measurement of optical density

Bacterial concentration was measured in culture by several methods that used in our case and which is most expedient is to make measurements of turbidimetry, that is to say, the optical density at 600 nm by a spectrophotometer

The suspension should be sufficiently dilute so that the optical density does not exceed a value of about 1.

## RESULTS AND DISCUSSION

### Lactic acid bacteria

#### Tests acatalase and test gram

All bacterial strains submitted to catalase test

**TABLE 1 : Test result gram stain and catalase isolated bacterial strains**

Selected lactic acid bacteria	Catalase	Gram stain
1	-	+
2	-	+
3	-	+
4	-	+
5	-	-
6	-	+
7	-	+
10	-	+

strains are catalase negative and gram more except number 5 gram negative strain that is what makes us to remember as gram positive and catalase negative microscopic observations are made and the strains that have the appearance of lactic acid are streaked on MRS medium (Man, Rogosa, Sharpe) solid.

To ensure their conservation lactic strains were streaked onto tubes containing nutrient agar slants (TSA Tryptic Soy Agar) at +4 ° C in the dark

#### pH

The monitoring of the various metabolic trait selected strains is provided by monitoring the pH.

PH changes were clearly differentiated one notices that the strains were 9 and 7 reduce the pH to a very large value that is respectively about 3.64 and 3.68 pH values against other strains n were not much difference and varied an average of 4.2.

We retain the two strains 9 and 7 for the preparation of our ferments if based only on the values of pH obtained but to confirm our perceived deduction we bring them to make other tests.

#### Acidity

Lactic acid bacteria are a group of microorganisms that are characterized by their ability to ferment carbohydrates into lactic acid, supports the preservation of food.

And bacteriocin production by inhibiting the development of pathogenic strains<sup>9,10</sup>.

Monitoring the acidity is a confirmation of the change in pH which is confirmed by the above mentioned values.

The acidity values stem 9 and 7 are the most important reports by other strains. The results men-

**TABLE 2: Change in the optical density of the pH and acidity lactic bacteria selected**

Selected lactic acid bacteria	pHf	PHf	DOi	DOf	Acidity
1	6,46	4,23	0,69	0,46	1,4
2	6,46	4,34	0,72	0,83	1,4
3	6,46	4,2	0,82	0,842	1,4
4	6,46	4,13	0,74	0,87	1,3
6	6,46	4,18	0,7	0,95	1,5
7	6,46	3,68	0,76	0,96	2,4
8	6,46	4,22	0,65	0,605	1,4
9	6,46	3,64	0,76	0,98	2,2
10	6,46	4	0,73	0,96	1,6

tioned above also converge well to the above selection obtained

**Optical density**

The optical density of the solution increases linearly with the number of bacteria.

Following the results depicted in TABLE # 2 we see clearly that stem 9 and 7 respectively reached the values 0.98 and 0.96 at dilutions of 1/5 an average increase of 32%.

For other strains increased from 1 % to 28 %.

We also observe that the strain # 10 arrives at an increase of 31 % with a pH of 4.

The optical density values of the further confirm the performance of the two above noted strains ; we retain for the preparation of the ferment

**CONCLUSION**

The biodegradable fraction of household waste represents 50% of the wet weight of the household trash. Many questions exist about the best treatment to be in place for this waste fraction.

The aim of that work is to highlight the economic and environmental interests of a sector of biological treatment. The feedback multiply and clearly reinforce the choice to integrate the management of organic waste in the overall elimination scheme. Relevance to install a biological treatment unit is reinforced by the massive fragrance different releases landfill.

Production sources of household and similar organic waste are many and varied. It follows that the

**TABLE 3 : Changes of pH and acidity of the optical density**

STEM	pHi	pHf	DOi	DOf	DL	AC
1	6.8	4.37	0.34	0.76	1/7	6.3
2	6.8	5.45	0.42	0.89	1/7	5.5
3	6.8	3.39	0.20	0.60	1/7	4.5
4	6.8	3.82	0.36	0.84	1/7	8
5	6.8	3.76	0.06	0.09	1/7	9
6	6.8	4.69	0.39	0.82	1/7	6
7	6.8	4.21	0.33	0.84	1/7	7
8	6.8	4.21	0.37	0.43	1/7	9
9	6.8	5.32	0.28	0.42	1/7	5.5

**DL: Dilution Factor**

tonnages likely biological treatment are high. Waste recovery through waste from the food industry, the fraction of organic waste in the biological treatment process of elimination to benefit<sup>[11,12]</sup>.

The challenges of establishing a biological treatment unit are important.

Establish a biological treatment unit often worries local government officials who fear the complaints of local residents. It is true that many facilities have led to conflicts with neighbors whose origins constant emission of foul odors. This era is now gone due to technological advances made since several years. Our presentation of the different modes of treatment tends to demonstrate that it is possible for all types of communities to engage in a chain of biological treatment of organic waste performance.

Production practices and spreading compost poor quality have created a lack of confidence in most industry players for conversion of organic waste.

It is up to local officials in charge of waste management that it is to give the necessary impetus to the development of biological treatment, full component of an overall scheme of disposal. Information campaigns and sorting ambassadors are all ways in which the community then has to accompany the citizen in a quality approach from the upstream sector.

The sustainability of the sector based on the alignment between supply and demand on the one hand and on the other hand, between the producers and users of finished products. The establishment of a consultative committee between all these actors is recommended to ensure product traceability.

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Quality<sup>[13]</sup>, tracking<sup>[14]</sup> and consultation are the watchwords which must be followed when setting up a chain of biological treatment of organic waste.

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