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Full Length Research Paper

Survey of the presence of *Lactobacillus plantarum* in the fermented maize dough hydrolysate used in the technology of the improvement of *lanhouin*

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Research works are currently going on to find out the real existence of *Lactobacillus plantarum* in the fermented maize dough hydrolysate and in other fermented maize products in West Africa sub region. This work aims to search for the presence of lactic acid bacteria in general and of the *L. plantarum* in particular, in fermented maize dough hydrolysate produced in Benin by the fermentation of the maize in water. The use of *L. plantarum* selective medium (LPSM) denoted the presence of characteristic yellow facultative-anaerobic colonies with a yellow aura in the hydrolysate. This result reveals therefore the presence of *L. plantarum* which turned around 10^5 to 10^6 CFU/ml.

Key words: Fermented maize dough hydrolysate, lactic acid bacteria, *Lactobacillus plantarum*.

INTRODUCTION

The use of lactic acid bacteria in the food industry has found widespread application in the world. Lactic acid bacteria, in the process of the fermentation of food, play a large role in the improvement of the health quality of the latter, according to Leisner (1994), cited by Leroi (2009). According to Liu et al. (2009), many food fermentation principles and practices are well established, and food companies can predictably produce consistently good quality fermented product.

Lactic acid bacteria are obtained from various sources in the food industry. By their heterofermentative nature, lactic acid bacteria are used in the monitoring of fermentation of food products (Nissen et al., 2009). The one often used in the control of the fermentation of fish and fish paste is *Lactobacillus plantarum*. But the use of the *Lactobacillus plantarum* for controlling the fermentation process is well appropriate (Annan et al., 2003). According to Halm et al. (2004), lactic acid bacteria do not affect the maturation process because they do not have proteolytic enzyme (protease). But they can destroy putrefying microflora, inducing accumulation of the products of protein hydrolysis that gives the taste and aroma of the salted food commodity.

With regard to the fermentation of fish, several technologies, especially Asian, raise the activity of *L. plantarum* by the supply of source of carbohydrate (such as rice) in the mode of fermentation of fish. In Benin, to value agriculture by-products, we intend to use the hydrolysate of fermented maize dough as a source of carbohydrate and lactic acid bacteria in the control of the fermentation of *lanhouin,* a fermented fish product used in Benin as a condiment (Dossou-Yovo et al., 2011).

Fish live naturally with a non-majority lactic flora (Leroi, 2009) in their gastrointestinal tract. According to Ringo et al. (1997), cited by Leroi (2009), Lactobacilli, notably *L. plantarum*, have been found in the Atlantic salmon (*Salmo salar*). The hydrolysate of fermented maize dough is suspected to contain lactic acid bacteria, in view of the

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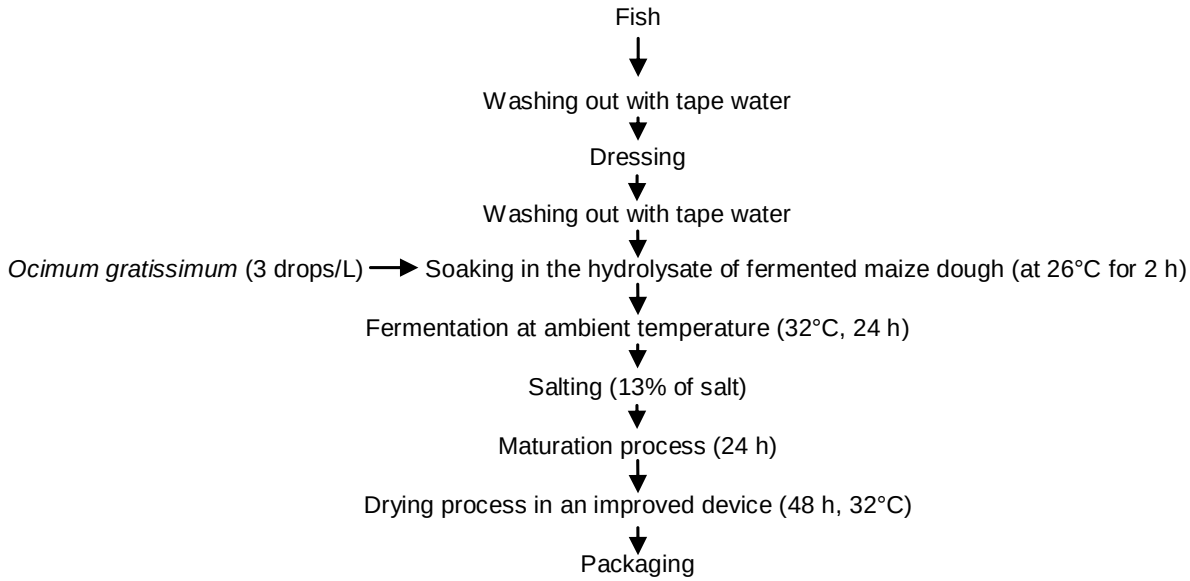

Figure 1. Flow chart of improved *lanhouin* production.

process of its obtaining. It appears therefore appropriate to characterize the hydrolysate of fermented maize dough to confirm its composition vis-à-vis the lactic acid bacteria and the *L. plantarum* in particular.

MATERIALS AND METHODS

The study took place in the Applied Biology and Microbiology laboratory of the Astrakhan State Technical University (ASTU), in Russia.

The study material consisting of the hydrolysate of fermented maize dough, and fermented, salted and dried fish flour from Benin was used to inoculate a selective culture media to identify firstly the population of lactic acid bacteria and in a second time to isolate the *Lactobacillus plantarum*. The use of the hydrolysate of the fermented maize dough is included in the flow chart (Figure 1) of the improved *lanhouin* production (Dossou-Yovo, 2002). *Lanhouin* is a product from fermented, salted and sun-dried fish. And in the view to have a product with very low water content, drying in a steam room was performed at 50 to 60°C.

Hydrolysate of fermented maize dough and fermented fish flour samples are collected according to the Russian standards GOST 25311-82 and GOST 26668-85.

Viability count of lactic acid microorganisms and their most probable number (MPN) were performed in accordance with Russian standards GOST R ISO 7218-2008 and GOST 10444.11-89 by deep sowing method on an agar with the hydrolyzed milk and a method of limiting cultivations on sterile skimmed milk (GOST 17681-82). The collection of pure culture of *L. plantarum* was obtained from the last 3 cultivations of sterile milk. In the last cultivations, we found lactic acid rod-shaped on the selective differential medium with bromocresol purple and ciprofloxacin according to Bujalance et al. (2006). On the given medium, colonies of *L. plantarum* are yellow colour with a yellow aura growth.

The selective differential medium designated LPSM (*L. plantarum* selective medium) for isolating and enumerating *L. plantarum* was as follows: the formulation per 0.4 L of medium was peptone (4 g), yeast extract (2 g), D-sorbitol (8 g), ciprofloxacin (1.6 g), sodium acetate (2 g), ammonium citrate (0.8 g), potassium phosphate (0.8 g), magnesium sulfate (0.04 g), manganesum sulfate (0.02 g), bromocresol purple (0.008 g), beef extract (4 g) and agar (6 g). The medium without ciprofloxacin was autoclaved for 15 min at 121°C and cooled at 50°C. Then ciprofloxacin was sterilized by filtration before being added to the cooled medium. The pH of the medium was 6.02 ± 0.1. The solidified LPSM was a purple color.

RESULTS AND DISCUSSION

Microbiological analysis of the hydrolysate of fermented maize dough and the fermented, salted and dried fish flour has shown the presence of viable heterofermentative lactic acid microorganisms which showed growth inside and on the surface of the agar with the hydrolyzed milk in the form of characteristic colonies same as with the study of Bujalance et al. (2006): small grayish colonies, superficial - Leuconoformings, light, deep - in the form of pieces of cotton - "spiders"; small round superficial colonies and deep colonies in the form of boat, sometimes with excrescence. It is noticed that in the hydrolysate, the number of typical colonies was 9 to17 × 10^5 CFU/ml, but in flour tests it is 2 to 3 times as lower (1 to 3 × 10^3 CFU/ml), which may probably be due to more rigid temperature mode of processing of the flour (drying in a steam room at 50 to 60°C). The microscopy of the painted and fixed preparations has shown the presence of gram-positive rod-shaped bacteria (Figure 2).

To find out the MPN of lactic acid microorganisms in investigated samples, signs of growth in all cultivations (to 5 inclusive), in the form of a clot and the formation of serum was found. The microscopy of clots of the sour milk has shown the presence of fixed non-spore forming

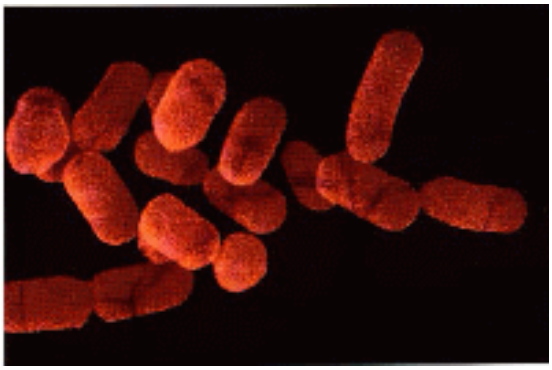

Figure 2. Gram-positive rod-shaped bacteria.

gram-positive long and short single rod-shaped and chains; the rod-shaped of the different length was in the form of separate or coupled cages or in the form of chains, and micrococcus of different size and located in an arrangement in space that allows them to carry the given microorganisms to heterofermentative lactic acid bacteria.

The subsequent seeding from the last 3 fermented test tubes with milk on the selective differential medium to obtain a pure culture of *L. plantarum*, has shown the presence of characteristic yellow facultative-anaerobic colonies with a yellow aura only in the sowed hydrolysate. The obtained results have allowed for calculating the number of *L. plantarum* which in initial hydrolysate is 10^5 to 10^6 CFU/ml. In the fermented fish flour, *L. plantarum* are not found out.

Conclusion

This study directed mainly on the hydrolysate of fermented maize dough, finally showed the presence of rod-shaped bacteria with all similitude to *L. plantarum*. But more study, especially a bio-molecular work is needed to confirm the real presence of *L. plantarum* in the hydrolysate of fermented maize dough used and its role in the control of the fermentation of fish to obtain improved *lanhouin* in Benin.

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REFERENCES

Annan N, Poll L, Sefa-Dedeh S, Plahar W, Jakobsen M (2003). Volatile compounds produced by *Lactobacillus fermentum*, *Saccharomyces cerevisiae* and *Candida krusei* in single starter culture fermentations of Ghanaian maize dough. J. Appl. Microbiol., 94: 462-474. DOI:10.1046/J.1365-2672. 2003.01852.

Bujalance C, Jimenez-Valera M, Moreno E, Ruiz-Bravo A (2006). A selective differential medium for *Lactobacillus plantarum*. J. Microbiol. Methods, 66: 572-577.

Dossou-Yovo P (2002). Biochemical justification for the improvement of the traditional methods of production of the *lanhouin* in Benin. Thesis submitted at the Technological University of Kuban State, Krasnodar, Russia, p. 129.

Dossou-Yovo P, Josse GR, Bokossa I, Palaguina I (2011). Survey of the improvement of fish fermentation for *lanhouin* production in Benin. Afr. J. Food Sci., 5(17): 878-883.

GOST 10444.11-89 Food products (1989). Russian standard of ''Methods of definition of lactic acid microorganisms''.

GOST 17681-82 Flour of animal origin (1982). Russian standard of ''Test methods''.

GOST 25311-82 Forage flour of animal origin (1982). Russian standard of ''Methods of the bacteriological analysis''.

GOST 26668-85 Food products and flavourings (1985). Russian standard: ''Methods of sampling for microbiological analysis''.

GOST R ISO 7218 Microbiology of foodstuff and forages for animals (2008). Russian standard: The general requirements and recommendations about microbiological researches.

Halm M, Osei-Yaw A, Hayford A, Kpodo KA, Amoa-Awua WKA (2004). Experiences with the use of a starter culture in the fermentation of maize for "Kenkey" production in Ghana. World J. Microbiol. Biotechnol., 12(5): 531-536.

Leisner J (1994). Characterization of lactic acid bacteria isolated from lightly preserved fish products and their ability to metabolize various carbohydrates and amino acids. Ph.D. Thesis. Technological laboratory, Lyngby, and The Royal Veterinary and Agricultural University of Copenhagen, Denmark.

Leroi F (2009). Bactéries lactiques et applications alimentaires. Partie 2: les produits de la mer. In : Physiologie, Métabolisme, Génomique et applications industrielles des bactéries lactiques (Economica). http://archimer.ifremer.fr/doc/00002/11340/

Liu ZY, Zhang ML, Zhang J, He L, Hou F (2009). Fermentation of bighead carp (*Aristichthys nobilis*) surimi and the characteristics of fermented bighead carp surimi products. J. Sci. Food Agric., 89:511-516. DOI: 10.1002/JSFA.3488.

Nissen L, Chingwaru W, Sgorbati B (2009). Gut health promoting activity of new putative probiotic/protective Lactobacillus spp. Strains: a functional study in the small intestinal cell model. Int. J. Food Microbiol., 135(3): 288-94.

Ringo E, Olsen RE, Overli O, Lovik F (1997). Effect of dominance hierarchy formation on aerobic microbiota associated with the epithelial mucosa of subordinate and dominant individuals of Arctic char, *Salvelinus alpinus* (L.). Aquacult. Resour., 28: 901-904.