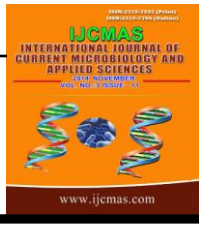
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 11 (2014) pp. 1-8**
http://www.ijcmas.com



Original Research Article

Isolation and Quantification of Lactic Acid Bacteria from Traditional Fermented Products in Benin

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Keywords

Fermented products, lactic acid bacteria, isolation, quantification

A B S T R A C T

In Africa, fermented food products are particularly used as weaning foods for young children, pregnant women and the seniors. In Benin, most of these cereals-based foods are manufactured and sold around the streets. These are ablo, dèguè, akpan, abotin, gowé etc ... This study focused on the isolation and enumeration of bacteria and yeast from twenty six (26) samples of traditional African fermented foods. Decimal dilution method allowed us to isolate a total of 42 different strains of microorganisms among which we counted 30 lactic acid bacteria. They confirmed their biological potential by expanding in selective medium MRS. When they were then subjected to screening, the medium of MRS-broth-starch agar, and eight (08) of the isolated strains tested showed their characters of amylolytic strains. The eight strains isolated were tested and finally cultivated at temperatures and pH ranging respectively from 30°C to 60°C and 3.0 to 6.5. These eight (08) amylolytic lactic acid bacteria (ALAB) synthesized enzyme amylase both inside and outside the bacterial cell with a very interesting activity value. The temperature limit between 40 and 45°C is best for the bacteria 26.2, B16 and B18 while the optimum pH for amylase synthesis is between 4.0 and 4.5 for the same microorganisms that have given the largest values of enzyme activity. The best strains 26.2, B16 and B18 secrete more enzymes in extracellular medium than intracellular.

Introduction

Fermentation is one of the oldest and economical production methods and food preservation in the world. For several decades, fermented foods are very important in the diet of African (Odunfa, 1985). In Africa, these fermented foods are

particularly used as weaning foods for small children (Kalui *et al.,* 2008). In Benin, these products are very much involved in the diet of the elderly and are often prepared with cereals (Bokossa *et al.,* 2013). During fermentation, the bacteria and fungi transform the raw material fermented food products. During the fermentation process the lactic acid bacteria (LAB) are capable of lowering the pH of the product to below 4.5. Which acidification is the hygienic point of view a major advantage because it prevents the growth of most pathogens (Tchekessi *et al.*, 2013). Thus, the preservative effect of lactic acid bacteria in the manufacture and storage of fermented foods is mainly due to that they create acidic conditions by converting carbohydrates into organic acids (lactic and acetic acids) in the food (Hiromi *et al.,* 1997) cited by Mavhungu (2006).

Several studies have shown that these lactic acid bacteria are probiotics. Probiotics are defined as live microorganisms, mainly bacteria, which are safe for human consumption and which have beneficial effects on health (Bonifait *et al.,* 2009). Grasson (2002) cited by Mavhungu (2006) demonstrated the capacity of lactic acid bacteria (LAB) to control a range of human pathogens including *Escherichia coli*, *Campylobacter jejuni* and *Clostridium perfringens*. The same author has shown that other effects of these probiotic bacteria reside in their ability to fight the tumors and to stimulate significantly the immune system. In addition, beneficial role of lactic acid bacteria in food preservation and Human Health was also demonstrated by Smahan in 2010. As regards amylolytic lactic acid bacteria, it could be of economic interest in the production of lactic acid from direct fermentation of starchy products (Xiaodong *et al.*, 1997) and could provide an alternative for treating vegetable wastes (Chamersee *et al.*, 1997).

Indeed, dèguè is a fermented beverage made from milk and cereals flour. It is a beverage widely consumed in Benin and other countries in West Africa. Today, due to the intensification of the phenomenon of street food, production and consumption of dèguè have grown in size and a rapid growth in Benin. Bokossa (2014) developed three (03) types of dèguès all having a pH below 4.5. The same author has shown that is a highly nutritious food that can contribute to food security in Benin. However, no study has been done on the screening, quantification and isolation of fermentative microorganisms from these fermented cereals products.

Materials and Methods

We have isolated from twenty-six samples of African fermented traditional food product some lactic acid bacteria by the decimal dilution method. The samples consist of cereals such as maize, millet and sorghum, as well as yogurt and other fermented foods prepared with these cereals and milk. The products studied were seeded on MRS media and incubated under anaerobic conditions at 30°C for 48hours. Each sample, we counted the total flora of lactic acid bacteria.

After 48 hours of incubation, we isolated 42 pure strains of microorganisms from which the cultural and morphological characteristics were studied. The new isolates were subjected to screening to determine their amylolytic activities by use of medium-starch agar MRS-broth. A total of eight (08) strains isolated and tested according to this method and showed their character of amylolytic strains. Then all these strains were submitted to the test, to verify their ability to synthesize the amylase enzyme and/or glucoamylase.

To confirm if this is pure cultures of bacteria lactic acid (LAB) isolated, we tested at two levels of the following criteria:

1-Morphologically: microscopic observation of individual cells, their shape, the color of their colonies.

2-Biochemically: we performed staining Gram's Method and observation of catalase activity.

Thus, the morphology of the cells was determined and biochemical tests (Gram coloration, presence or absence of the enzymatic activity of catalase in a solution of 3% H_2O_2) were performed.

Screening of amylolytic lactic acid bacteria (ALAB)

Qualitative analysis of the amylolytic activity of the isolated strains was performed by culturing of the isolates of ALAB in selective MRS (solid and liquid) medium containing 10g/L of starch as sole carbon source. In liquid medium, was added to the acid and indicator that changes color upon acidification of the medium. The detection of the amylolytic activity of isolated ALAB was performed with a culture of 24 hours on MRS agar-starch; starch is used as the sole carbon source. It was used for qualitative analysis of the amylolytic activity of the isolates on solid culture medium, the test method of agar-diffusion. After incubation at 37°C for 48 hours, the plates were poured with a solution of iodine and potassium iodine in w/v (0.15% I_2 and 1.5% KI) forming a blue starch-iodine complex. In the presence of amylolytic activity is observed in round pushed bacterial colonies, a clear zone due to the absence of starch consequently its assimilation.

The qualitative analysis of the presence of the amylolytic activity in isolated LAB was tested in a liquid culture medium, MRS-broth liquid starch. For this purpose, we used cultures of 24 hours; these bacteria were incubated at 37°C for 48 hours. In the presence of amylolytic activity, it was observed a color change of the culture medium due to their acidification. As a positive control, in all our quality tests, it was used a reference strain amylolytic *Lactobacillus paracasei* DSM 23505 B41.

Determination of glucoamylase activity of ALAB

The glucoamylase activity of isolated ALAB was demonstrated by their ability to degrade starch. This activity was determined by measuring the amount of reducing sugars by the method of 3.5-DSK. To conduct investigations we used a culture of 24hours of ALAB. The glucoamylase activity of the isolated bacteria was determined at a temperature of 55°C and pH 5.5 (0.1 mol/L phosphate buffer solution of citrate). The synthesized quantity of enzymes for hydrolysis of starch was measured. One unit of glucoamylase activity is the amount of enzyme required to liberate one (1.0) micromol of glucose from soluble starch at 1% for a (1.0) minute to the temperature and the appropriated pH.

Determination of α-amylase activity of ALAB

The α-amylase activity was determined in isolated ALAB. These bacteria have shown their ability to utilize starch as the sole carbon source in a solid culture medium and/or modified by the liquid according to the method described per Agati *et al.* (1998) and Giraud *et al.* (1993). The enzymatic activity of the isolates was determined at different temperatures values between 30 and 60°C and pH values between 3.0 and

6.5 to mark profiles of temperature and pH of the action of α-amylase by ALAB. One unit of enzyme is considered the required amount of enzyme capable for decomposing 10 mg starch during 30 min.

Results and Discussion

Quantification and isolation of lactic acid bacteria or not from traditional fermented African

African traditional fermented foods and beverages prepared from cereals such as maize, millet and sorghum are the best sources of potentially isolating amylolytic lactic acid bacteria (ALAB). For this purpose, 26 fermented products were used to isolate LAB. In each product, it was determined the total bacterial flora (Table 1). A total of 42 different bacteria were isolated and counted. Among these bacteria, 30 were confirmed as LAB grown on selective medium appropriated MRS. Apart from LAB, other microorganisms have been isolated based on their morphology and by microscope, and they belong to the group of yeasts.

The results presented in Table 1 show that the number of lactic acid bacteria in tested products varied from 3.176-9.505Log CFU/mL.

The microorganismes content of the grains (millet, maize, sorghum) and flours from these cereals were situated between 3.176-5.362Log CFU/mL. Contrary to grains, in fermented milk products, the number of lactic acid bacteria was significantly higher (7.5 to 9.5 log CFU/ml).
The results can be described as logical in the sense that the natural yogurt added to milk as ferment naturally contains bacteria. We have not recorded any microorganism in the maize grains, white sorghum grains and millet flour. However, yeast colonies were observed. This shows that in obtaining of these fermented products with taste and specific flavor intervene a mixture of culture of bacteria and yeast.

Determination of glucoamylase activity isolated ALAB fermented products obtained based cereal grains

Table 2 shows the results of glucoamylase activity of isolated ALAB from fermented products obtained based cereal grains.

Biosynthesis of amylase enzyme from isolated LAB

To verify the power of biosynthetic of isolated LAB, we proceeded to the culture during the last 48hours on medium containing starch. The activity of synthesized amylase enzyme was determined not only outside of the cell and in the culture medium but also inside the cell after disintegration of the cell wall at different temperatures and pH. The isolated LAB presented different amylasics activities. According to the temperature (Table 3), the value of the extracellular activity in the culture medium varies from 0.00U/mL to 14.75U/mL. The bacterium 26.2 presented the higher extracellular activity at the temperature of 45°C and at pH 5.0. The same observations were made by Agati *et al.* (1998) on another fermented product of Benin called "Ogi". Thus, these authors have shown that some ALAB of strains of OgiE1 develop their amylolitic activity at optimum temperature of 45°C and pH 5.0. According to Table 3, all isolated ALAB have their greatest enzymatic activity between 40 and 45°C. The ALAB 26.2 (13.066 U/mL), 26.1 (7.891 U/mL), B18 (8.181 U/mL) and B16 (7.549 U/mL) have higher values of temperature and these values have been obtained at 40 or 45°C.

Table.1 Number of lactic acid bacteria (LAB) isolated in tested products

№	Products	Number of LAB, Log CFU/mL
1	Yogurt nature	7.591
2	Fermented milk powder	9.431
3	Dèguè millet	9.041
4	Dèguè red sorghum	9.505
5	Dèguè white sorghum	8.845
6	Dèguè maize	9.230
7	Millet pellets	7.301
8	Maize pellets	8.369
9	Red sorghum pellets	9.380
10	White sorghum pellets	7.380
11	Millet grains	5.362
12	Maize grains	-
13	Red sorghum grains	4.903
14	White sorghumgrains	-
15	Millet flour	-
16	Maize flour	5.079
17	White sorghum flour	3.176
18	Red sorghum flour	9.255
19	Reconstituted milk powder (80%) mixed with soy milk (20%) and fermented	9.176
20	Fermented cow's milk	7.863
21	Dèguè mil from cow's milk	7.602
22	Dèguè red sorghum from cow's milk	7.556
23	Dèguè white sorghum from cow's milk	7.716
24	Dèguè maize from cow's milk	7.949
25	Dèguè Abokpan	8.322
26	Dèguè Akpan	8.033

Legend: LAB: lactic acid bacteria

Table.2 Glucoamylase activity of ALAB isolated from fermented products

№	ALAB isolated	Extracellular activity GLA, U/mL	Intracellular activity GLA, U/g
1.	3.1	0.251	0.562
2.	3.2	0.317	1.541
3.	26.1	-	0.256
4.	26.2	-	-
5.	21.1	-	-
6.	B16	0.456	1.235
7.	B17	-	-
8.	B18	-	-

Legend: ALAB: Amylolytic lactic acid bacteria; GLA: Glucoamylase
U/mL: Unit per milliliter; U/g: unit per gram

Table.3 Amylase Activity of isolated ALAB at different temperatures

ALAB	Extracellular activity Amyl, U/mL							Intracellular activity Amyl, U/g						
isolated	30ºC	35ºC	40ºC	45ºC	50ºC	55ºC	60ºC	30ºC	35ºC	40ºC	45ºC	50ºC	55ºC	60ºC
3.1	0.683	0.769	3.536	1.213	2.528	1.161	0.410	0.333	0.999	3.177	1.076	2.280	0.974	0.000
3.2	1.179	1.281	3.399	1.742	1.503	1.008	0.649	0.948	1.127	2.869	1.793	0.974	0.077	0.000
26.1	1.144	4.663	4.202	7.891	0.939	0.478	0.137	0.820	1.998	2.946	4.484	2.229	1.307	0.205
26.2	1.196	10.231	7.430	13.066	5.226	1.315	0.188	0.974	5.611	6.584	7.071	6.072	2.485	0.666
21.1	0.444	0.529	1.452	3.450	3.433	1.623	0.239	0.102	0.384	1.127	2.998	3.177	0.999	0.000
B_{16}	2.220	4.595	7.549	4.031	2.682	1.196	0.222	0.897	1.819	3.126	2.690	2.331	0.999	0.000
B_{17}	2.818	3.245	6.456	3.006	1.879	0.803	0.000	0.974	0.999	1.742	1.665	0.794	0.307	0.000
B_{18}	1.281	4.441	7.310	4.236	2.528	1.008	0.137	1.460	2.639	6.917	5.303	2.383	0.897	0.000

Legend: ALAB: Amylolytic lactic acid bacteria; Amyl: Amylase; U/mL: Unit per milliliter; U/g: unit per gram

Table.4 Amylase activity of isolated ALAB at different pH values of the culture medium

ALAB	Extracellular activity Amyl, U/mL								Intracellular activity Amyl, U/g							
isolated	pH 3.0	pH 3.5	pH 4.0	pH 4.5	pH 5.0	pH 5.5	pH 6.0	pH 6.5	pH 3.0	pH 3.5	pH 4.0	pH 4.5	pH 5.0	pH 5.5	pH 6.0	pH 6.5
3.1	1.776	2.340	3.997	5.636	3.331	2.664	0.529	0.000	0.999	1.050	1.793	1.998	3.305	2.383	0.743	0.026
3.2	1.503	3.160	5.209	5.534	3.211	2.664	0.888	0.000	0.820	1.307	2.075	2.562	2.895	1.614	0.692	0.231
26.1	2.613	3.143	3.314	5.414	7.191	5.739	1.008	0.154	0.615	0.717	2.101	2.716	4.458	2.434	0.589	0.128
26.2	1.537	2.135	2.835	7.771	14.757	5.244	1.401	0.000	0.769	1.409	2.639	4.048	7.686	4.740	1.332	0.026
21.1	0.769	2.682	3.467	4.150	4.492	3.484	0.837	0.068	0.000	0.666	3.074	3.587	4.355	2.485	0.922	0.026
B_{16}	0.752	2.921	3.723	7.139	4.526	3.194	1.161	0.222	0.666	1.588	2.126	3.279	2.511	1.281	0.820	0.026
B_{17}	1.657	1.759	2.613	6.149	7.259	4.253	1.059	0.085	0.000	1.537	1.640	4.202	2.998	2.331	0.922	0.256
B_{18}	0.854	1.469	1.588	8.181	4.748	2.887	0.991	0.000	0.410	0.589	1.665	6.943	4.612	4.302	1.179	0.154

Legend: ALAB: Amylolytic lactic acid bacteria; Amyl: Amylase; U/mL: Unit per milliliter; U/g: unit per gram

So, we can say that the optimum action of the secreted amylase enzyme by the bacteria temperature is between 40 and 45°C. Our results are comparable to those obtained by Pompey *et al.* (1993) and Guiraud *et al.,* 1991, which showed that *Lactophilus amylovorus, Lactophilus amylophilus Lactophilus plantarum A6* and present their amylolytic activities at an optimal temperature between 40-50°C. The lowest activity is presented by the bacterium 3.1 (1.213U/mL).

As regards the pH (Table 4), the most interesting values are recorded enzymatic activity at pH 4.5 and 5.0. These results are contrary to those obtained by Pompey *et al.* (1993) and Guiraud *et al.,* 1991, which showed that the amylasic pH of *Lactophilus amylovorus, Lactophilus amylophilus* and *Lactophilus plantarum A6* is situated between pH 5.0-6.0. The extracellular activity of isolated LAB 26.2 (14.757 U/mL) at 45°C and pH 5.0 is superior to any other activity of all isolated LAB. The temperature and pH are two important parameters influencing the biosynthesis of glucoamylase and amylase enzymes secreted by these amylolytic lactic acid bacteria.

Conclusion

The present study allowed isolating from our fermented food products eight (08) different amylolytic lactic acids bacteria which have developed a strong ability of synthesis of amylase enzyme and glucoamylase. The limits of temperature and pH respectively from 40 to 45°C and 4.5 to 5.0, are two parameters indicating the optimum conditions for biosynthesis of enzyme amylase.

Acknowledgements

The authors wish to thank the Ministry of Higher Education and Scientific Research (MESRS) of Benin for financial support.

References

Agati V., J.P. Guyot, J. Morlon-Guyot, P. Talamond and Hounhouigan, D.J. 1998. Isolation and characterization of new amylolytic strains of *Lactobacillus fermentum* from the fermented maize doughs (mawe and ogi) from Benin. Journal of Applied Microbiology 85, 512-520.

Bokossa, A., 2014. Production et caractérisation physico-chimique, microbiologique et sensorielle de trois catégories de "Dèguè", une boisson locale fermentée à base de lait, Mémoire de Master, Faculté des Sciences et Techniques, Université d'Abomey-Calavi, Bénin, 84p.

Bokossa Yaou, I., C. K. C. Tchekessi, J. Banon, C. Agbangla, Adeoti K. et Dossou-Yovo, P. 2013. Etude socio-économique de production d'une pâte traditionnelle fermentée "gowé" fabriquée à base de maïs au Bénin. Journal de la Recherche Scientifique de l'Université de Lomé (Togo), Série A, 15(3), 347-358.

Bonifait, L., F. Chandad et Grenier, D. 2009. Les probiotiques en santé buccale : mythe ou réalité? Journal of the Canadian Dental Association 75(8), 585-590.

Chamersee, M., S. L. Chakrabarty, B. D. Chattopadhay and Mandal, R. K. 1997. Production of lactic acid by direct fermentation of starchy wastes by an amylase producing *Lactobacillus*. Biotechnology Letters 19, 873-874.

Giraud, E., L. Gosselin, B. Marin, J. L. Parada and Raimbault, M. 1993. Purification and characterization of an extracellular amylase from

Lactobacillus plantarum strain A6. Journal of Applied Bacteriology 75, 276-282.

Giraud, E., A. Brauman, S. Keleke, B. Lelong and Raimbault, M. 1991. Isolation and physiological study of an amylolytic strain of *Lactobacillus plantarum*. Applied Microbiology and Biotechnology 36, 379-383.

Kalui, C. M., J. M. Mathara, P. M. Kutima, C. Kiiyukia and Wongo, L. E. 2008. Partial characterization and identification of lactic acid bacteria involved in the production of ikii: a traditional fermented maize porridge by the Kamba of Kenya. Journal Tropical of Microbiology and Biotechnology 4(1), 3-15.

Mavhungu, N. J. 2006. Isolation and Caracterization of Lactic Acid Bacteria from « Ting » in Northern Province of South Africa. Master Microbiology, University of Pretoria, South Africa, 84 p.

Odunfa, S. A., 1985. African fermented foods. In: Wood BJB, ed. Microbiology of foods. London: Elsevier, 155-191.

Pompeyo, C. C., M. S. Gomez, S. Gasparian and Morlon-Guyot, J. 1993. Comparison of amylolitic properties of *Lactophilus Amylovorus* and of *Lactophilus Amylophilus*. Applied Microbiology and Biotechnology 40, 226-269.

Smahan, A. A., 2010. Beneficial Role of Lactic Acid Bacteria in Food Preservation and Human Health: A Review. Research Journal of Microbiology 5, 1213-1221.

Tchekessi, C. K. C., I. Bokossa Yaou, J. Banon, C. Agbangla, K. Adeoti, P. Dossou-Yovo et Assogba, E. 2013. Caractérisations physico-chimiques et microbiologiques d'une pâte traditionnelle "*gowé*" fabriquée à base de maïs au Bénin. Journal de la Recherche Scientifique de l'Université de Lomé (Togo), Série A, 15(2): 377-387.

Xiaodong, W., G. Xuan and Rakshit, S. K. 1997. Direct fermentative production of lactic acid or cassava and other starch substrates. Biotechnology Letters 9, 841-843.