Scientific Research Publishing

Advances in Microbiology, 2020, 10, 563-574
https://www.scirp.org/journal/aim
ISSN Online: 2165-3410
ISSN Print: 2165-3402

First Development of a Biotechnological Ferment Based on a Consorsium of the Genus *Bacillus* for the Optimization of the Fermentation Process of Cassava Tubers

Josabeth Ickofa[1,2,3], Christian Aimé Kayath[1,2*], Michel Dzondo Gadet[3,4]

[1]Laboratoire de Biologie Cellulaire et Moléculaire (BCM), Faculté des Sciences et Techniques, Université Marien N'GOUABI, Brazzaville, République du Congo
[2]Institut national de Recherche en Sciences Exactes et Naturelles (IRSEN), Avenue de l'Auberge Gascogne, Brazzaville, Congo
[3]Laboratoire de l'ingénierie moléculaire et Sensorielle Alimentaire (IMSA/ENSP), Université Marien N'GOUABI, Brazzaville, Congo
[4]Centre de Recherche et d'Initiation des Projets de Technologies, Cité Scientifique, Avenue de l'Auberge Gascogne, Congo, Brazzaville
Email: *chriskayath@yahoo.fr

How to cite this paper: Ickofa, J., Kayath, C.A. and Gadet, M.D. (2020) First Development of a Biotechnological Ferment Based on a Consorsium of the Genus *Bacillus* for the Optimization of the Fermentation Process of Cassava Tubers. *Advances in Microbiology*, **10**, 563-574.
https://doi.org/10.4236/aim.2020.1010041

Received: September 21, 2020
Accepted: October 27, 2020
Published: October 30, 2020

Abstract

Due to its nutritional values, cassava has become an unavailable food and is one of the essential foods in the Republic of Congo. Fermentation of tubers is still traditional. Fiftyrod-shaped spore-forming bacteria were screened and carried out in batch mode for the fermentation abilities of cassava tubers in order to develop biotechnological starter. The Penetrometry Index (PI) has been used to screen bacteria and 16SrRNA as well as *fibE*one step multiplex PCR which were used to molecularly identify isolates. Emulsification Index, Proteolytic as well as amylolytic, and cellulolytic activities of some strains were quantitatively evaluated for prooving orgaleptic characterics. As results *Bacillus subtilis* (MT994787), *Bacillus subtillis* (MT994789), *Bacillus tequilensis* (MT994788), *Bacillus safensis*, and *Bacillus subtilis* have been identified. Single isolates were able to ferment tubers in 48 h and 72 hours meanwhile *Bacillus* consortia were able to shift fermentation of tubers from 48 hours to 24 hours. The consortium could be used as the major bacterial starters. Strains were associated with the ability to secrete biomolecules including biosurfactants, protease, amylase and cellulase.

Keywords

Consorsium, Fermentation Process, Cassava, Strains

1. Introduction

The traditional fermentations of the cassava retting process are a major concern in rural areas in the Republic of Congo. Fermentation processes are based on natural ferments which create an infernal expectation of the fermentation time. The expected products generally have a non-standardized quality [1]. Fermentation does not present greater acceptance in the sensorial analysis for taste, aroma and general acceptance. Nowadays the work of microorganisms has been well documented to considered like an indisputable input of fermentation including the genera *Bacillus* [2], *Lactobacillus* [3] [4], *Lactoccoccus* [4], *Saccharomyces* [5] and *Candida* [6] and molds as well. The *Saccharomyces cerevisiae* is the only commercially available probiotic yeast used in controlled fermentations of bread and other dairy products from pastries [6] even if *Bacillus* GRAS species are on its way to be accepted like a probiotic and prebiotic bacteria [7].

Manihot esculenta, Cranzt, commonly called cassava [8], manioc, yuca, macaxeira, mandioca, aipim, and agbeli, is a woody shrub of the spurge family, Euphorbiaceae is extensively cultivated as an annual crop in Republic of Congo for its edible starchy tuberous root and all other derived products [9]. This plant represents nearly 80% of local consumption [1].

Bacteria of the genus *Lactobacillus* have already and intensively been used as a starter culture [10]. It is often difficult to accept that during a traditional fermentation there is only one genus of bacteria. Strains are usually in the form of anecosystem consortium [11]. Without citing the other genera, bacteria of the genus *Bacillus* are ubiquitous in acid or alkaline fermentations [2] and they should probably play several roles still unclear since the fermentative capacity seems to be associated only with yeasts and lactic acid bacteria. In addition *Bacillus* species have been used as a starter [12] for improving acetoin and tetramethylpyrazine in Baoning bran vinegar [13] and for bettering the production of okpehe, a traditional African fermented condiment in Nigeria [14]. It has been demonstrated that *Bacillus* species possess important characteristics for further development of starter cultures and its organoleptic characteristics [15].

By the time of drafting this paper no study has separately and specifically shown the genus *Bacillus* roles in the direct fermentation of cassava tubers and retting as well. The processing and production of cassava are rudimentary in the Republic of Congo. Developing a biotechnological ferment could be of great added value among the responses to be given to achieve food self-sufficiency. In this way, the objective of this work is to isolate and to evaluate the fermentation potential of *Bacillus* species from cassava tubers to select those favorable to be used as starter cultures for the elaboration of a cassava fermented food and derived products.

2. Methods

2.1. Isolation of Strains with the Ability to Ferment Tubers

Samples from fermented tubers have been collected. Dilutions were done and

microorganism suspension was streaked on Mossel supplemented with polymixin B. The Petri dishes were incubated at 37˚C for 24 h to 48 h. Each colony of different appearance was separately isolated. Purification of the isolates was rigorously done by successive cultures. Purity was estimated by using a microscope for morphological characterization. Gram status was determined by using 3% KOH. Sporulation, hydrogen peroxide (H_2O_2), and oxidases tests were used for biochemical characterization.

Three (3) pieces of freshly cut cassava tubers around 2 cm^3 in size and weighing 12.30 g each were added to the jars under aseptic conditions containing 100 mL of distilled water sterilized at 121˚C for 15 minutes. Then a volume of 3 mL of overnight culture was inoculated into the different jars. The optical density was taken before and after seeding. Physicochemical parameters such as softening, O.D., pH were respectively read using a graduated penetrometer, a spectrophotometer, and a pH meter. After 16 hours of incubation at room temperature, physicochemical parameters were read successively three times a day at a regular interval of 3 hours, during the 5 days of fermentation. The selection of the isolates was made on the basis of the complete softening time of the pieces of cassava tubers, which must be less than 72 hours with which the values of the O.D. and the pH are associated. At the end of the selection, 3 classes of isolates were retained; namely classes of isolates which fermented cassava tubers in 24, 48 and 72 hours.

Tuber penetrometer resistance is an effective and reliable method for evaluating cassava tubers strength. A mecanic penetrometer has been used to introduce in fermented tubers. During the fermentation process the penetrometry Index (PI) have been assessed. The values were established according to the texture of the fermented cassava tubers. A score of ten (10) was associated with the tuber whose the penetrometer completely has been penetrated and broke the tubers. An index of seven (7) to eight (8) was associated with the tuber with penetration of the stem creating cracks. An index of five (5) was associated with the tuber that the penetrometer entered but did not break. Zero (0) was associated with the tuber whose peak strength and maximum stress were not allowable.

2.2. The Production of Enzymes and Biosurfactant

The Proteolytic, amylolitic and cellulolytic activities of some *Bacillus* strains were assessed for the ability to secrete proteases, amylase and cellulase in the extracellular environment as described and modified [16]. The E24 was investigated by adding crude oil with LB medium in 1:1 ratio (v/v). The solution was vortexed for 5 min and incubated for 24 h. Emulsification percentage was calculated through the height of emulsion layer. In addition, E24 was determined for gasoline, diesel fuel or SAE 140. All the experiments were performed in triplicates. E24 = Height of emulsion layer/Total height of solution ×100.

2.3. Molecular Identification of Microorganisms

The recent molecular identification using the *fibE* gene encoding for the fibrinolytic enzyme has been used for targeting strains like *Bacillus amyloliquefaciens*, *B. subtilis*, *B. pumilus*, *B. licheniformis*, *B. altitudinis*, *B. mojavensis*, *B. safensis*, and *B. atrophaeus*. Extraction and purification of isolate genomic DNA were performed according to the NucleoSpin Microbial DNA (Macherey-NAGEL) kit. Briefly, isolates were grown in 5 mL LB broth for 24 h at 37˚C with stirring. The DNA purity was assessed by electrophoresis on agarose gel and by the ratio of optical densities 260/280 nm. 5 µL of each amplification product was mixed with 2 µL of loading buffer (BIOKE). Mixtures were subjected to electrophoresis on 1% agarose gel (w/v). The 10 kb 2-Log (BIOKE) was used as a molecular weight marker. The housekeeping 16S rRNA gene has been amplified by PCR (Thermal Cycler, Bio-Rad) by using universal primers fD1 (5'-AGACTTTGATCCTGGCTCAG-3' and rP2 (5'-ACGGCTACCTTGTTACGACTT-3'). 5 µL of each amplification product was mixed with 2 µL of loading buffer (BIOKÉ). Mixtures were subjected to electrophoresis on 1% agarose gel (w/v). The 10 kb 2-Log (BIOKÉ) was used as a molecular weight marker. The PCR products were purified using the solution of Gel Extraction kit (Omega Bio-tek), and the purified products were subjected to sequencing by the Sanger technique (3130 × l Genetic Analyser (Applied Biosystems)). The sequences obtained were aligned with the software BioNumerics 7.5 (Applied Maths, Belgium) and corrected manually to resolve discrepancies between the sense and antisense strands. Sequences were compared with homologous sequences contained in the sequence databanks through NCBI (National Center for Biotechnology Information (https://blast.ncbi.nlm.nih.gov/Blast.cgi) using the BLASTn program based on the identification criterion. All sequences have been stored in NCBI GenBank data.

3. Results

3.1. Screening of Bacteria with the Ability to Ferment Cassava Tubers

Appropriate dilutions of each collected sample were done on Mossel medium. 50 bacteria with *Bacillus* orientation were isolated. Morphological and cultural assessment revealed that all the isolates were Gram-positive, spore-forming and rod-shaped bacteria (data not showed). They were also catalase-positive and aerobic. Those bacteria have been assessed for their ability to quickly ferment tubers either in 24 hours, or in 48 hours or in 72 hours. Amond 90 isolates, 21 have been able to ferment tubers. Strains have been highlly showed a good penetrometry index (PI) including S3 (8), S7 (10), S8 (8)n S9 (10), S10 (S7), S13 (8), S18 (7), S19 (7), S20 (5), S24 (10), S26 (8), S32 (7), S35 (8), S38 (5), S40 (5), S42 (10), S44 (8), S46 (5), S48 (5), S49 (10 and S50 (10) (**Figure 1(a)**). Twelve (12) Isolates with the ability to easily fermentalone tubers were mixed in consortia. In single as results none bacterium has found to ferment in 24 h, nine

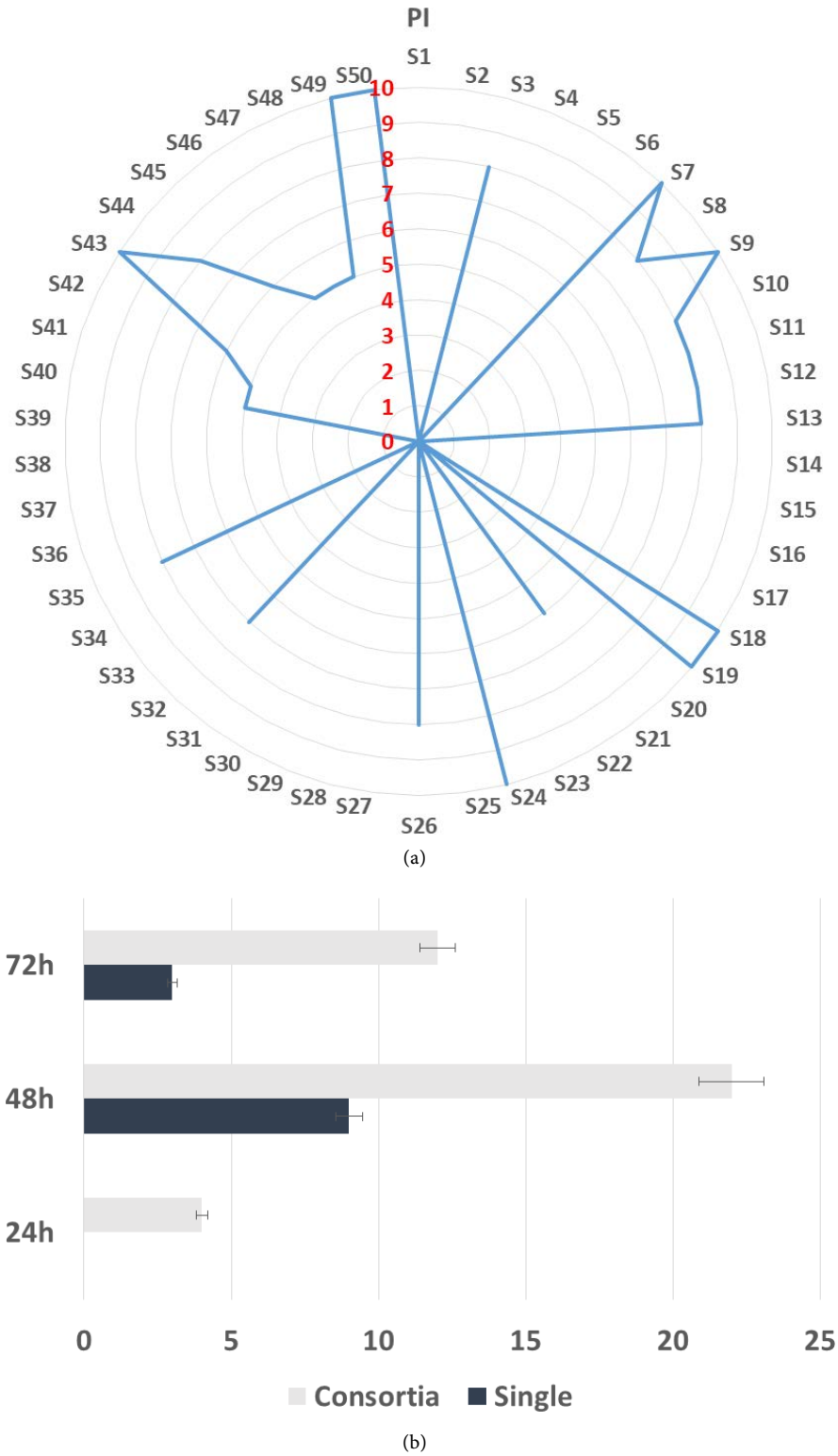


Figure 1. (a): Screening of isolates to ferment cassava tubers. S1, S2, ···, S50: Isolates. (b): Screening of single bacteria and consortia to ferment cassava tubers.

(9) in 48 h and three (3) in 72 h. Associations S7 + S35, S7 + S18, S7 + S19 and S7 + S26 were reduced the duration fermentation shifting from 48 hours when

testing alone to 24 hours in consortia and the penetrometry index were high to PI (10) scale (Figure 1(b)).

3.2. Identification of Isolated Bacteria

Isolates with good profiles based on fermentation ability were selected for the extraction of genomic DNA. A total of twenty one isolates were selected: S3 (8), S7 (10), S8 (8) S9 (10), S10 (S7), S13 (8), S18 (10), S19 (10), S20 (5), S24 (10), S26 (8), S32 (7), S35 (8), S38 (5), S40 (5), S42 (10), S44 (8), S46 (5), S48 (5), S49 (10 and S50 (10). The revelation was made on 1% agarose gel by using BET. After the amplification using an one step of multiplex PCR with the specific primers, results showed that only two pairs of specific primers allowed the amplification of the *fibE* gene allowed to identifiy *B. subtillis* and *B. Safensis.* This includes S7 and S35 (Table 1). The identification of other isolates with *Bacillus* sp oriented phenotype have been confirmed using 16S rRNA gene, *Bacillussubtillis* (Gen bank: MT994787), *Bacillus subtillis* (Gen bank: MT994789) and *Bacillus tequilensis* (Gen bank: MT994788) have been identified by corresponding to S18, S19 and S26, respectively. S32, S49 and S50 could not be identified this was associated to *Bacillus* spp (Table 1). The ability of Single Isolates to ferment cassava tubers has been highlighted in Figure 2.

3.3. The Abilities of Isolates to Secrete Biomolecules

Exploring bacterial communities with biomolecule production was purposed in this study. The isolates with a good fermentation capacity were subjected to be assessed for secretion of Biomolecules such as biosurfactants, cellulases, amylases, pectinases and proteases. By exploiting proteolytic activity we showed that isolates were able to secrete proteases in the extracellular medium. The diameter of clear zone around the wells indicates proteolytic digestion of the skimmed has been calculated (Figure 3(a)). Amylolytic and Cellulolytic activities have been carried out at 37˚C for enzymatic production and rate have

Table 1. Identification of Isolates by using 16S rRNA and One Step Multiplex PCR.

	Strains	Methods
S7	*Bacillus Safensis*	Classical test of Microbiology One Step Multiplex PCR
S18	*Bacillus subtilis* (Gen bank: MT994787)	Classical test of Microbiology 16S rRNA
S19	*Bacillus subtillis* (Gen bank: MT994789)	Classical test of Microbiology 16S rRNA
S26	*Bacillus tequilensis* (Gen bank: MT994788)	Classical test of Microbiology 16S rRNA
S32	*Bacillus* sp	Classical test of Microbiology
S35	*Bacillus subtillis*	Classical test of Microbiology One Step Multiplex PCR
S43	*Bacillus* sp	Classical test of Microbiology
S49	*Bacillus* sp	Classical test of Microbiology
S40	*Bacillus* sp	Classical test of Microbiology

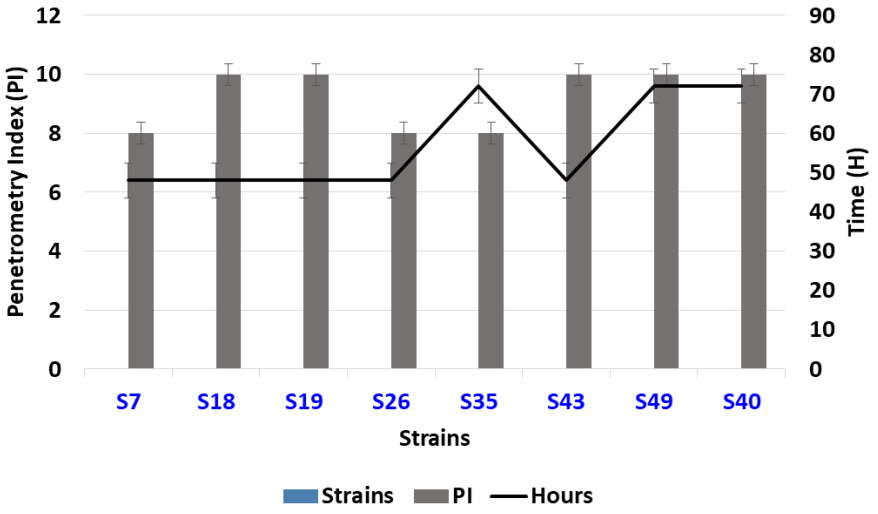
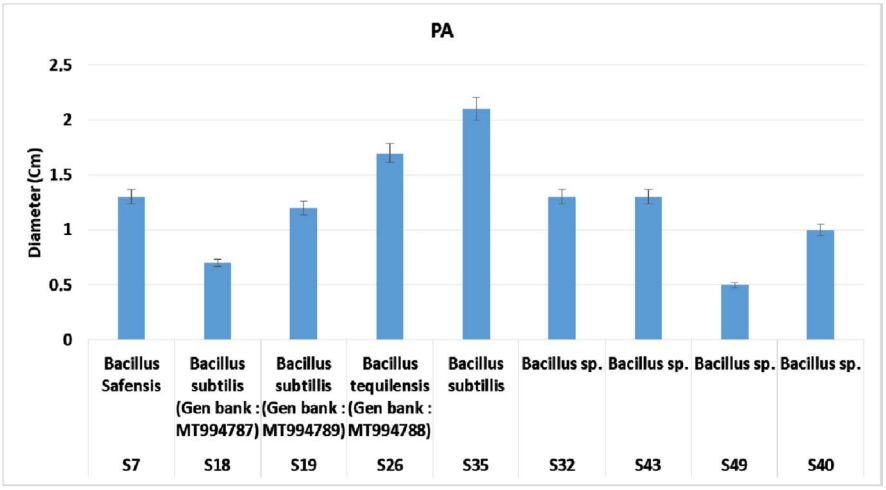


Figure 2. Ability of Isolates to ferment cassava tubers. S7: *Bacillus Safensis*, S18: *Bacillus subtilis* (Gen bank: MT994787), S19: *Bacillus subtillis* (Gen bank: MT994789), S26: *Bacillus tequilensis* (Gen bank: MT994788), S32: *Bacillus* sp, S35: *Bacillus subtillis*, S43: *Bacillus* sp, S49: *Bacillus* sp, S40: *Bacillus* sp.

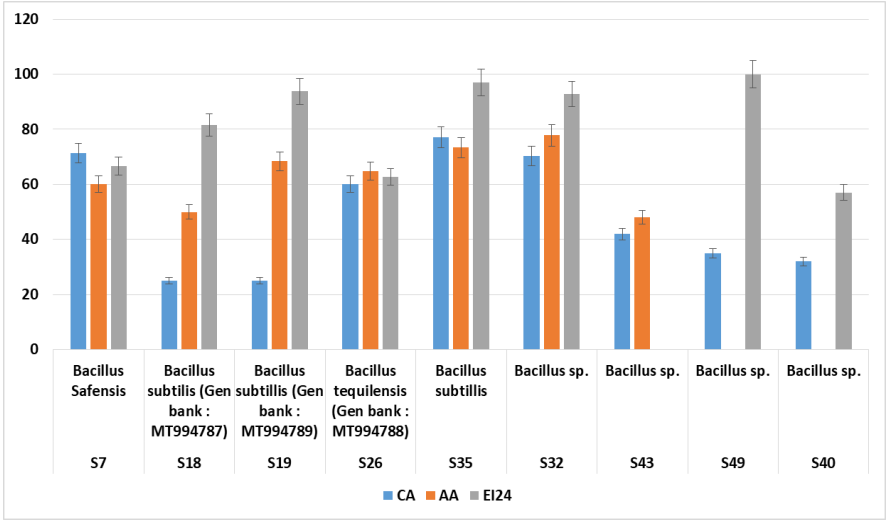
been done (**Figure 3(b)**). To highlight if identified strains could involve in the production of with more successful, a qualitative and quantitavie test called emulsion index in 24 hours (E24) has been conducted by inoculating precultures in flasks containing the nutrient broth. Incubation has been done overnight at 37˚C. As results the so-called starters were able to secrete biosurfactants by mixing with either gasoline. The average emulsion index (EI24) for gasoline are illustrated in **Figure 3(b)**.

4. Discussion

In this *in vitro* work we showed that Bacillus species could be used to ferment tubers from cassava crop. We found for the first time that *Bacillus subtilis* (Gen bank: MT994787), *Bacillus subtillis* (Gen bank: MT994789), *Bacillus tequilensis* (Gen bank: MT994788) and Bacillus Safensis could be used as starter in the cassava fermentation and retting. Some *in vitro* fermentation studies in terms of selection and evaluation of *Bacillus* strains as starter cultures have been demonstrated specially for the production of okpehe, a traditional African fermented condiment [14]. *Bacillus subtilis* and *Bacillus amyloliquefaciens* are widely used to produce fermented foods from soybeans and locust beans in Asian, and West African countries [17] [18]. Immobilized Cells of *Bacillus circulans* ATCC 21783 on palm curtain for fermentation and Daqu (a traditional fermentation starter for the production of baijiu and vinegar) and its derived products have been demonstrated [19] [20]. In addition *Bacillus* strains were further used as starters and tested for their ability to ferment several foods [21]. Bacteria belonging to the genus *Bacillus* isolated in this work are able to ferment alone the tubers between 40 hours and 72 hours. This includes *Bacillus subtilis* (Gen bank: MT994787), *Bacillus subtillis* (Gen bank: MT994789), *Bacillus tequilensis*



(a)



(b)

Figure 3. Ability of strains to secrete Biomolecules. (a): PA: proteolytic activity, (b): CA: Cellulolytic activity, AA: Amylolytic activity, EI24: Emulsification Index.

(Gen bank: MT994788), *Bacillus Safensis* and *Bacillus subtillis* that were isolated by using 16 rRNA gene and *fibE* one step multiplex PCR [22]. When Bacteria have been mixed in pairs, they systematically reduce the duration of fermentation for only 24 hours, testifying various interactions including mutualism and symbiosis between the strains. It's worthy to recognize that in traditional fermentation bacterial ecosystem as well as yeast are co-cultivated [23] [24]. Solid-state Co-cultivation of *Bacillus subtilis* and *Bacillus mucilaginosus* have been showed to stimulate cell growth [24].

In this work we showed that *Bacillus subtilis* (Gen bank: MT994787), *Bacillus subtillis* (Gen bank: MT994789), *Bacillus tequilensis* (Gen bank: MT994788) and *Bacillus safensis* were able to produce enzymes (cellulase, protease and amylase) and biosurfactants as well. The production of enzymes and biosurfactants by the isolated bacteria could give great added value. A couple of organoleptic

characteristics are the work of these biomolecules. Incorporation of lipopeptide MSA31 biosurfactant in muffin showed improved organoleptic qualities compared to positive and negative control [25]. Then the genus *Bacillus* is known for its ability to produce extracellular enzymes such as amylases [26], pectinases [27] [28] [29], cellulases [30] [31] [32], proteases [33] [34] and other biomolecules as well [35].

5. Conclusion

The use of bacteria of the genus Bacillus as a starter for Fermentation of cassava tubers improves and shortens the fermentation time to 24 h when bacteria are placed in consortium. This study opens the way for an important characterization of a local ferment which will make fermentation possible in short time to compensate the lack of cassava tubers in the Republic of Congo. The stater cultures are being inverstigated.

Acknowledgements

We are grateful to Institut national de Recherche en Sciences Exactes et Naturelles (IRSEN), Congo, Brazzaville for continuous encouragements of trainees.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Kayath, C.A., Nguimbi, E., Goma-Tchimbakala, J., Mamonékéné, V., Lebonguy, A.A. and Ahombo, G. (2016) Towards the Understanding of Fermented Food Biotechnology in Congo Brazzaville. *Advance Journal of Food Science and Technology*, **12**, 593-602. https://doi.org/10.19026/ajfst.12.3317

[2] Ouoba, L.I.I., Vouidibio Mbozo, A.B., Thorsen, L., Anyogu, A., Nielsen, D.S., Kobawila, S.C., *et al.* (2015) *Lysinibacillus louembei* sp. nov., a Spore-Forming Bacterium Isolated from Ntoba Mbodi, Alkaline Fermented Leaves of Cassava from the Republic of the Congo. *International Journal of Systematic and Evolutionary Microbiology*, **65**, 4256-4262. https://doi.org/10.1099/ijsem.0.000570

[3] Tsuji, A., Kozawa, M., Tokuda, K., Enomoto, T. and Koyanagi, T. (2018) Robust Domination of *Lactobacillus sakei* in Microbiota during Traditional Japanese Sake Starter Yamahai-Moto Fermentation and the Accompanying Changes in Metabolites. *Current Microbiology*, **75**, 1498-1505. https://doi.org/10.1007/s00284-018-1551-8

[4] Du, R., Ge, J., Zhao, D., Sun, J., Ping, W. and Song, G. (2018) Bacterial Diversity and Community Structure during Fermentation of Chinese Sauerkraut with *Lactobacillus casei* 11MZ-5-1 by Illumina Miseq Sequencing. *Letters in Applied Microbiology*, **66**, 55-62. https://doi.org/10.1111/lam.12824

[5] Zhu, L.X., Wang, G.Q., Xue, J.L., Gou, D.Q. and Duan, C.Q. (2017) Direct Stamp of Technology or Origin on the Genotypic and Phenotypic Variation of Indigenous *Saccharomyces cerevisiae* Population in a Natural Model of Boiled Grape Juice Fermentation into Traditional Msalais Wine in China. *FEMS Yeast Research*, **17**,

fow108. https://doi.org/10.1093/femsyr/fow108

[6] Amorim, J.C., Piccoli, R.H. and Duarte, W.F. (2018) Probiotic Potential of Yeasts Isolated from Pineapple and Their Use in the Elaboration of Potentially Functional Fermented Beverages. *Food Research International*, **107**, 518-527. https://doi.org/10.1016/j.foodres.2018.02.054

[7] Jezewska-Frackowiak, J., Seroczynska, K., Banaszczyk, J., Jedrzejczak, G., Zylicz-Stachula, A. and Skowron, P.M. (2018) The Promises and Risks of Probiotic Bacillus Species. *Acta Biochimica Polonica*, **65**, 509-519. https://doi.org/10.18388/abp.2018_2652

[8] Giles, J.A.D., Oliosi, G., Rodrigues, W.P., Braun, H., Ribeiro-Barros, A.I. and Partelli, F.L. (2018) Agronomic Performance and Genetic Divergence between Genotypes of *Manihot esculenta*. *The Anais da Academia Brasileira de Ciências*, **90**, 3639-3648. https://doi.org/10.1590/0001-3765201820180099

[9] Pinto-Zevallos, D.M., Pareja, M. and Ambrogi, B.G. (2016) Current Knowledge and Future Research Perspectives on Cassava (*Manihot esculenta* Crantz) Chemical Defenses: An Agroecological View. *Phytochemistry*, **130**, 10-21. https://doi.org/10.1016/j.phytochem.2016.05.013

[10] Jampaphaeng, K., Ferrocino, I., Giordano, M., Rantsiou, K., Maneerat, S. and Cocolin, L. (2018) Microbiota Dynamics and Volatilome Profile during Stink Bean Fermentation (Sataw-Dong) with *Lactobacillus plantarum* KJ03 as a Starter Culture. *Food Microbiology*, **76**, 91-102. https://doi.org/10.1016/j.fm.2018.04.012

[11] Janssen, D., Eisenbach, L., Ehrmann, M.A. and Vogel, R.F. (2018) Assertiveness of *Lactobacillus sakei* and *Lactobacillus curvatus* in a Fermented Sausage Model. *International Journal of Food Microbiology*, **285**, 188-197. https://doi.org/10.1016/j.ijfoodmicro.2018.04.030

[12] Wang, P., Wu, Q., Jiang, X., Wang, Z., Tang, J. and Xu, Y. (2017) *Bacillus licheniformis* Affects the Microbial Community and Metabolic Profile in the Spontaneous Fermentation of Daqu Starter for Chinese Liquor Making. *International Journal of Food Microbiology*, **250**, 59-67. https://doi.org/10.1016/j.ijfoodmicro.2017.03.010

[13] Zhang, L., Huang, J., Zhou, R. and Wu, C. (2017) Evaluating the Feasibility of Fermentation Starter Inoculated with *Bacillus amyloliquefaciens* for Improving Acetoin and Tetramethylpyrazine in Baoning Bran Vinegar. *International Journal of Food Microbiology*, **255**, 42-50. https://doi.org/10.1016/j.ijfoodmicro.2017.05.021

[14] Oguntoyinbo, F.A., Sanni, A.I., Franz, C.M. and Holzapfel, W.H. (2007) *In Vitro* Fermentation Studies for Selection and Evaluation of Bacillus Strains as Starter Cultures for the Production of Okpehe, a Traditional African Fermented Condiment. *International Journal of Food Microbiology*, **113**, 208-218. https://doi.org/10.1016/j.ijfoodmicro.2006.07.006

[15] Ramos, C.L., de Sousa, E.S., Ribeiro, J., Almeida, T.M., Santos, C.C., Abegg, M.A., *et al*. (2015) Microbiological and Chemical Characteristics of Taruba, an Indigenous Beverage Produced from Solid Cassava Fermentation. *Food Microbiology*, **49**, 182-188. https://doi.org/10.1016/j.fm.2015.02.005

[16] Fulazzaky, M.A., Abdullah, S. and Salim, M.R. (2016) Supporting Data for Identification of Biosurfactant-Producing Bacteria Isolated from Agro-Food Industrial Effluent. *Data Brief*, **7**, 834-838. https://doi.org/10.1016/j.dib.2016.03.058

[17] Kimura, K. and Yokoyama, S. (2019) Trends in the Application of Bacillus in Fermented Foods. *Current Opinion in Biotechnology*, **56**, 36-42. https://doi.org/10.1016/j.copbio.2018.09.001

[18] Amoa-Awua, W.K., Terlabie, N.N. and Sakyi-Dawson, E. (2006) Screening of 42 Bacillus Isolates for Ability to Ferment Soybeans into Dawadawa. *International Journal of Food Microbiology*, **106**, 343-347. https://doi.org/10.1016/j.ijfoodmicro.2005.08.016

[19] Wang, J., Hu, Y., Qiu, C., Fan, H., Yue, Y., Jiao, A., *et al*. (2018) Immobilized Cells of *Bacillus circulans* ATCC 21783 on Palm Curtain for Fermentation in 5 L Fermentation Tanks. *Molecules*, **23**, 2888. https://doi.org/10.3390/molecules23112888

[20] Yan, Z., Zheng, X.W., Han, B.Z., Han, J.S., Nout, M.J. and Chen, J.Y. (2013) Monitoring the Ecology of Bacillus during Daqu Incubation, a Fermentation Starter, Using Culture-Dependent and Culture-Independent Methods. *Journal of Microbiology and Biotechnology*, **23**, 614-622. https://doi.org/10.4014/jmb.1211.11065

[21] Anisha, A.H., Anandham, R., Kwon, S.W., Gandhi, P.I. and Gopal, N.O. (2015) Evaluation of Bacillus spp. as Dough Starters for Adhirasam—A Traditional Rice Based Fermented Food of Southern India. *Brazilian Journal of Microbiology*, **46**, 1183-1191. https://doi.org/10.1590/S1517-838246420140409

[22] Ruiz-Barba, J.L., Caballero-Guerrero, B., Maldonado-Barragan, A. and Jimenez-Diaz, R. (2010) Coculture with Specific Bacteria Enhances Survival of *Lactobacillus plantarum* NC8, an Autoinducer-Regulated Bacteriocin Producer, in Olive Fermentations. *Food Microbiology*, **27**, 413-417. https://doi.org/10.1016/j.fm.2009.10.002

[23] Benoit, I., van den Esker, M.H., Patyshakuliyeva, A., Mattern, D.J., Blei, F., Zhou, M., *et al*. (2015) Bacillus Subtilis Attachment to *Aspergillus niger* Hyphae Results in Mutually Altered Metabolism. *Environmental Microbiology*, **17**, 2099-2113. https://doi.org/10.1111/1462-2920.12564

[24] Dai, J.Y., Yang, Y., Dong, Y.S. and Xiu, Z.L. (2020) Solid-State Co-Cultivation of *Bacillus subtilis*, *Bacillus mucilaginosus*, and *Paecilomyces lilacinus* Using Tobacco Waste Residue. *Applied Biochemistry and Biotechnology*, **190**, 1092-1105. https://doi.org/10.1007/s12010-019-03146-3

[25] Kiran, G.S., Priyadharsini, S., Sajayan, A., Priyadharsini, G.B., Poulose, N. and Selvin, J. (2017) Production of Lipopeptide Biosurfactant by a Marine Nesterenkonia sp. and Its Application in Food Industry. *Frontiers in Microbiology*, **8**, 1138. https://doi.org/10.3389/fmicb.2017.01138

[26] Singh, V., Sharma, R. and Sharma, P. (2015) Isolation, Screening and Optimization of Amylase Producing Bacillus sp. from Soil. *Asian Pacific Journal of Health Sciences*, **2**, 86-93. https://doi.org/10.21276/apjhs.2015.2.3.19

[27] Yu, P. and Xu, C. (2018) Production Optimization, Purification and Characterization of a Heat-Tolerant Acidic Pectinase from Bacillus sp. ZJ1407. *International Journal of Biological Macromolecules*, **108**, 972-980. https://doi.org/10.1016/j.ijbiomac.2017.11.012

[28] Mahto, R.B., Yadav, M., Sasmal, S. and Bhunia, B. (2019) Optimization of Process Parameters for Production of Pectinase Using *Bacillus subtilis* MF447840.1. *Recent Patents on Biotechnology*, **13**, 69-73. https://doi.org/10.2174/1872208312666180917094428

[29] Mohandas, A., Raveendran, S., Parameswaran, B., Abraham, A., Athira, R.S.R., Mathew, A.K., *et al*. (2018) Production of Pectinase from *Bacillus sonorensis* MPTD1. *Food Technology and Biotechnology*, **56**, 110-116. https://doi.org/10.17113/ftb.56.01.18.5477

[30] Kundu, A. and Majumdar, B. (2018) Optimization of the Cellulase Free Xylanase

Production by Immobilized Bacillus Pumilus. *Iranian Journal of Biotechnology*, **16**, e1658. https://doi.org/10.21859/ijb.1658

[31] Sun, L., Cao, J., Liu, Y., Wang, J., Guo, P. and Wang, Z. (2017) Gene Cloning and Expression of Cellulase of *Bacillus amyloliquefaciens* Isolated from the Cecum of Goose. *Animal Biotechnology*, **28**, 74-82. https://doi.org/10.1080/10495398.2016.1205594

[32] Hur, S.J., Lee, S.Y., Kim, Y.C., Choi, I. and Kim, G.B. (2014) Effect of Fermentation on the Antioxidant Activity in Plant-Based Foods. *Food Chemistry*, **160**, 346-356. https://doi.org/10.1016/j.foodchem.2014.03.112

[33] Yao, Z., Kim, J.A. and Kim, J.H. (2019) Characterization of a Fibrinolytic Enzyme Secreted by *Bacillus velezensis* BS2 Isolated from Sea Squirt Jeotgal. *Journal of Microbiology and Biotechnology*, **29**, 347-356. https://doi.org/10.4014/jmb.1810.10053

[34] Heo, K., Cho, K.M., Lee, C.K., Kim, G.M., Shin, J.H., Kim, J.S., *et al.* (2013) Characterization of a Fibrinolytic Enzyme Secreted by *Bacillus amyloliquefaciens* CB1 and Its Gene Cloning. *Journal of Microbiology and Biotechnology*, **23**, 974-983. https://doi.org/10.4014/jmb.1302.02065

[35] Radhakrishnan, R., Hashem, A. and Abd Allah, E.F. (2017) Bacillus: A Biological Tool for Crop Improvement through Bio-Molecular Changes in Adverse Environments. *Frontiers in Physiology*, **8**, 667. https://doi.org/10.3389/fphys.2017.00667