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# Enzyme profiles of potential starter cultures for the fermentation of baobab seeds

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**The extracellular enzymatic activity of ten (10) strains of predominant bacteria involved in Maari process has been investigated using the APIZYM (BioMérieux, France) commercial system, with the objective of determining the differences in the enzymatic profiles of the various species. Variable enzymatic activity was recorded showing the specific activity of each species during the fermentation of baobab seeds. Almost all isolates possessed phosphatase activity. All aerobic mesophilic bacteria (AMB) lacked trypsin. No lactic acid bacterium (LAB) was able to produce α-galactosidase. Naphthol-AS-BI-phosphohydrolase was produced by all isolates. The enzymatic pattern of these potential starter cultures can be used for predicting their suitability for baobab seeds fermentation and for monitoring their stability.**

**Key words:** Enzymatic activity, baobab seeds, fermentation, starter cultures.

## INTRODUCTION

Spontaneous fermentation of baobab seeds is a processing technique applied in Burkina Faso and in others countries in West Africa including Benin, Mali and Nigeria for the production of Maari, an indigenous condiment (Parkouda et al., 2010). The spontaneous nature of the process results in varying product quality which invariably depends heavily on the producer skill and processing conditions. Several microorganisms are known to be involved in the fermentation of baobab seeds. The dominance of *Bacillus* species, especially *Bacillus subtilis* is reported by Parkouda et al. (2010). Other microorganisms including *Enterococcus* sp. and *Pediococcus* sp. have also been reported to be associated with the fermentation of baobab seeds. In our previous work, we reported that during the processing of the baobab seeds, carbohydrates and lipids content decrease but not protein content (Parkouda, 2010). These changes are likely to be explained by the cooking and loss in the cooking water; and the metabolic activities of the microorganisms involved in the fermentation (Kpikpi et al., 2009; Yagoub et al., 2004). Indeed, the raw materials used to produce the alkaline condiments are substrates in which *Bacillus* spp. grow and produce metabolites and enzymes recognized to have beneficial effects on health (Achi, 2005; Dahal et al., 2005; Wang and Fung, 1996). Mbajunwa et al. (1998) and Njoku et al.

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(1990) demonstrated the ability of *B. subtilis* to soften the tissue of African oil beans leading to the desired texture of *ugba* and suggested that the strain may possess pectic and proteolytic enzymes that readily hydrolyzed the pectin and protein components of the beans. The APIZYM system (bioMérieux, France) is a semi-quantitative micro-method for analysing enzymatic activities of microorganisms, which has been used to characterize many microorganisms (Gruner et al., 1992). For the purposes of quality control and standardization of Maari, there is the need to exploit starter cultures in Maari production. One of the technological properties that could be used to select microorganisms to be used as starter cultures, is the enzymatic activity. The objective of this study was then to assess the enzymatic profiles of the potential starter cultures for Maari production. The information would contribute to the development of starter cultures with predictable characteristics, which could be used in small-scale and commercial production of Maari for improved and consistent quality.

## MATERIALS AND METHODS

Ten microorganisms isolated from fermented baobab seeds and previously characterized (Parkouda et al., 2010), were assessed of their extracellular enzymatic activities using APIZYM system kits (APIZYM BioMérieux, France) following the manufacturer's instructions. The microorganisms had been previously identified as *Bacillus coagulans* BL174, *Bacillus licheniformis* B6, *Bacillus subtilis* B3, *B. subtilis* B122, *B. subtilis* B222, *Staphylococcus sciuri* AB41, *Enterococcus avium* LB70, *Pediococcus acidilactici* L74, *Enterococcus casseliflavus* L142 and *Enterococcus faecium* L9. This APIZYM system consisted of a strip composed of 20 micro-cupules, which contain 19 substrates and one control, to detect 19 different enzymatic activities: Alkaline phosphatase, Esterase C4, Esterase lipase C8, Lipase C14, Leucine arylamidase, Valine arylamidase, Cystine arylamidase, Trypsin, α-Chemotrypsin, Acid phosphatase, Naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase.

The aerobic mesophilic bacteria consisting of *B. coagulans* BL174, *B. licheniformis* B6, *B. subtilis* B3, *B. subtilis* B122, *B. subtilis* B222, and *S. sciuri* AB41, were grown in Brain Heart Infusion broth (Fluka, Steinheim, Germany) while the lactic acid bacteria, made up of *E. avium* LB70, *P. acidilactici* L74, *E. casseliflavus* L142 and *E. faecium* L9 were propagated in MRS broth (Merck, Darmstadt, Germany). Cells were harvested by centrifugation at 5000 ×g for 10 min and washed 3 times in 5 ml of sterile saline solution containing 8·5 g $l^{-1}$ NaCl and 1·5 g $l^{-1}$ Bactopeptone (DIFCO), pH 7. Following the last washing step, an initial suspension of each culture was made and adjusted to McFarland turbidity standard (5 to 6) using API suspension medium (API, Biomerieux, France). Aliquots of 65 µL of each culture suspension were added to one of the 20 reaction cupules in the APIZYM strip. The strips were incubated at 37°C overnight after which the reactions were terminated by addition of one drop each of the APIZYM reagents A and B according to the manufacturer's instructions. The experiments were performed in duplicate. Enzymatic activity was recorded as positive if a score of 1 or greater was obtained after assessment of the colour intensity using the manufacturer's colour chart.

## RESULTS AND DISCUSSION

The APIZYM profile of the representative strains previously isolated from Maari is shown in Table 1. Generally, the enzymatic activities varied significantly among the strains and species. *B. coagulans* seemed to produce 18 of the tested enzymes, as against 16, 7 and 6 for *B. subtilis* B222, B122 and B3, respectively; and 8 and 10 for *B. licheniformis* and *S. sciuri*. *E. faecium* L9 produced 18 from the 19 tested enzymes. *E. casseliflavus* L142, *E. avium* LB70 and *P. acidilactici* L74, produced comparatively few. Alkaline phosphatase, which cleaves orthophosphoric monoesters to orthophosphates and alcohols in alkaline conditions, was present in all tested isolates except for *P. acidilactici*. The isolates (exception for *B. subtilis* B3) produced both alkaline and acid phosphatases, two enzymes that have similar functions but have pH optima of 8.0 to 9.0 and 5.0, respectively, thus indicating an extensive phosphatase activity by these isolates (Waltman II et al., 1982). All the *Bacillus, Enterococcus* and *Staphylococcus* strains tested produced esterase (C4) and esterase lipase (C8), while *P. acidilactici* displayed negative results for these activities. Lipase (C14) activity was only observed for *E. faecium* L9, *B. coagulans* BL174, *B. subtilis* B222 and *S. sciuri* AB41. In a previous study, Antai and Ibrahim (1986) attributed oil degradation in African locust beans during fermentation to enzymes produced by *Staphylococcus* or *Leuconostoc* species.

Variability between species and within *B. subtilis* species in lipase and esterase activity was also previously reported (Ouoba et al., 2003b). Lipase activity has been identified as a property that may not be desirable during the production since it may result in rapid rancidity of the product (Wagenknecht et al., 1961). However, adequate lipolytic activity could probably be a good characteristic, because liberation of free fatty acids was required for the development of desired aroma characteristics (Beaumont, 2002; Odunfa and Adesomoju, 1986).

All *Bacillus* isolates produced α-glucosidase, which hydrolyzes α(1-6) linkages at branch points of dextrin. Production of β-glucosidase, which hydrolyzes the β(1-4) linkages of glucosides such as cellulose or plant starch, was species and strains dependent. These results are in line with previous studies which reported that *Bacillus* spp. are producers of amylase, galactanase, galacto-sidase, glucosidase and fructofuranosidase, enzymes involved in degradation of carbohydrates during alkaline fermentation (Aderibigbe et al., 1990; Kiers et al., 2000; Omafuvbe et al., 2000; Sarkar et al., 1997). None of the *Bacillus* strains produced trypsin; trypsin activity was observed for *E. casseliflavus* L142 and *E. faecium* strains. No tested lactic acid bacteria was able to produce *α*-galactosidase.

**Table 1.** Enzyme activity of *Bacillus, Enterococcus, Staphylococcus* and *Pediococcus* strains isolated from *Maari* (potentially starter culture) determined with the APIZYM test.

| Parameter | *Bacillus coagulans* BL174 | *Bacillus licheniformis* B6 | *Bacillus subtilis* B3 | *Bacillus subtilis* B122 | *Bacillus subtilis* B222 | *Staphylococcus sciuri* AB41 | *Enterococcus avium* LB70 | *Pediococcus acidilactici* L74 | *Enterococcus casseliflavus* L142 | *Enterococcus faecium* L9 |
|---|---|---|---|---|---|---|---|---|---|---|
| Esterase (C4) | + | + | + | + | + | + | + | - | + | + |
| Esterase Lipase (C8) | + | + | + | + | + | + | + | - | + | + |
| Lipase (C14) | + | - | - | - | + | + | - | - | - | + |
| Leucinearylamidase | + | + | + | - | + | + | - | + | + | + |
| Valinearylamidase | + | - | - | - | + | - | - | + | - | + |
| Cystinearylamidase | + | - | - | + | + | - | - | + | + | + |
| Trypsin | - | - | - | - | - | - | - | - | + | + |
| α-Chymotrypsin | + | - | - | - | + | - | - | - | + | + |
| Alkaline phosphatase | + | + | + | + | + | + | + | - | + | + |
| Acid phosphatase | + | + | - | + | + | + | + | + | + | + |
| Naphthol-AS-BI-phosphohydrolase | + | + | + | + | + | + | + | + | + | + |
| α-Galactosidase | + | - | - | - | + | - | - | - | - | - |
| β-Galactosidase | + | - | - | - | + | - | - | - | + | + |
| β-Glucuronidase | + | - | - | - | + | + | - | - | - | + |
| α-Glucosidase | + | + | + | + | + | + | - | - | - | + |
| β-Glucosidase | + | + | - | - | + | + | - | + | + | + |
| N-Acetyl- β-glucosaminidase | + | - | - | - | - | - | - | - | + | + |
| α-Mannosidase | + | - | - | - | - | - | - | - | - | + |
| α-Fucosidase | + | - | - | - | + | - | - | - | - | + |

NB: '+' refers to positive reaction and indicates presence of enzyme in concentrations of >5 nmol, '- 'refers to negative reaction

The need to select the most appropriate starter cultures for the production of condiment is important in order to obtain the most desirable product and achieve the much needed product consistency to aid acceptability and industrialization of the traditional fermented foods in West Africa (Sanni, 1993). Isolate showing an interesting enzymatic activity profile would be important in developing a starter culture. The present observations seem to provide the basis for selecting isolates for the development of a starter culture, which could improve product quality and consistency. *Bacillus* species, especially *B. subtilis,* were found to dominate the alkaline fermentation of seeds for food condiments production and to show interesting technological properties as reviewed by Parkouda et al. (2009). The possible role of *Enterococcus* strains that were repeatedly isolated during Kinema production, a similar condiment produced by fermentation of soybeans, was also investigated (Sarkar et al., 1994).

The usefulness of the enzymatic profile to differentiate bacteria, together with its possible interest for the bacteria ability to degrade some subtracts were previously evaluated (Ouoba et al., 2003a, b, 2007; Azokpota et al., 2006; Kpikpi et al., 2009).

The observations in this study show variability in the enzymatic capabilities among the different isolates, indicating contributions of each of the isolates to the fermentation of Baobab seeds in Maari production. This suggests that, an appropriate starter culture for Maari production would be made up of a consortium of species. Based on their predominance and their ability to

produce different extracellular enzymes, *B. subtilis* B222, *S. sciuri* AB41 and *E. faecium* L9 could be selected as suitable starter cultures but their safety aspects needto be study.

## Conflict of Interests

The author(s) have not declared any conflict of interests.

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