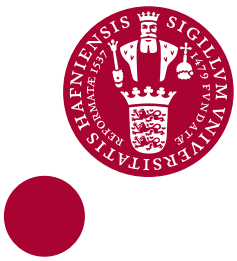

The biodiversity of aerobic endospore-forming bacterial species occurring in Yanyanku and Ikpiru, fermented seeds of Hibiscus sabdariffa

Agbobatinkpo, Pélagie Bidossessi; Thorsen, Line; Nielsen, Dennis Sandris; Azokpota, Paulin; Akissoe, Noèl; Hounhouigan, Joseph; Jakobsen, Mogens

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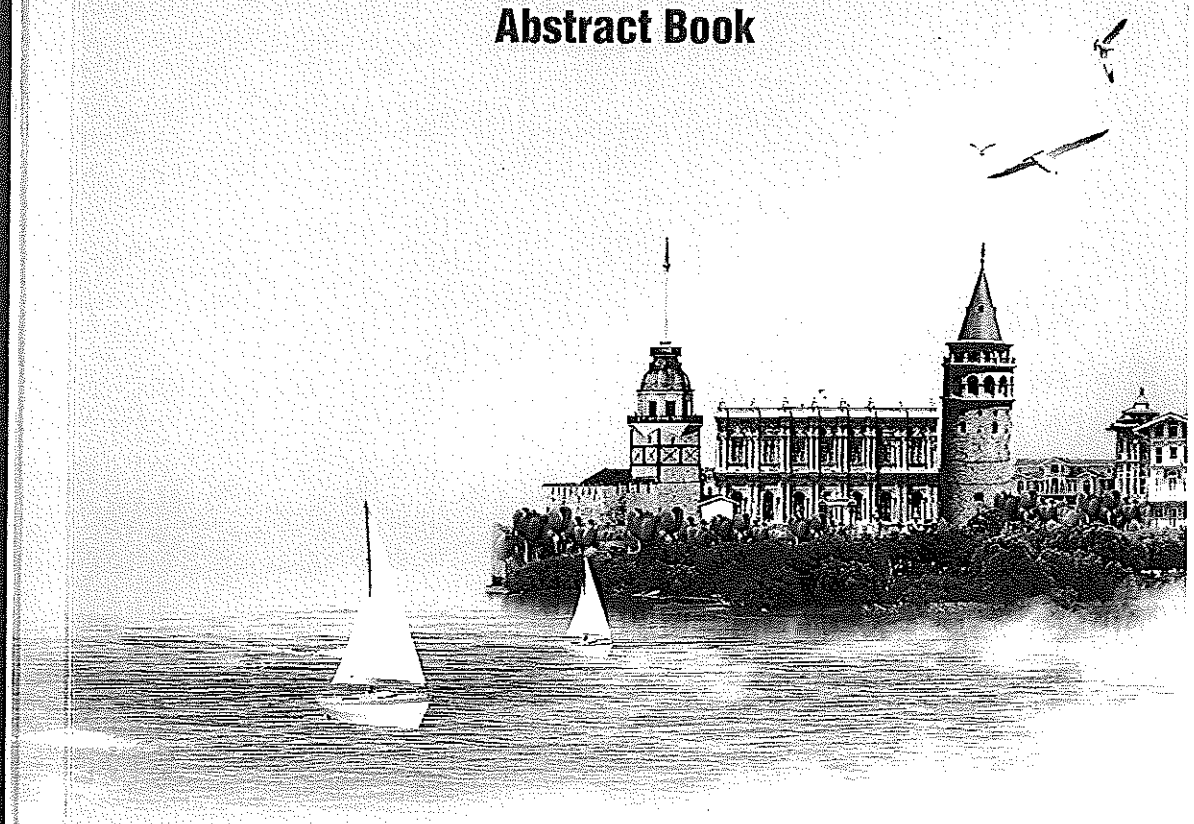

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id bacteria associated with fermented beverage from

RISTIMUÑO[2], Pier Sandro
sa AZNAR[1]
Burjasot, SPAIN

di Microbiologia-Centro Ricerche

: beverage prepared from maize in the
cteria (LAB) species present during
: analyzed by molecular methods.
rmentation process using maize flour
rst step, manually shaped buns were
ıe sugar, water and fermented (18-20
d with fresh maize flour and water,
ıs were separated before the final
ɔial counts were in the range of 10^4-
mong assayed media. A total of 146
ing steps were identified by internal
orphic DNA (RAPD) profiles as
ı (20%), *Lactobacillus* (23%) and
species-specific PCR revealed *E.*
. lactis and *W. viridescens* as the
uction process showed the presence
cing exhibited the highest diversity
roides and *E. mundtii*. From pre-
b. brevis and *Leuc. mesenteroides*
d as the dominant species in the
ely *Leuc. lactis* while the jelly-like
ng concentration, both species but
les revealed intraspecies diversity,
motolerant *Enterococcus faecium*
Leuc. mesenteroides (13%) that
: indigenous foods may provide
vhich can be exploited as new
ɔnal quality of traditional Andean

P-471

The biodiversity of aerobic endospore-forming bacterial species occurring in Yanyanku and Ikpiru, fermented seeds of *Hibiscus sabdariffa*

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Yanyanku and Ikpiru are two fermented products from Malcavene bean (Hibiscus sabdariffa) used as functional additives for African locust bean seeds (*Parkia biglobosa*) fermentation in Benin. In the present study, a total of 343 Aerobic Endospore-Forming Bacteria (AEFB) isolated from Yanyanku and Ikpiru produced in northern and southern Benin, at different production sites were identified using phenotypic and molecular biology based methods, including 16S rRNA, gyrA and gyrB genes sequencing. Detection of genes encoding cytotoxin K (cytK1, cytK2), haemolysin BL (hblA, hblC, hblD) and a genetic determinant for the emetic toxin cereulide EM1 were also performed. Regardless of geographical location and production site, it was the same five-six predominant species that were involved in the fermentation of *H. sabdariffa* for the production of Yanyanku and Ikpiru. The predominant species were of the genus *Bacillus* and included *B. subtilis* (19-41% of isolates), *B. cereus* (6-41%), B. amyloliquefaciens (9-20%), B. safensis (6-23%), B. licheniformis (4-25%), and *B. altitudinis* (0-19%). Other species occurred sporadically and included *B. flexus, B. circulans* (<1% each), as well as species of the genera (5.5% of isolates) *Lysinibacillus*, *Brevibacillus*, *Aneurinibacillus* and *Paenibacillus*. Sequencing of the gyrA gene showed to be an efficient marker for differentiation of *B. pumilus* and *B. safensis*. All the *B. cereus* isolates lacked the gene encoding the cytotoxin K-1 but 91% of them harboured the gene encoding the cytotoxin K-2 and 6% the emetic specific gene fragment EM1. The genes encoding haemolysin BL hblA, hblC, hblD were present in 15%, 34% and 35% of *B. cereus* isolates, respectively. None of *B. cereus* from functional additives Yanyanku and Ikpiru harboured all the toxin genes investigated. This study is the first to identify the AEFB of the functional additives Yanyanku and Ikpiru to species level and perform a safety evaluation based on toxin gene detections. The results can be used to initiate selection of suitable startercultures for the controlled fermentation of Yanyanku and Ikpiru in order to provide a stable and safe product.