



ASSOCIATION OF APPLIED BIOLOGISTS

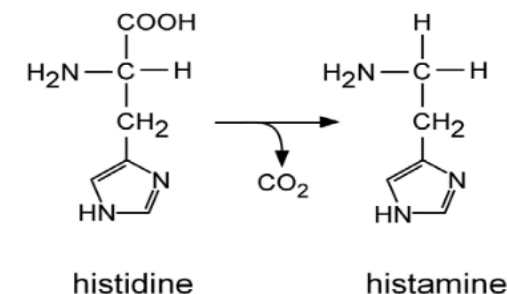


Legume Science and Practice 2

A three day virtual conference on 1st – 3rd September 2021



Biogenic amines in African locust bean (*Parkia boglobosa*) fermented seeds



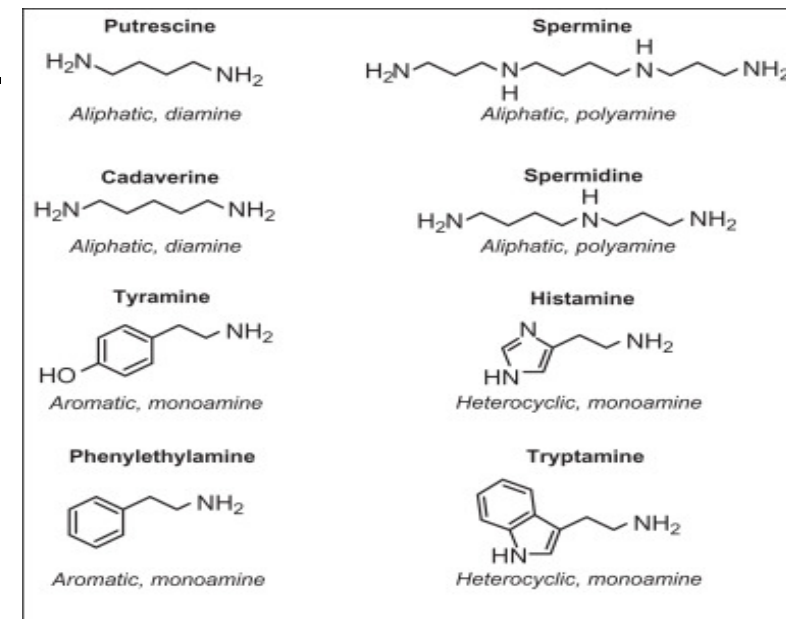
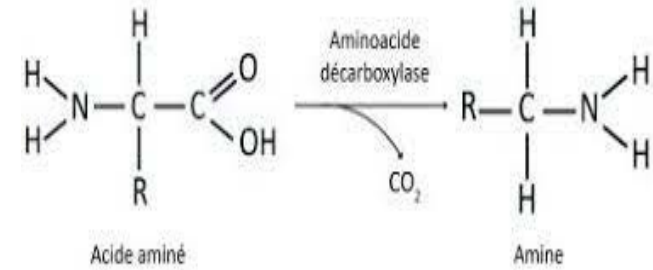
NADEGE T KONE, **MICHEL Y ESSE**, **TAGRO GUEHI** (Université Nangui Abrogoua, Côte d'Ivoire), **CAROLINE STRUB** (Polytech Montpellier, France), **NAWEL ACHIR** (Institut Agro, France), **JOËL GRABULOS**, **CHRISTIAN MESTRES** & **ELODIE ARNAUD** (CIRAD, France)



Introduction

Biogenic amines (BA)

- ✓ Low molecular weight nitrogenous bases.
- ✓ Can accumulate at high concentration in food due to microbial decarboxylation of their precursor amino acids.
- ✓ Most common BA found in foods : **histamine, tyramine, cadaverine, 2-phenylethylamine, spermine, spermidine, putrescine and tryptamine.**
- ✓ High BA content in protein rich fermented foodstuffs (meat, fish, soya, cocoa beans).
- ✓ Consumption of food with high BA content is of public health significance because of their toxic effects (allergic reactions, disorders in neurone, respiratory and cardiovascular systems).

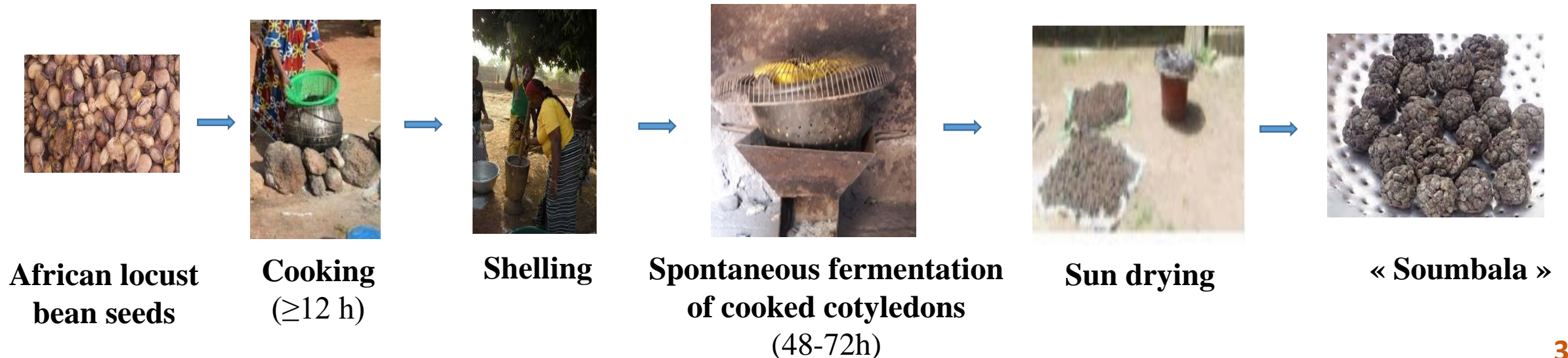


Introduction

What are African locust bean seeds and “Soumbala”?

- ❑ **African locust bean** (*Parkia* species) is a perennial tree legume which plays an important part in traditional agriculture. *Parkia* trees are found in Africa, South-East Asia and tropical South-America (Campbell-Platt, 1980).
- ❑ **Soumbala** is the name for processed African locust bean seeds used as most important indigenous food condiment very prized by the West African populations (Reddy, 2018).

Processing of African locust bean in “Soumbala”



Problematic

BA cause harmful toxicological reactions and intoxications to consumers and may give undesirable flavour compounds to *Soumbala*.

Main Objective

Evaluation of the presence of biogenic amines and the ability of *Bacillus* strains isolated from *Soumbala* to show amino acids decarboxylation activities as well

Material & methods

Plant material : African locust bean seeds



Raw seeds

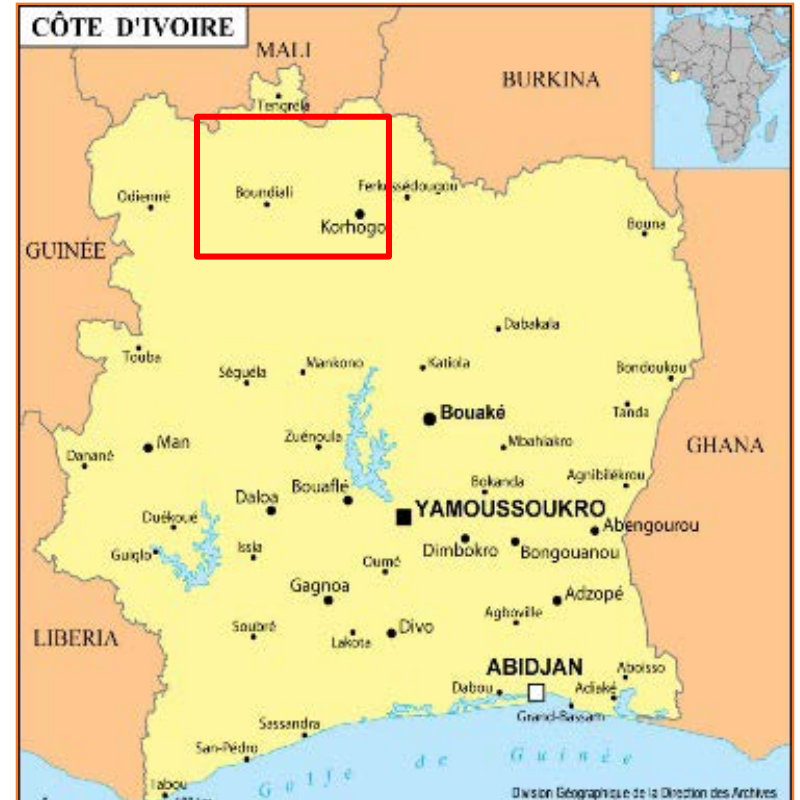


Soumbala and samples during fermentation

Sampling

- ✓ Samples were collected among 6 producers in 3 north regions of Côte d'Ivoire (2 producers / region)

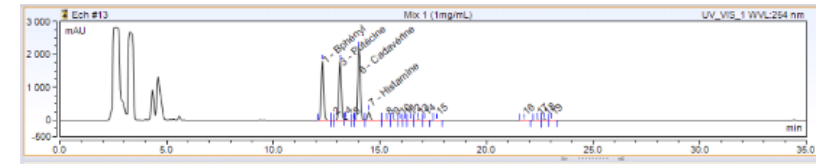
Total: 36 samples collected



Material & methods

1. Identification and quantification of biogenic amines contents

Acidic extraction, derivatization and analysis by High Performance Liquid Chromatography (Yoon *et al.*, 2015).



2. Isolation and biochemical identification of *Bacillus* strains

- ❖ Presumptive *Bacillus* isolates obtained by subculture of fermented *Parkia biglobosa* seeds incubated (PCA, 37°C, 24h) and stored at - 80° C.
- ❖ Cultural and microscopic identifications.
- ❖ Biochemical identification with catalase and oxidase tests and API 50 CH / B strips (Freitas *et al.*, 2008).



3. Decarboxylase activities of isolated *Bacillus* strains

Taylor's medium used for screening decarboxylases (ODC, LDC, HDC, PHDC, ADH) (Lopez-Sabater *et al.*, 1994)



Results and discussion

1. Biogenic amines content

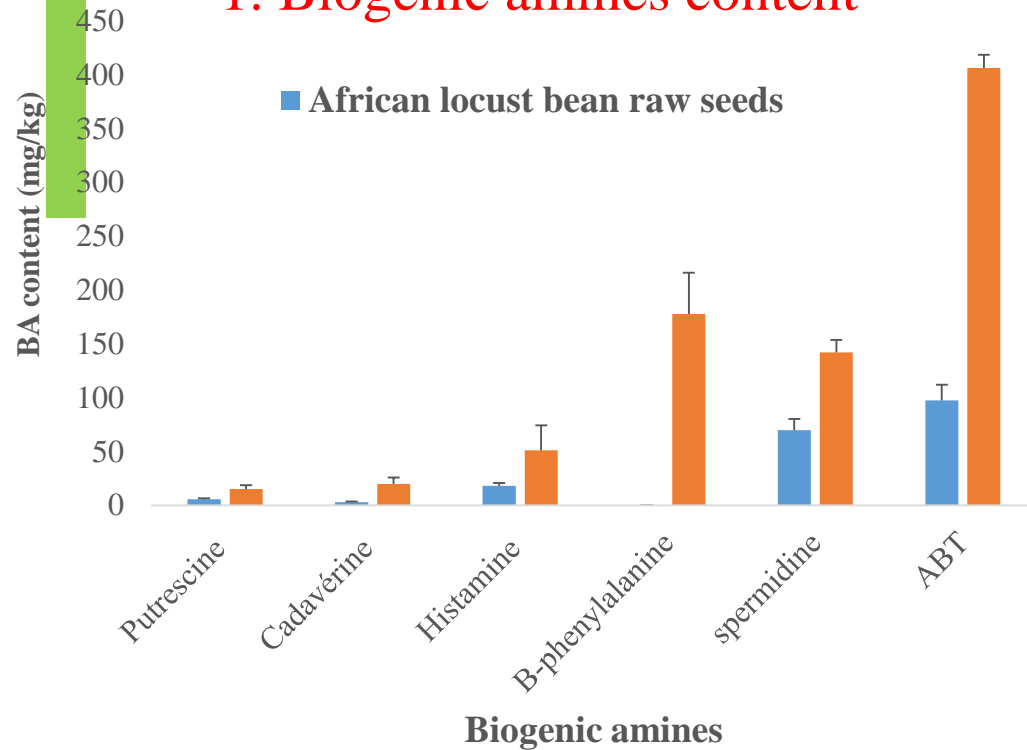
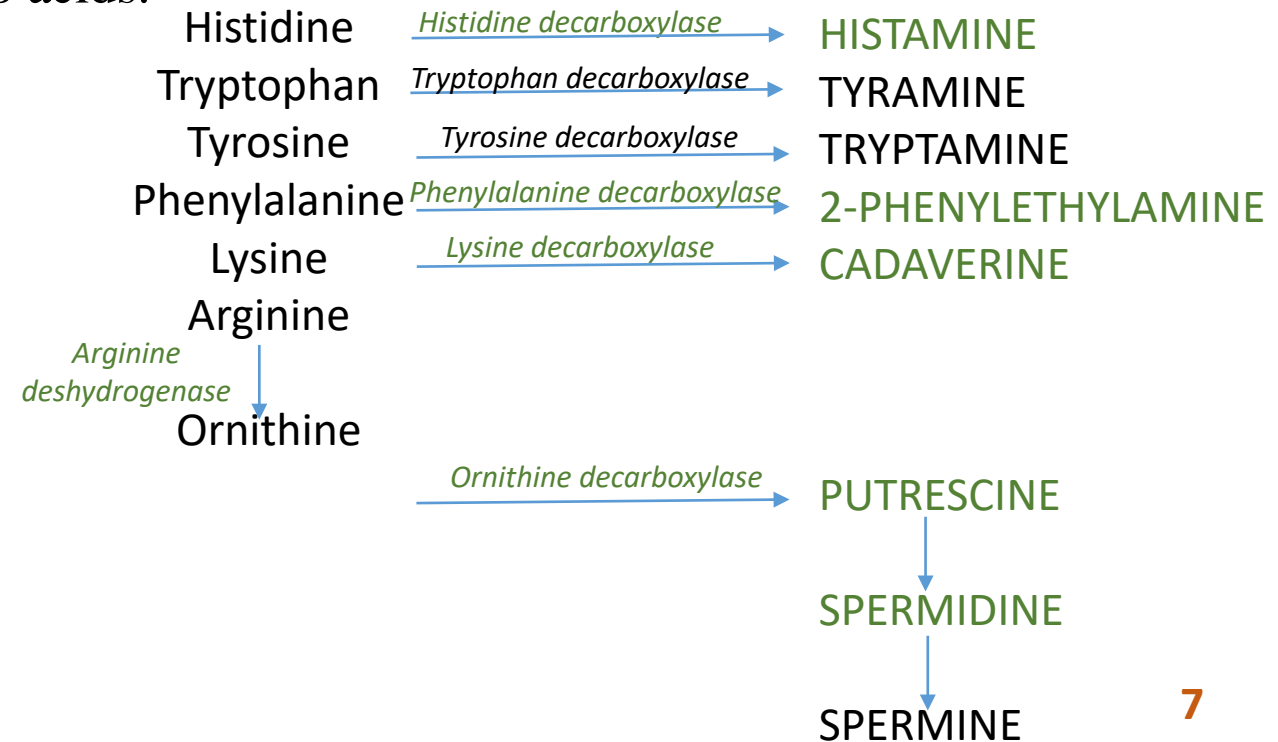


Figure 1. Content and identification of biogenic amines found in raw and fermented African locust bean seeds

Reglementation for histamine content in fish :

- n = 9
- mean = 100 mg/kg
- Max = 200 mg/kg
- 2 samples max between 100 & 200 mg/kg

- ◆ Spermine, tyramine and tryptamine were not found.
- ◆ BA are naturally present in biological organisms (responsible for vital functions) ([Swider et al., 2019](#)).
- ◆ The increase of BA content in Soumbala compared to the raw *Parkia biglobosa* seeds could be due to the microbial decarboxylation of amino acids.



Results and discussion

2- Cultural and morphological characteristics of Bacillus isolates

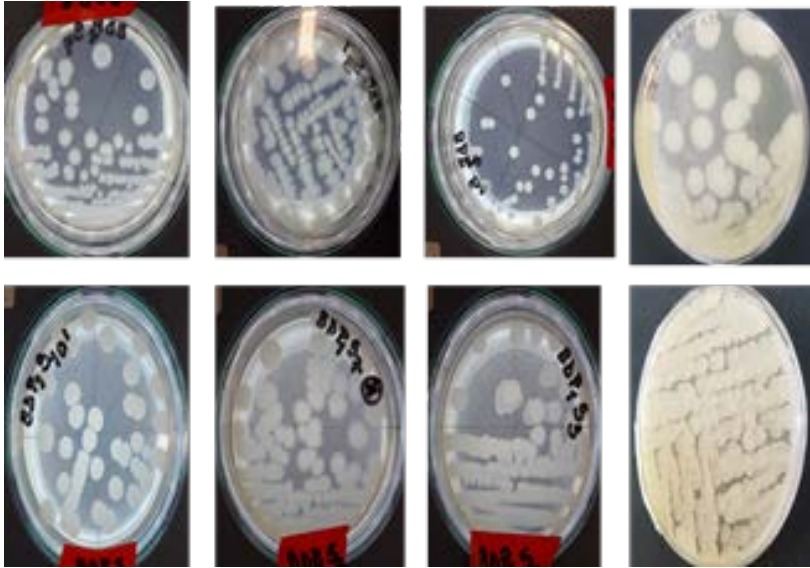


Figure 2. Cultural observation of the bacterial colonies

A total of 60 *Bacillus* sp. were isolated

- Colony circular or slightly serrated, beige,
- Opaque or translucent, dry or sticky,
- flat or flat with rounded center, $3 < \phi \leq 9$ mm

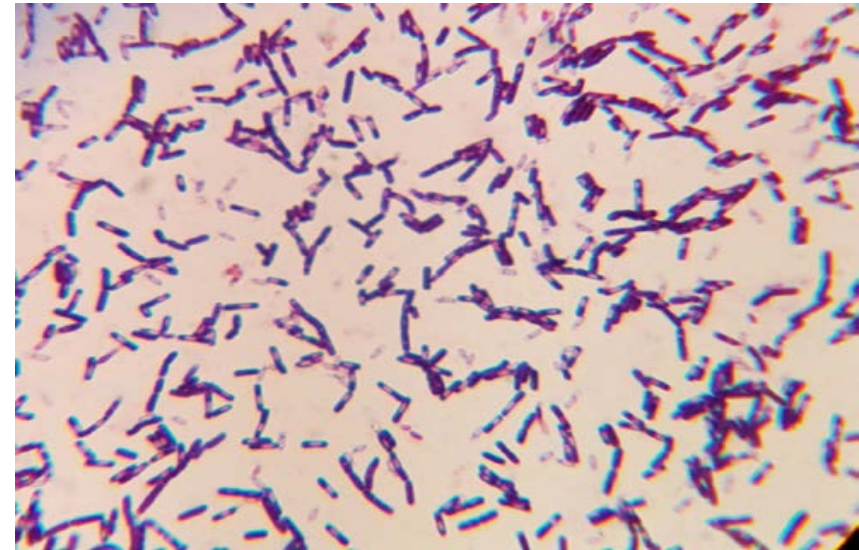


Figure 3. Microscopic characteristics from GRAM staining

- ✓ 52 isolates *Bacillus* Gram (+) mobile, sporulated, Catalase (+) and Oxidase (+)

Results and discussion

3- Biochemical identification of *Bacillus* sp. isolates

52 presumptive *Bacillus* isolates belonging to 11 species : *B. subtilis* (16 strains, 31%), *B. lentus*, *B. pumilus*, *B. cereus*, *B. firmus*, *B. megaterium*, *B. circulans*, *Geobacillus stearothermophilus*, *Brevibacillus non reactive*, *B. licheniformis*, *Aneurinibacillus aneuriniliticus*.

- ✓ Large diversity of *Bacillus* species found during spontaneous fermentation of African locust bean seeds.
- ✓ *Bacillus subtilis* dominant as previously reported ([Parkouda et al., 2009](#)).

4- Potential biogenic amines producing *Bacillus* isolates

- ◆ 69% of strains (36) produced decarboxylases and 31% (16 strains) did not.

Conclusions & perspectives

This study was performed to analyze the occurrence of biogenic amines in African locust bean seeds and in Soumbala after fermentation and the ability of strains to produce decarboxylases.

- ♦ Five BAs (putrescine, cadaverine, histamine, 2-phenylethylamine, spermidine) were found in *Parkia biglobosa* seeds with higher content in fermented seeds compared to raw seeds.
- ♦ 52 presumptive *Bacillus* isolates were isolated and identified belonging to 11 *Bacillus* species. 16 *Bacillus* strains showed decarboxylase producing abilities and very probably cause the increasing of BAs content in Soumbala.
- ❖ Use of non decarboxylase producing *Bacillus* strain as a starter to produce Soumbala with low biogenic amines content
- ❖ Study of conditions (pH, T°, salt) unfavourable for the production of decarboxylases or their activity

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Legumes
Translated

aab
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Cropping and the Environment Group

esa
ÉCOLE SUPÉRIEURE
D'AGRICULTURES
Angers Loire

THANK YOU FOR YOUR KIND ATTENTION

