Original Research Article
Isolation of *Aspergillus sp* from some decomposing fruits and vegetables from banana rhyzosphere soil for citric acid production

ABSTRACT

Aims: The general objective of this study is to isolate certain strains capable of producing citric acid.

Place and Duration of Study: Sample collection was carried out on two types of organic matter. Sampling was done from February to April 2021 in 3 municipalities in Abidjan with 10 samples per site.

Methodology: To carry out this work, isolation and purification of Aspergillus sp strains were carried out. Macroscopic and microscopic identifications of the mold isolates were carried out. The search for aflatoxin-producing molds was carried out. The analysis ended with a screening of molds capable of synthesizing citric acid.

Results:*Aspergillus niger*, *Aspergillus sp1* and *Aspergillus sp2* showed no fluorescence, while *Aspergillus flavus*, *Aspergillus candidus* and *Aspergillus fumigatus* showed fluorescent spots, indicating the presence of aflatoxin.

Conclusion: Aspergillus isolates capable of producing citric acid were isolated during this study. *Aspergillus niger*, *Aspergillus sp1* and *Aspergillus sp2* do not produce aflatoxin and have the capacity to synthesize citric acid.

Keywords: Aspergillus, aflatoxin, citric acid, organic matter

1. INTRODUCTION

Microbes are microscopic organisms widely distributed in nature, mainly encompassing varieties of species such as bacteria and fungi [1]. Within these microorganisms, a remarkable diversity is exploited in the industrial field to produce a varied range of

compounds, including citric acid (CA) beingone of the most important [2]. However, most microorganisms used for this purpose are not capable of generating commercially viable profits, due to the limited accumulation of CA, a product of energy metabolism, requiring conditions of major disequilibrium to occur in substantial quantities [3]. With the advancement of biotechnology, considerable efforts have been made to improve CA production by exploiting new genetically modified strains or optimizing existing strains through mutagenesis. However, despite this progress, gaps persist, both in terms of substrates and mutated strains [4,18,19,20], highlighting the need for extensive research to overcome these obstacles. The genus Aspergillus, a widely distributed family of fungi comprising several species with significant industrial applications, offers interesting potential for the production of CA by fermentation [5]. Among these species, *Aspergillus niger* stands out for its ability to synthesize CA, an organic acid of major economic importance used in various industries, including beverages, food, detergents, cosmetics and pharmaceuticals [6]. . However, despite the economic benefits associated with CA production by *Aspergillus niger* (A.niger), several challenges remain. In particular, the ability of some Aspergillus strains to produce aflatoxin (AF), a toxic and carcinogenic mycotoxin, poses food safety risks and limits AC production [7]. This constraint raises the crucial need to select and detect aflatoxin-free Aspergillus strains, thereby ensuring the quality and safety of the final products. In this context, this study is to characterize the diversity of Aspergillus species present in decomposing organic matter, such as vegetables and fruits, as well as in banana plantation soil, while emphasizing the detection of Aspergillus strains that do not produce aflatoxins. By integrating multidisciplinary approaches from microbiology, genomics and analytical chemistry, this research aims to identify non-toxic Aspergillus strains present in these environments andto identify beneficial strains for AC production, while minimizing the risks associated with aflatoxin.

2. MATERIAL AND METHODS

2.1Sampling

Sample collection was carried out on two types of organic matter. These are fruits (orange, lemon) and vegetables (onion and garlic), all rotting and rhizospheric banana soils. As for the soil, samples were taken from the rhizosphere of banana trees. As for fruits and vegetables, they were collected randomly in three communes of Abidjan (Yopougon, Koumassi and Bassam) near the market trash bins and then transported to the laboratory in an appropriate container for analysis. Forty samples were collected, i.e. 10 samples per site and per municipality.

2.2 Microbiological analysis technique

2.2.1 Isolation of fungal species

Isolation of fungal species from soil was carried out according to the standard method [8]. A layer of soil of approximately 5 cm was removed from the banana plantation soil. Approximately 10 g was diluted in 90 mL of buffered peptone water to obtain the inoculum. Successive dilutions were made from the inoculum. To do this, 1 mL of the inoculum is diluted in 9 mL of sterile distilled water. This operation is repeated until the desired dilution is obtained. 1 mL of each retained dilution is introduced into Petri dishes then 20 mL of potato agar medium (PDA) is added then homogenized.As for fruits and vegetables, the collection of species was done at the laboratory level using an air bio-collector (PACK TRIO LOW MONO HEPA-100 L/min). Each Petri dish is placed successively in the bio-air collector. 100 L/min of air from the jars containing the fruits and vegetables are captured and distributed uniformly in a Petri dish. Approximately 6 Petri dishes of 90 mm diameter each containing 20 ml of PDA agar medium were used. All plates were incubated at 30°C for 3 to 5 days.

2.2.2 Purification and conservation of strains

The strains identified as presumed filamentous fungi were re-isolated and purified again on Sabouraud medium with Chloramphenicol. Purification consisted of streaking each strain identified on the Petri dishes to ensure that there was no contamination. After 24 hours of incubation at 37°C, the strains obtained are pure. They are stored in test tubes containing CEZAPEK agar for further work. Thus 110 isolates of filamentous fungi belonging to the genus Aspergillus sp were preserved for further work.

2.3 Macroscopic and microscopic identification of fungi

The isolates obtained were subcultured again on Sabouraud Chloramphenicol agar. Macroscopic identification was done by eye and was based essentially on cultural characteristics. After culturing the isolates for 24 hours at 37°C on Sabouraud Chloramphenicol medium, several aspects of the vegetative system are observed. Moreoverthe microscopic identification of *Aspergillus sp* colonies was carried out on the basis of their morphological characteristics and lactophenolic blue coloring of cotton, following standard methods of[9].

2.4 Testing for aflatoxins

The capacity of the isolated mushrooms to produce aflatoxin was detected in the laboratory according to the method of [10]. Each strain was inoculated into the center of the solidified agar medium in glass Petri dishes and incubated at 30°C for 3 days. To observe the color

change of the colonies after incubation, the dishes were placed upside down and 0.2 ml of 25% ammonia solution was placed in the lid of the Petri dish. The boxes were incubated for 3 days at 30°C and then the boxes were taken out of the incubator and turned over. Immediately after introducing the ammonia solution into the petri dish, exposure to ammonia vapor made it possible to detect aflatoxin-producing strains. If the base color of the colony changes to a red pink or yellow orange color with different degree, it shows that the fungus has the ability to produce aflatoxins.

2.5 Qualitative screening of citric acid production

A qualitative screening of AC production by isolated Aspergillus cultures was carried out according to the standard method of[11]**.**Czapek-Dox agar medium supplemented with bromocresol purple (10 ml) was poured into individual sterile Petri plates in triplicate and allowed to cool to room temperature. Approximately 0.5 ml of the Aspergillus conidia suspension was transferred to each of the Petri dishes. Plates were incubated at 30 ± 1°C for 3–5 days. Fungal colonies producing yellow halo areas on the plates were considered positive for AC production[12]**.**

3. RESULTS

This section discusses the results obtained and discussions of the study. The analysis focused on the search for Aspergillus in the rhyzospheric soil of banana plants and in rotting fruits and vegetables. It also presents Aspergillus producing aflatoxin and citric acid.

3.1Diversity of the fungal flora of the different samples

Around 110 fungal isolates were isolated and purified. This is the genus Aspergillus isolated from fruits, vegetables and banana plantation soil. A total of 70 Aspergillus sp isolates were found in fruits and vegetables while 40 Aspergillus sp isolates were isolated from banana plantation soil. Furthermore, the 110 isolatesweregroupedinto 6 species.

Aspergillus candidus *Aspergillus Sp1*

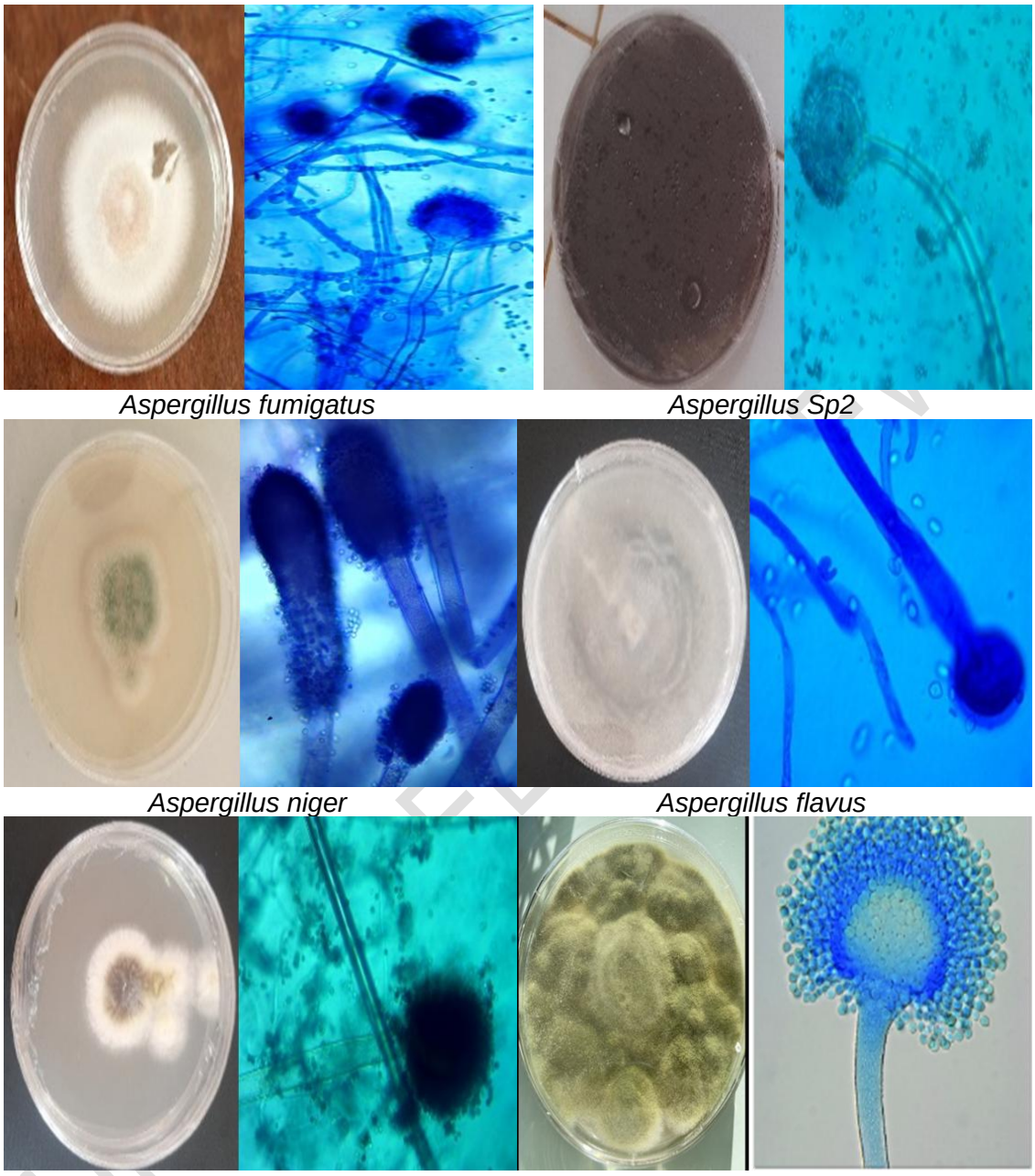

Figure1: Different isolated filamentous fungi

3.2Ability of isolates to produce aflatoxin

Table I mentions the result of the aflatoxin-producing strains. A. candidus, and A. flavus showed a high capacity for aflatoxin production while A. fumigatus showed a moderate capacity for aflatoxin production on CEA medium. As for A. niger, A. sp1 and A. sp2 did not show the ability to produce aflatoxins.

Table I:Result of screening for FA-producing strains using the ammonia vapor method

Mushrooms	CEA
Aspergillus candidus	++++
Aspergillus fumigatus	-
Aspergillus niger	ND
Aspergillus sp1	ND
Aspergillus sp2	ND
Aspergillus flavus	++++

ND=not detected; Color intensity: low (-); strong (+)

3.3Ability of isolates to produce citric acid

Approximately 6 isolated Aspergillus strains were screened for their CA production capacity on Czapek-Dox agar medium. Among the 6 fungal isolates A. niger, A.sp1 and A.sp2 showed the largest yellow halo area and were selected for further quantitative estimation studies. The A.fumigatus isolate showed a medium yellow halo zone. While A.candidus and A.flavus isolates showed a very weak yellow halo area.

Table II:Citric acid producing isolates

Mushrooms	Czapeck-dox-bcp
Aspergillus candidus	-
Aspergillus fumigatus	++
Aspergillus niger	++++
Aspergillus sp1	++++
Aspergillus sp2	++++
Aspergillus flavus	-

AC production (+); low production (-)

4. DISCUSSION

In the present study, A. Sp was isolated and identified fromof the rhyzospheric soil of banana trees, vegetables and rotting fruits. These materialswere evaluated for the production of citric acid. Isolation of species of A. niger from soil samples was reported by [13]; [6].

The analysis of the different samples revealed the presence of many different fungal species. Macroscopic identification made it possible to partially identify molds of the genus Aspergillus sp. Furthermore, the observation of parameters such as the structure of the thallus, the relief of the colonies, the size of the colonies and the color of the colonies made

it possible to partially confirm the presence of A. niger, a mold having an interest in the production of acid. citric. These results obtained are similar to those of[9]; [14].

As for microscopic identification,examination at objective 40 is sufficient to highlight most of the important elements to identify such as hyphae, conidiophores, conidia and spores. Also the microscopic description of the isolates in this study is identical to the work carried out by[9]; [15]**.**

All fungal isolates obtained in this study were grouped into 6 different Aspergillus species using this analysis. Indeed, rhyzosphere soil isolates from banana, rotting fruits and vegetables, share 3 species in common. These are Aspergillus niger; Aspergillus sp1 and Aspergillus sp2. As for Aspergillus candidus, Aspergillus flavus and Aspergillus fumigatus, it was specific to the banana rhyzospheric soil sample.

Regarding the qualitative screening of citric acid production, six isolated Aspergillus sp were screened for their citric acid production capacity on Czapek-Dox agar medium supplemented with bromocresol violet. Indeed, bromocresol violet, is an indicator dye, changes with pH. When*Aspergillussp*produces citric acid in the medium it diffuses through the agar medium and therefore reacts with the dye and changes the purple color of the dye to yellow [16].

The results showed the ability of certain isolates to produce aflatoxins in the CEA medium, and the percentage of isolates positive for this test is 30%. The isolation of A. flavus and A. candidus showed a high capacity of aflatoxin production while A. fumigatus showed a moderate capacity on the production of aflatoxins at the CEA medium while isolates of A.niger n did not show the ability to produce aflatoxins. These results are compatible with[17]who found some isolates of the fungus Aspergillus isolated, the ability to produce aflatoxins.

There is a relationship between the production of aflatoxin and that of AC. Indeed, the more aflatoxin the mold produces, the lower the AC performance will be. The molds A. candidus, A. flavus and A fumigatus which were detected positive in the aflatoxin screening, we obtained a low yield in terms of the coloring of the Czapeck medium.

5. CONCLUSION

The identification of Aspergillus Sp strains is essential for the determination of citric acid-producing strains. Macroscopic and microscopic examinations made it possible to identify the isolated mycelial strains. Several species of Aspergillus in samples of rhyzospheric soil, rotting vegetables and fruits were isolated in this study. Aflatoxin-producing strains were isolated and those that could be used for the production of citric acid.

REFERENCES

1.LoisonL. From microbe to gene, a new reading of the history of French genetics. Bulletin of history and epistemology of life sciences. 2012; 19, 203-215.https://doi.org/10.3917/bhesv.192.0203(French)

2. Show PL, Oladele KO, Siew QY, Zakry FAA, Lan JCW, Ling TC. Overview of citric acid production from Aspergillus niger, Frontiers in Life Science, 2015;8(3):271–283,http://dx.doi.org/10.1080/21553769.2015.1033653

3. Dhillon GS. Valorization of agro-industrial waste by fungal bio-productionan important platform product (citric acid) with simultaneous extraction of chitosan,Thesis presented for obtaining the degree of Philosophiae doctor (Ph.D.), University of Quebec, Canada, 2012;469p. (Google scholar)

4. Kouassi KEA. Contribution to the valorization of agricultural by-products into bioproducts. Doctoral thesis, Agroresource Sciences, National Polytechnic Institute of Toulouse,2018;(Toulouse, France), 122p. (Google scholar)

5. Sawant O., Mahale S., Ramchandran V., Nagaraj G. & Bankar A. Fungal citric acid production using waste materials: a mini-review, Journal of Microbiologic, Biotechnology and Food Sciences, MES Abasaheb Garware College, Department of Microbiology, India, 2018;8 (2) 821-828, doi: 10.15414/jmbfs.2018.8.2.821-828

6. Behera BC: Citric acid from Aspergillus niger: a comprehensive overview, Critical Reviews in Microbiology, 2020; DOI: 10.1080/1040841X.2020.1828815

7. Saba S., Shahzad A., Atif J. & Akhtar A.Aflatoxin Contamination, Its Impact and Management Strategies: An Updated Review,*Toxins (Basel)*,2022; 27;14(5):307. doi:10.3390/toxins14050307.

8. Iqbal J, Haq IU, Javed MM., Hameed U, Khan AM, Parveen N, ... & Khan, TS. Isolation of Aspergillus niger strains from soil and their screening and optimization for enhanced citric acid production using cane molasses as carbon source. J Appl Environ Biol Sci, 2015; 5(4), 128-137.

9. Diguta CF. Ecology of molds present on grape berries. Food and Nutrition. University of Burgundy; UniversitateaBucureşti, 2010. NNT: 2010DIJOS045. tel-00597399. French.

10. Saito M. & Machida S. A method for rapid identification of aflatoxin-producing strains of Aspergillus flavus and Aspergillus parasiticus using ammonia vapor, Mycoscience, 1999;40:205-208.

11. Ali S. Studies on the submerged fermentation of citric acid by Aspergillus niger in a stirred fermentor, Ph.D. Thesis. 2004; University of Punjab Lahore, Pakistan

12. Kareem SO, Akpan I., Alebiowu OO Production of citric acid by Aspergillus niger using pineapple waste. Wrong. J. Microbiol. 2010;6:161–165.13.

14. Dufresne P & St-Germain G. Identification of mushrooms of medical importance, Public Health Laboratory of Quebec, Institut de santépublique Québec, Canada, 2021; 64p. French.

15. Ruchi S. Pathogenicity of Aspergillus niger in plants. review article, Cibtech Journal of Microbiology, 2012; 1:47-51.

16. Ali S., Ashraf H. & Ikram U. Enhancement in citrate production by alcoholic limitation. J. Biol. Sci. 2002; 2:70–72.

17. Amadi JE & Adeniyi O. Production of mycotoxins by fungi isolated from stored cereals. African Journal of Biotechnology, 2009;8(7):1219-1221. French.

18.Chebon, S., Wanyoike, W., Bii, C., Gathumbi, J., &Ogoyi, D. (2015). Incidence of Aflatoxigenic Fungi and Aflatoxins in Maize Cultivated Under Rain-Fed and Irrigation Farming Systems in Kenya. Microbiology Research Journal International, 11(6), 1–11. https://doi.org/10.9734/BMRJ/2016/18051

19.Babaei, A., Manafi, M., &Tavafi, H. (2013). Effect of Aloe vera Extracts on Production of Aflatoxin B1 and Extracellular Proteins by Aspergillus flavus. Annual Research & Review in Biology, 4(6), 978–984. https://doi.org/10.9734/ARRB/2014/5308

20.Saleem, A. R. (2017). Mycobiota and molecular detection of Aspergillus flavus and A. parasiticus aflatoxin contamination of strawberry (Fragaria ananassa Duch.) fruits. Archives of Phytopathology and Plant Protection, 50(19-20), 982-996.

DEFINITIONS, ACRONYMS, ABBREVIATIONS

AC: citric acid
PDA: Potatoes dextrose agar
CEA: coconut extract agar