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(RESEARCH ARTICLE)



## Comparative study of two isolation methods of Cyanobacteria in Côte d'Ivoire

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### Abstract

Cyanobacteria are prokaryotic organisms found in the freshwaters of most countries of the world. Interest in these organisms has increased in recent years because of their ability to produce toxins that are harmful to higher organisms. The objective of this study was to compare two methods of isolation of cyanobacteria in Côte d'Ivoire. Monthly water withdrawals were carried out from June 2016 to March 2018 on the Aghien Lagoon (Côte d'Ivoire) and analyzed using two methods, namely the isolation method on modified BG11 agar and the direct binocular method. A total of 1682 cyanobacteria were isolated, 1593 (94.70%) cyanobacteria were obtained by direct isolation and 89 (5.30%) by seeding isolation. The 1682 cyanobacteria were purified by successive transplanting on the agar BG11 modified, 563 cyanobacteria (33.47%) were obtained. Of this total, 92.36% (n = 520 cyanobacteria) were obtained by the direct method and 7.64% (n = 43 cyanobacteria) by the isolation method on modified BG11 agar. In order to maintain the growth of purified cyanobacteria, the 563 cyanobacteria were transferred to liquid modified BG11 medium. However 38 (6.74%) cyanobacteria had a positive growth on this medium of which 5.76% (n = 30) came from the direct method and 17.02% (n = 8) from the isolation method on modified BG11 agar. This study has shown that the isolation method that has resulted in better yield has been isolation by isolation method on modified BG11 agar.

**Keywords:** Aghien; Cyanobacteria; Isolation; Culture

### 1. Introduction

Freshwater is the main source of drinking water for most of the world's human populations [1]. Increased eutrophication of these rivers will result in massive development of phytoplankton species, mainly cyanobacteria [2-3].

Cyanobacteria are photosynthetic prokaryotic microorganisms naturally present in the aquatic environment (less than 20000 cells / mL), without any problem being associated with it [4]. However, problems arise when there is proliferation of cyanobacteria [5]. This problem is all the more serious when the species responsible for these blooms can produce toxins. The contamination of freshwater by these toxins limits their use because they are often the cause of deadly intoxication of herds of domestic or wild animals, fish, birds and humans [6-8]. In contrast, many studies have shown that cyanobacteria are a new source of various bioactive compounds [9-10].

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Thus, for carrying out studies on their secondary metabolites, the provision of viable strains of cyanobacteria is important. This motivated the creation of numerous collections including that of the Pasteur Institute of Paris (IPP), the National Institute for Environmental Sciences (NIES) of Japan, the Institute of Hydrobiology (FACHB) of China and the University of Texas (UTEX) in the United States. However, in Africa, the collections are almost non-existent.

In Côte d'Ivoire, only studies of Coulibaly et al. [11] were conducted on the isolation and culture of cyanobacteria by testing different media, namely Conways, BG11 and Bold. Our study consisted in comparing two methods of isolation of cyanobacteria on modified BG11 medium in Côte d'Ivoire in order to set up a collection.

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## 2. Material and methods

### 2.1. Sampling

Monthly water withdrawals were carried out over a 22-month period from June 2016 to March 2018 on the Aghien lagoon (Côte d'Ivoire). Samples were collected using 20 µm porosity plankton net with a collector. This consisted of filtering 5 liters of surface water with the aid of the net, then recovering in the collector 100 ml of lagoon water containing the cyanobacteria. The contents of the collector are then transferred to a one-liter bottle. This process was performed several times until the bottle was filled. The samples thus obtained were stored in a cooler containing cold accumulators and then transported to the laboratory for analysis.

### 2.2. Isolation and culture of cyanobacteria

The culture medium used for the isolation and culture of cyanobacteria was liquid and solid modified BG11 medium. These media were prepared according to the formulas and indications of Coulibaly et al. [11] at pH 7.1. Two isolation methods were performed:

#### 2.2.1. First method: Isolation by seeding

500 µL of water from the lagoon were diluted successively in 1000 µl of the modified liquid medium BG11, then 100 µl of each dilution was spread on a Petri dish containing agar medium BG11 modified according to the described method by Rippka et al. [12].

#### 2.2.2. Second method: Direct isolation under a binocular loupe

A quantity of 2 µl of water from the lagoon was successively diluted in 4 µl of the modified liquid medium BG11. These micro-dilutions were observed with a binocular microscope, the least concentrated was identified and cyanobacteria taken one by one using a micropipette. Each colony was transferred to a well. This process was repeated until the 96-well plate, each containing 10 µl of the modified BG11 liquid medium, was filled.

#### 2.2.3. Incubation

The Petri dishes and the 96-well plates were sealed with Parafilm and placed in a culture room at  $25 \pm 1^\circ \text{C}$  where they were subjected to a light intensity (white light quality MAZDA FLUOR M2TF 36W / LJ 1.2 m long, 32 Watt) producing a daylight-like light of  $175.56 \pm 1$  lux. Petri dishes and plates were observed daily with a binocular magnifying glass to monitor the growth of cyanobacteria.

#### 2.2.4. Successive transplanting

After several weeks of growth, the cyanobacteria colonies on the Petri dishes were taken one by one under the binocular microscope. The agar containing the colony was cut with a scalpel blade sterilized by the Bunsen flame and transplanted streaks onto a new Petri dish.

Similarly, when the development of cyanobacteria was observed in the wells, they were collected one by one under the binocular microscope with a micropipette then transferred to a Petri dish containing the agar-modified BG11 medium.

The transplanting was repeated a ten times in order to obtain pure colonies of cyanobacteria. The cyanobacteria thus obtained were transferred into the Erlenmeyer flasks with plugs containing a volume of 10 ml of the liquid modified BG11 medium, incubated in a room at  $25 \pm 1^\circ \text{C}$  with a brightness of  $175.56 \pm 1$  lux.

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### 3. Results

#### 3.1. Number of strains isolated according to isolation mode

A total of 1682 cyanobacteria were isolated from the 126 lagoon water samples collected during this study. Of the total cyanobacteria obtained, 94.70% (n = 1593) were isolated by the direct method and 5.30% (n = 89) by the isolation method on modified BG11 agar.

#### 3.2. Number of cyanobacteria obtained after successive transplanting

Of the 1682 cyanobacteria isolated a total of 563 purified cyanobacteria were obtained after repeating a few times successive transplanting on the agar medium. 520 cyanobacteria or 92.36% were obtained by the direct method and 43 (7.64%) by the isolation method on modified BG11 agar.

#### 3.3. Number of cyanobacteria obtained after transfer to the liquid medium

The 563 cyanobacteria obtained after the various successive subcultures were transferred to the liquid medium. After three months on the liquid medium, 38 (6.75%) cyanobacterial strains had positive growth. Of these, 30 strains came from direct isolation at a rate of 5.76% and 8 (17.02%) by isolation method on modified BG11 agar.

#### 3.4. Cyanobacteria rate obtained after growth according to isolated cyanobacteria

Of a total of 1682 cyanobacteria isolated, 38 had a positive growth, a rate of 2.25% of isolated cyanobacteria. 8.98% (n = 8) obtained from the 89 cyanobacteria isolated by the isolation method on modified BG11 agar and 1.88% (n = 30) obtained from 1593 cyanobacteria isolated by the direct isolation method.

#### 3.5. Cyanobacteria colonies lost during the study

In this study, 1682 cyanobacteria were isolated by two methods. In order to obtain pure strains, successive subcultures were carried out, 563 cyanobacteria were obtained and transferred to a liquid medium, ie a 66.52% loss of cyanobacteria. 38 cyanobacteria had positive growth on this medium, a total loss of 97.74%.

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### 4. Discussion

Several studies have been carried out on the isolation of cyanobacteria and their toxic or beneficial effect [13, 10], but very few concerning exclusively isolation and culture [11]. The objective of this work was to compare two modes of isolation of cyanobacteria from the Aghien lagoon samples from June 2016 to March 2018.

A high isolation rate was observed for the direct isolation mode under a binocular loupe. This could be due to the fact that the cyanobacteria were spotted then taken one by one and put in culture. While the isolation on agar, 100 µL was taken for seeding, this quantity could contain a large microbial flora and algae variety whose rapid growth would significantly prevent the isolation of cyanobacteria [14].

Thus, during the various purification tests by the successive transplanting technique, a significant loss of cyanobacteria was observed. This could be due to contamination by some bacteria or fungi in agar favored by the favorable growing conditions. Meseck et al. [15] observed a 13% decrease in the growth rate of cyanobacteria in the presence of a bacterial community, while other authors observed total inhibition of growth [16, 17]. The loss of cyanobacteria during successive transplanting could also be explained by the very loss of cyanobacteria during the transfer from one agar to another.

In this study, 38 cyanobacteria had positive growth on the medium used. Some studies have reported proportions inferior to those obtained in this study. This is the case of the studies conducted by Oufdou and Oudra [10] in Morocco which isolated and cultured 10 cyanobacteria in samples taken from 4 different sites. In France, Wilame and Hoffman [18] isolated 11 cyanobacteria in samples collected from 8 sites in the Artois-Picardie basin. However, other studies have reported higher frequencies. Berger [13] isolated 57 cyanobacteria in May 2002 samples.

This difference in result could be attributed to the mode of isolation used, to the composition of the culture medium or to the different variations of the luminous intensity of the incubation room. Indeed, all the studies cited above have used culture media different from that used in this study but an identical isolation protocol. Oufdou and Oudra [10] and [13] used the seeding method followed by the different subcultures, but different culture media, namely the liquid

and solid media BG13 and Z8 with or without nitrogen. On the other hand, Wilame and Hoffman [18] used the BG11 culture medium supplemented with antibiotics and the seeding method followed by the different transplantation.

This growth rate of cyanobacteria on the modified BG11 medium could be explained by the development of fungi and other bacteria present in the Erlenmeyers thus contaminating the different cultures. This development of fungi and other bacteria sometimes inhibits the development of cyanobacteria. This fact was noticed by Roger and Reynaud [19] in France and [20] in Burkina Faso.

The physicochemical conditions of the culture medium could prevent the development of cyanobacteria. Thus, several authors have shown that besides the physicochemical conditions suitable for a good functioning, certain species excrete in the environment where they live toxic substances preventing the development of competing species. This was shown by Tassigny and Lefebvre [21] for non-thermal freshwater algae.

Another fact that could influence the growth of cyanobacteria is the light intensity of the growing room. For this purpose, all cyanobacteria do not develop in the same range of light intensity, some require a low intensity while others a high intensity.

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## 5. Conclusion

Interest in cyanobacteria has increased in recent years given the many secondary metabolites they can synthesize. This study compared two modes of cyanobacterial isolation in Côte d'Ivoire and found that the seeding isolation method was the most conclusive. However, isolation and cultivation of cyanobacteria are difficult methods given their slow growth and rapid contamination by certain microorganisms.

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## Compliance with ethical standards

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### *Disclosure of conflict of interest*

The authors declare that there are no conflicts of interest.

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