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ISOLATION AND CHARACTERIZATION OF ENDOGENOUS MANGOSTEEN (*GARCINIA MANGOSTANA*) YEASTS THAT CAN BE USED IN THE FERMENTATION INDUSTRY

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Abstract:

This study aimed to isolate and characterize endogenous yeast strains of mangosteen (*Garcinia mangostana*). Thus, we harvested the fruits of the mangosteen in the Moungo department, Littoral region of Cameroon. Isolation was carried out on SDA (Sabouraud Dextrose Agar) 0.05% chloramphenicol agar medium. The identification was made using the API gallery. A total of 30 yeasts strains were isolated. Among these isolates, three were selected on the basis of their ability to produce and resist alcohol, and then characterized. According to the biochemical characterization using Gallery Api 20 C AUX Biomérieux, these isolates L1H1, L1G and L1I2 isolated from the arils of mangosteen belong respectively to the species *Saccharomyces cerevisiae 2*, *Saccharomyces cerevisiae 1*, *Cryptococcus luteinis*. These isolates were identified

as the most efficient not only in terms of ethanol production but also in the resistance to it. Therefore, it was concluded that these isolates could well adapt to biotechnological uses.

Key words: Isolation, mangosteen, yeasts, fermentation

1. Introduction

Fruits are a very important food group in the human diet, along with vegetables, due to their richness in nutrients and their multiple health benefits. The United Nations General Assembly intends to promote the beneficial effects of fruits and vegetables on health and nutrition, and their role in a healthy and balanced diet and lifestyle (UN, 2020). Thus, the year 2021 has been named the "International Year of Fruit and Vegetables" to promote the health and nutrition benefits of fruits and vegetables and the need to reduce their loss and waste (UN, 2020; FAO, 2021). Global fruit production was estimated at about 868 million tons in 2018 with 17.4 million tons in Central Africa (FAO, 2021). However, they face multiple problems, including a very high loss rate. This is in the order of 15 to 50% in sub-Saharan Africa and is mainly due to their perishable nature induced by their microbial flora. The development of the latter leads to the fermentation of sugars present in the fruits, thus causing their rotting. Hence the need to control the endogenous flora of fruits. Among these fruits we find the mangosteen. Of its scientific name Garcinia mangostana, the mangosteen is a fruit with a pinkish pericardium containing 6 to 8 quarters of white arils in which one or more seeds are found (CIRAD, 2013). Native to Southeast Asia, mangosteenaccounts for 5.21% of million tons of global tropical fruit production in 2018 (FAO, 2020). It is commonly referred to as the "queen of fruits" as it is recognized as one of the best tropical fruits due to its high concentration of secondary metabolites (Holm et al., 2016). However, the work done so far on this species has provided information on its botanical and agronomic aspects (FAO, 2020). In addition, other works have focused on the phytochemistry of mangosteen, the anticancer, antitumor, pharmacological and medicinal properties of the extracts of this fruit (Tangponga, et al., 2011; Manimekalai et al., 2015; Melia et al., 2019). Despite its multiple properties the mangosteen is a little known fruit in our society and so far exploited only for consumption (for its nutritional properties). However, it presents difficulties of conservation in time and space. This is a real problem, hence the search for alternatives of exploitation.

As a fruit, the mangosteen constitutes an important reservoir of microorganisms, particularly yeasts, whose control would constitute a way for its conservation, transformation and valorization. Indeed, yeasts, common inhabitants of fruits, are endowed with biotechnological characteristics (Mananjara et al.,2016). The present work therefore aims to isolate and characterize endogenous yeasts with biotechnological potential from mangosteen.

2. MATERIAL AND METHOD

2.1. Sampling

In this study, ripe mangosteen fruits were collected from IRAD in Njombé's plantations in the Moungo Department, Littoral Region of Cameroon. The arils of these fruits constituted the basic

material for the isolation of endogenous yeasts. These were collected in sterile containers, aseptically ground and incubated at 30°C for 72 h for spontaneous fermentation.

2.2. Isolation of yeast strains

The stock solution obtained underwent decimal dilutions. Isolation was performed on SDA (Sabouraud Dextrose Agar) 0.05% chloramphenicol agar (Cissé et al., 2009) to inhibit bacterial growth from dilutions 10^{-6} and 10^{-9} at 30°C for 48h. The pure isolates obtained were purified and stored in cryotubes at -4°C.

2.3. Demonstration of the fermentative power of isolated yeasts

Yeast isolates were first subjected to an ethanol production test. They were inoculated (10%v/v) in triplicate at a concentration of 10^6 cells/ mL in 50mL of sterile 8°Brix mangosteen juice and incubated at 30°C for 72h. Incubation flasks were sealed with silicone caps to ensure anaerobiosis, and CO_2 could be evacuated through a sterile plastic piping installed and soaked in water, passing through the caps **(Yamaoka et *al*., 2014)**. The quantity of alcohol produced was measured using a hydro-alcohol meter.

2.4. Ethanol tolerance

Yeasts isolates capable of producing alcohol were subjected to a second test based on the resistance of the latter. Liquid YPD (yeast extract peptone dextrose) medium supplemented with alcohol at different levels was used. After sterilization of the YPD medium, absolute ethanol was added at concentrations ranging from 6 to 15% (v/v). Each isolate was inoculated with 1% (v/v) in 30 mL of medium. Incubated at 30°C for 48h, cell growth was assessed by spectrophotometric reading (600nm) and then by cell counting under a light microscope **(Armanul et *al*., 2017)**.

2.5. Basic identification

Isolates identification was based on the determination of cultural, morphological and biochemical characteristics. Commercial API (Analysis Profile Index) 20C AUX kit (BioMérieux, France) was chosen for the identification of the selected strains according to the manufacturer's instructions. API 20 C AUX kit is a standardized system containing a combination of 20 fermentation substrates and used for the identification of yeasts **(Guiraud et *al*., 2003) .**

2.6. Statistical Analysis

Results were expressed as mean ± standard error (M ± ES) of three replicates (n=3). In between groups comparison was made by the ANOVA test using Statgraphics Centurion Version 17.1.8 software.

3. RESULT

3.1. Isolation of endogenous yeasts

The morphology of vegetative yeast cells was observed on solid medium in petri dishes after 48h of incubation at 30°C **(Kurtzman and Fell 1998)**. A total of 30 yeasts were isolated from mangosteen fruits. These isolates were found to be different in both colony shape and size (Fig1).

Figure 1: yeasts isolated on SDA medium

3.2. Ethanol production

All isolated yeasts were subjected to a double screening. The first was based on their fermentative capacity, as shown in Figure 2.

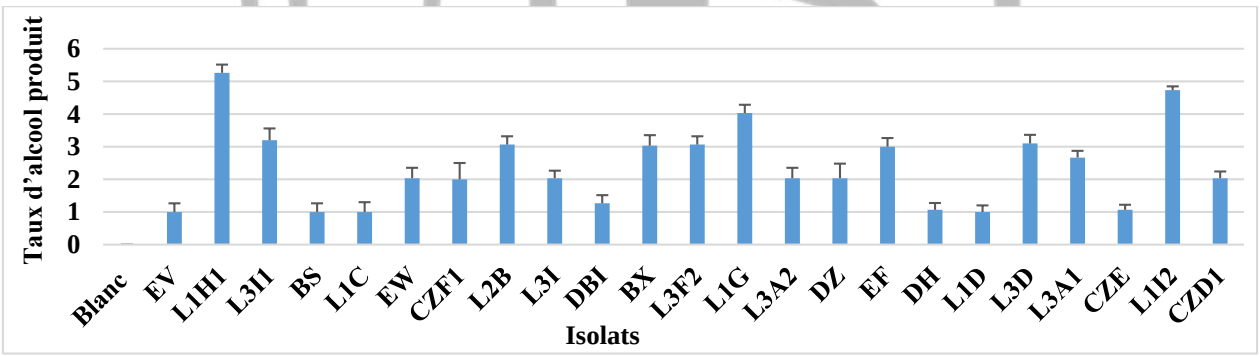

Figure 2: Alcohol production rate according to the different yeasts

Of the 30 isolates tested, 23 achieved alcoholic fermentation with alcohol levels ranging from 1° to 5.3° after 3 days of fermentation in a medium containing 8°Brix sugars.

3.3. Ethanol tolerance

Isolates capable of producing ethanol were screened based on ethanol tolerance. Figure 3 shows that all these isolates were capable of growing with 16% ethanol in the medium. However, L1H1, L1G and L1I2 isolates showed higher growth rates.

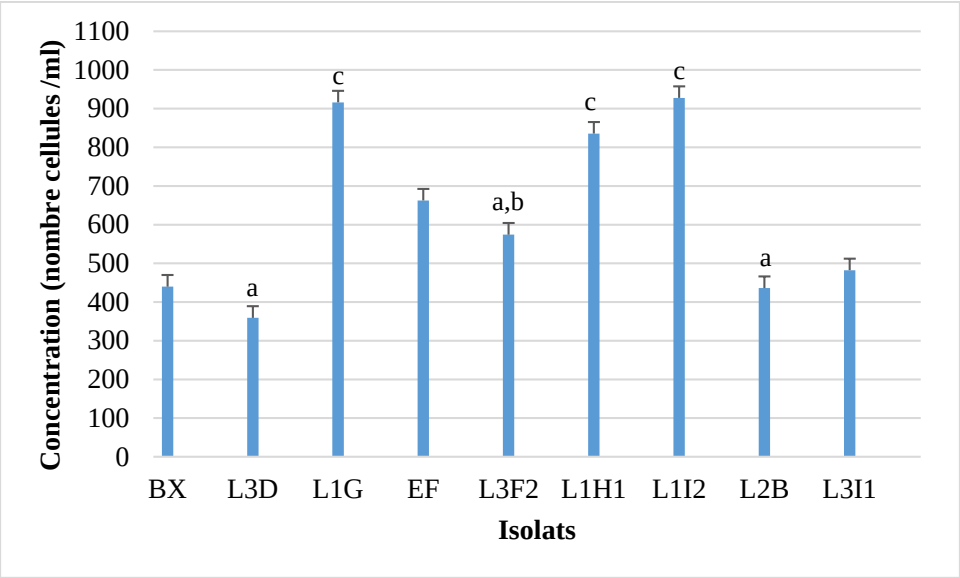

Figure 3: Growth of yeast in a medium with 15% alcohol

3.4. Identification of isolated yeast strains

Isolates L1H1, L1G and L1I2 showed an ovoid morphology with one, two or three buds under the microscope's light. These buds, visible after 48 h of incubation at 30°C confirmed the nature of these isolates (Figure 1b). Based on the whitish color, their ovoid and rounded morphology and the presence of multipolar buds, these isolates are indeed yeasts. However, the identification table provided by bioMérieux on the sugar assimilation test using API 20C AUX galleries revealed that isolates L1G is revealed to be *Saccharomyces cerevisiae 1*, isolate L1H1 is *Saccharomyces cerevisiae 2* and isolate L1I2 is *Cryptococcus laurentti*.

Table I: Results of biochemical characteristics of strains L1H1, L1I2 and L1G

	0	GLU	GLY	2KG	ARA	XYL	ADO	XLT	GAL	INO	SOR	MD	NAG	CEL	LAC	MAL	SAC	TRE	MLZ	RAF
L1H1	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	+	+	+	-	+
L1G	-	+	+	-	-	-	-	-	+	+	-	-	-	-	-	+	+	+	-	+
L1I2	-	+	-	-	+	-	-	-	+	-	-	-	+	+	+	+	+	-	-	+

4. Discussion

Yeasts are ubiquitous eukaryotic microorganisms found in a wide variety of ecological niches, mainly in water, soil, air and on plant and fruit surfaces. Mangosteens collected in the Moungo region of Cameroon allowed the isolation of 30 yeast isolates. These yeasts are part of

the endogenous flora of the mangosteen. Indeed, fruits in general and the mangosteen in particular are reservoirs of multiple bioactive compounds such as amino acids, organic acids, vitamins and minerals. Their water content makes them suitable for the growth and development of yeasts **(Luzón et *al*., 2021)**. In this natural environment, the yeasts can carry out their metabolism and fermentative activity in a satisfactory way, since they have the necessary nutrients and substrates to cause the decomposition of the fruits **(Sergi, 2020)**. However, the endogenous flora of fruits depends not only on its biochemical composition but also on its degree of ripeness, season and harvest area **(König et *al*., 2013)**.

Not all yeasts have the same metabolism. Alcohol production results from the transformation of sugars present in the environment. The ability to carry out this transformation varies from one yeast to another depending on the species to which they belong. Thus, we find those with little or no fermentative activity. These yeasts are classified as "oxidative", "apiculate" or non-Saccharomyces yeasts **(IFV, 2019; Sergi, 2020)**. During fermentation, these yeasts are primarily responsible for the quality and flavor of the finished product **(Pranita et *al*., 2016)**. On the other hand, the remaining 23 metabolized the sugars present in the medium at varying degrees to produce ethanol. Indeed, yeast species behave differently towards various sugars as they contain different types of enzymes and thus metabolism **(Pranita et *al*., 2016)**. From a microbiological point of view, ethanol and carbon dioxide produced during alcoholic fermentation are waste products of microbial metabolism. Thus their accumulation in the medium becomes toxic and therefore inhibits their growth **(Da Silva et *al*., 2013)**. Various studies show that several yeasts tolerate concentrations ranging from 10 to 15% of ethanol in the medium. This result is in line with that of Panneerselvam et *al*., 2011, who showed that as soon as the alcohol content reaches 16 to 20%, there is no longer any growth of yeasts. According to Gbohaida et *al* (2016), fermentative capacity and the ability to resist ethanol are two related traits. Indeed, the more ethanol the yeast produces, the better its ability to resist ethanol. However, yeast isolates can produce and tolerate different levels of alcohol in the medium ranging from 5 to 20% ethanol (Luisa et al 2010). This tolerance depends on the composition of the cytoplasmic membranes of the cells and in particular the lipids on the one hand and the environmental culture parameters on the other hand. **(Boulal et *al*., 2016)**.

Based on the ability of the yeast isolates to assimilate a series of sugars, the use of the API 20C AUX gallery revealed the identity of each isolate of which L1H1 was *Saccharomyces cerevisiae 2*, isolate L1G was *Saccharomyces cerevisiae 1* and isolate L1I2 was *Cryptococcus laurentii*. The membership of L1G and L1H1 to the same genus and species corroborates the morphological similarity found at the microscopic level. All three strains are fermentative species **(Hidalgo et *al*, 2012; Sanchez, 2008; Bhadra et *al*, 2007)**, which explains their presence in mangosteen fruit because the fruit is rich in sugars and fermentative yeasts are known for their affinity with sugar-rich media **(Bhadra et *al*, 2007; Panneerselvam et *al*, 2011)**. Several works performed on yeast isolation from fruits have already shown the existence of these three strains on fruits such as mango, persimmon, dates, prickly pear, pineapple, grape, etc. **(König et al., 2013; Hidalgo et *al*, 2012; Bhadra et *al*, 2007; Mounir et *al*, 2016)**. The *Saccharomyces* Sp is used as a model as a basic and applied research tool are part of the normal endogenous flora of many fruits **(Mananjara et *al* 2016)**.

5. Conclusion

This study on the isolation of the endogenous levuriform flora of *Garcinia mangostana* harvested at Njombé in the Moungo region of Cameroon showed that the mangosteen is a reservoir of a variety of yeasts including Saccharomyces and Cryptococcus genus. These yeasts are of great interest and can be used for biotechnological purposes in fermentation process.

Conflict of interest

The authors declare that there is no conflict of interest in this work.

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