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Development of a "probiotic" supplement from palm wine yeasts

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Abstract

Numerous microbial species have been used as probiotic agents. These microorganisms include bacteria (*Bacillus, Bifidobacterium, Enterococcus, Escherichia coli, Lactobacillus, Lactococcus, Streptococcus*) and yeasts (*Saccharomyces*). Yeasts used as natural pre- or probiotics and added to animal feed can produce beneficial effects on their performance. This work therefore aims to develop a "probiotic" supplement from palm wine yeasts. The microorganisms chosen for this study are yeasts, more specifically the yeasts from palm wine. For this purpose, some palm wine will be collected and then fermented. Corn (bran and crushed grains) will be added to the fermentate to obtain the "probiotic" supplement from palm wine yeast. This supplement will be added to chicken feed in upcoming experiments.

Keywords: Microorganisms; Probiotics; Yeasts; Palm Wine.

1. Introduction

The concept of "probiotic" originated from the work of Metchnikoff (1908), where the idea developed was that live lactic bacteria ingested could have a beneficial effect. Over time, this notion has led to several definitions. Today, it is specified that probiotics are "live microorganisms that, when administered in adequate amounts, confer a health benefit to the host beyond the initial nutritional effect" (Bernardeau and Vernoux, 2009; Seppo and al., 2021). Numerous microbial species have been used as probiotic agents. These microorganisms belong to bacteria of the genera *Bacillus*, *Bifidobacterium*, Enterococcus, *Escherichia coli*, *Lactobacillus*, *Lactococcus*, *Streptococcus*, and yeasts of the genus *Saccharomyces* (Bernardeau and Vernoux, 2009; Ansari and al., 2023; Hosoda and al., 2023). Yeasts are the most ancient and well-studied microorganisms. They have a high content of Lysine (6 to 9%) and Threonine, but are low in sulfur-containing amino acids (Methionine and Cysteine) (Goldberg, 1985; NRC, 1994; Frohn, 2023). Their nucleic acid content is relatively low: 4 to 10%. They are a good source of B vitamins and also provide vitamin E and provitamin D. The use of yeasts in animal feed improves production parameters (Churchill and al., 2000; Upendra and Yathiraj, 2003; Miazzo and al., 2011; Mialon and al., 2021; Dunière and al., 2023). Palm wine is a highly appreciated beverage in Ivory Coast. It is obtained from three species of palm trees. Immediately after extraction, the unfermented sap is clear, sweet, and contains about 10-12% sugar, mainly sucrose (Ogbulie and al., 2007; Hai and al., 2024; Sumerta and al., 2025). During fermentation, the level of sucrose decreases as it is converted into ethanol and other products by natural microorganisms. The dominant yeast species during this fermentation are *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* (Odunfa and Oyewole, 1998; Lee, 2023). However, these microorganisms can coexist with other yeast species such as *Kloeckoera apiculata*, *Candida krusei*, and other *Candida spp*., *Pichia spp*. (Amoa-Awua and al., 2007).

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The objective of this work is to develop a "probiotic" supplement using palm wine yeasts. The effect of this supplement will be tested in various experiments on the zootechnical performance of chickens.

2. Material and methods

2.1. Materials

2.1.1. Biological Materials

Our biological materials consist of:

Yeasts

The source of our yeasts is palm wine obtained from the market in the Abobo district (Abidjan, Ivory Coast). This palm wine will be used for the cultivation of our yeasts.

Technical Materials

Our technical materials consist of

- A 15 horsepower electric hammer mill
- A 400 kg electric mixer
- An incubator measuring 94 cm x 52 cm x 141 cm = 689,208 cm^3
- A world-class balance with a capacity of 20 kg; precision of 0.1 g
- A 100 L barrel
- A microscope
- Petri dishes, flasks, slides, and cover slips
- OGA (Oxytetracycline Glucose Agar), Sabouraud + chloramphenicol, OGA + TTC, and Sabouraud + chloramphenicol + TTC agar plates
- Liquid media OGA and Sabouraud
- An API 20 C gallery
- An automated reading and interpretation system: ATB expression

2.2. Methods

2.2.1. Yeast Cultivation

For the cultivation of palm wine yeasts, the following procedure was used. Palm wine is added to warm sugared water. The mixture is thoroughly homogenized. The resulting mixture is placed in a tightly sealed barrel and left to ferment for 3 days. Fifty percent corn bran and 50% crushed corn grains were added to the ferment. The mixture was then kneaded and dried. The resulting product will be used as a "probiotic" supplement and incorporated into a base feed.

2.2.2. Identification of Yeast Strains and Species in Palm Wine

The goal is to identify yeast strains and species in palm wine. A total of 10 samples of 300 ml each were collected for our study. The analysis method used is the classical bacteriology approach, through culture and isolation on solid media. The pH of each sample was measured and neutralized if necessary before inoculation. Inoculation was performed on OGA (Oxytetracycline Glucose Agar) and Sabouraud + chloramphenicol agar plates by streaking, and then incubated at 25°C for 72 hours. Subsequently, the appearance of colonies on the different media was checked. Presumptive yeast colonies on OGA and Sabouraud + chloramphenicol appear creamy. They were then examined fresh between slides and cover slips under a microscope to confirm the actual presence of yeasts. The yeast colonies were then transferred into liquid OGA or Sabouraud media, and on OGA + TTC or Sabouraud + chloramphenicol + TTC agar plates for 48 hours at 25°C, to allow for better development for studying morphological, physiological, and biochemical characteristics. The previously isolated colonies are identified through biochemical characterization using the API 20 C rapid identification gallery (Biomérieux Auxanogram) and an automated reading and interpretation system: ATB expression.

2.2.3. Identification of Yeast Strains and Species in the PWC-Supplement (Palm Wine + Corn)

The isolation and identification of yeasts in the palm wine + corn supplement were carried out according to classical methods of verification in liquid media and isolation on solid media, specifically OGA and Sabouraud + chloramphenicol

agar. Ten (10) g of the supplement were revitalized in 90 ml of liquid OGA and liquid Sabouraud media in 250 ml flasks sealed with cotton wool. The flasks were incubated at 25°C for 72 hours in the incubator. After the incubation period, inoculation of the broths was performed on OGA and Sabouraud + chloramphenicol agar plates for the isolation and identification of presumptive yeasts. Identification was performed using the API 20 C gallery (Biomérieux Auxanogram) and an automated reading and identification system.

3. Results and discussion

3.1. Identification of Yeast Strains and Species in Palm Wine

The results obtained confirm that palm wine contains the following yeast species:

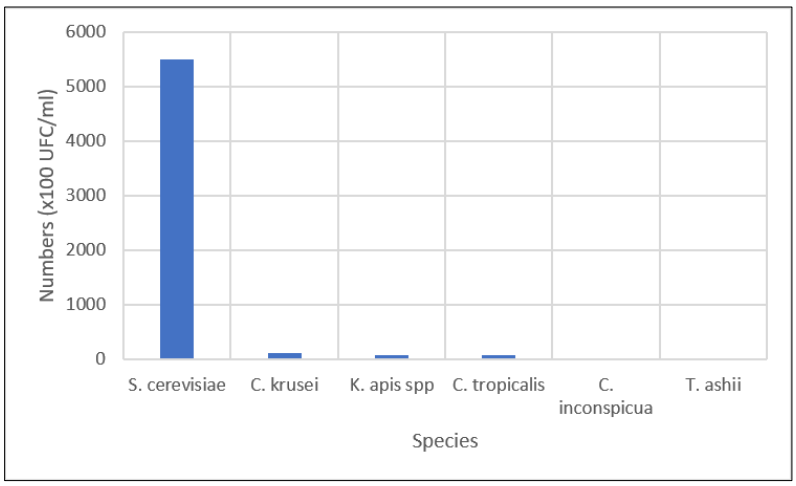

Figure 1 Yeast Species Present in Palm Wine

The analyzed palm wine contains different species of yeasts. There are six (6) species in total, with the most numerous being *Saccharomyces cerevisiae*. These results are consistent with those of **Law and al.** (**2011**), **Karamoko** (**2012**), and **Yao** (**2014**), who observed the presence of these six (6) species in addition to some others (*Hansenula anomala*, *Pichia farinose*, etc.) (**Tra Bi and al., 2023**). *Saccharomyces cerevisiae* is reported as one of the two most dominant species present in palm wine (**Odunfa and Oyewole**, **1998**; **Amoa-Awua**, **2006; Sumerta and al., 2025**).

3.2. Identification of Yeast Strains and Species in the PWC Supplement (Palm Wine + Corn)

The results obtained are

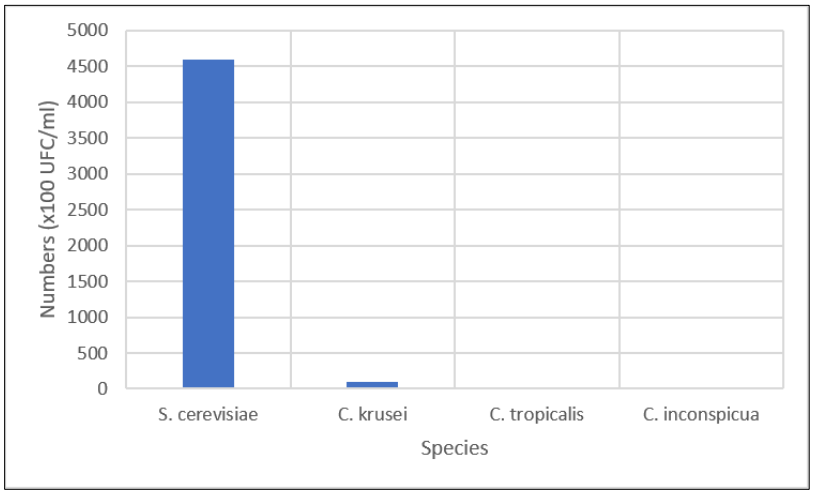

Figure 2 Yeast Species Present in the PWC Supplement

The PWC supplement also contains different species of yeasts. However, it only includes four (4) species. In the VM supplement, *Saccharomyces cerevisiae* is also present in large numbers. Yeasts used as probiotics are strains of *Saccharomyces cerevisiae* (**Toma and al.**, **2005; Maicas, 2020**). *Saccharomyces cerevisiae* constitutes a very high-quality protein source, as they are rich in lysine, tryptophan, threonine, and B vitamins, but poor in sulfur-containing amino acids (**Boze and al.**, **1992**; **Nguyen**, **2016**; **Parapouli and al., 2020**; **Ballet and al., 2023**). Therefore, the PWC supplement can serve as a "probiotic" supplement.

4. Conclusion

At the end of this study and based on the results obtained, the supplement developed can be considered a probiotic. Indeed, it contains a large quantity of *Saccharomyces cerevisiae*, a yeast commonly used as a probiotic in animal feed. Thus, for a proper evaluation of the developed "probiotic" supplement, experiments will be conducted to determine the effect of this "probiotic" supplement on the zootechnical performance of broiler chickens. Subsequently, further experiments may be conducted with the developed "probiotic" supplement on other types of poultry (laying hens, guinea fowl, quail, etc.) and other animals (pigs, fish, etc.).

Compliance with ethical standards

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K.Y.K.C. and B.L.G. designed the study. K.Y.K.C. contributed to data analysis and manuscript writing. All authors have read and approved the final version of the manuscript.

Disclosure of conflict of interest

There are no conflicts of interest.

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