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ISOLATION AND CHARACTERIZATION OF NEW YEASTS STRAINS FROM BARLEY SAMPLES

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Abstract

Due to their diversity and versatility, yeasts are considered of current industrial interest, as they can easily cover a wide range of industrial applications such as baker`s yeast, brewer`s yeast, nutritional yeast, distillation yeast, wine yeast or probiotic yeast. The purpose of this article is to isolate and characterize new barley yeast strains to obtain yeast biomass for the development of new fish feed recipes. Using the technique of decimal dilutions and inoculation by the "lawn" technique on DRBC Agar medium, the isolated strains were tested specifically to identify they're taxonomically. Colonies considered representative of a particular species or genus were isolated in pure culture and maintained on YPD Agar culture medium and cryopreserved in 20% glycerol at -20°C. 11 yeast strains belonging to the genera Saccharomyces, Candida, Cryptococcus, Torulaspora, Metschnikowia pulcherrima, Pichia, etc. were isolated. Further research will focus on the use of isolated strains of Saccharomyces cerevisiae mixed with strains of other non-Saccharmomyces to obtain yeast biomass as a potential source for fish feed.

Key words*: biomass, barley, isolation, yeasts strains.*

INTRODUCTION

The studies undertaken on yeasts, intensified in recent decades, are justified not only by the importance of these microorganisms as an experimental model, to which researchers in various specialties have turned their attention: cytology, microbiology, cell biology, genetics, but also by their applications in industry.

Yeats are without a doubt the most important group of microorganisms used by man since the beginning of his history as a social being.

From this point of view, biotechnology has always been contemporary with human history, first through its aspects - now called traditional - of making bread or wine, until today when the development of genetics and molecular biology led to the development of modern biotechnology (Banu C., 2000). Today, based on selection activities, we are witnessing worldwide the development of a special industry that produces yeasts in dry and granulated form.

These yeasts from different parts of the globe cannot always be successfully applied in other areas, which is why we believe it would be beneficial for the yeasts used to be based on the yeasts in the areas where they are to be used

Saccharomyces cerevisiae is undeniably the most studied and one of the most widely used eukaryotes in a wide variety of industrial processes, such as wine, food and ethanol production. Despite of the efficient adaptation of the various *S. cerevisiae* strains used in those processes, there is still a great potential of either optimizing existing strains, or exploiting the immense natural reservoir of environmental isolates (Parapouli et al., 2020).

Many researchers found yeasts in large numbers in a wide variety of natural habitats as different as leaves, flowers, sweet fruits, tree exudates, grains, roots fleshy fungi, insects, dung, soil. In assessing yeast strain for industrial use, specific physiological properties are required (Tikka et al., 2014).

Of all kinds of yeast, only a few species are used commercially. Typical commercial yeast applications include alcoholic beverages (beer, wine and spirits), soft drinks (root beer, kvass, kombucha, kefir, mauby), bread and food baking, bioremediation (to generate carbon dioxide for plant growth in the aquarium), food additives and flavoring agents, scientific research, and genetic engineering prophecies (Shurson, 2018). *Saccharomyces cerevisiae* is traditionally used in the food industry for the

production of alcoholic beverages, such as beer, wine, and sake, as well as for bread fermentation. More recently, *Saccharomyces cerevisiae* has also been used in the bioethanol industry and for the production of heterologous compounds, such as human insulin, hepatitis vaccines, and human papillomavirus vaccines (Hou et al., 2012).

Notwithstanding the fact that *S. cerevisiae* remains by far the most widely used industrial yeast species to date, other, so-called nonconventional yeasts, such as *Pichia, Torulospora*, have also claimed their stake as valuable contributors to industrial fermentation processes. Within non-Saccharomyces species, some cultures showed features of technological interest. Strains of *M. pulcherrima* showed the highest β-glucosidase activity and proved to be able to produce high concentrations of succinic acid. Strains of *M. pulcherrima* and *H. uvarum* showed a low fermentation power (about 4%), while *L. thermotolerans*, *Star. Bacillaris*, and *P. kudriavzevii* of about 10% (Aponte & Blaiotta, 2016).

Additionally, demands for increased productivity, wider substrate range utilization, and production of nonconventional compounds in industry, as well as changing in consumer preferences, led to a great interest in further improving the currently used industrial strains and the selection or development of strains with novel properties. It is also noteworthy that some *S. cerevisiae* strains lack the α-galactosidase enzyme and therefore cannot utilize melibiose, a disaccharide accumulated after the breakdown of raffinose when molasses are used for biomass production (Zhou et al., 2021).

The purpose of this research was to isolate and characterize new barley yeast strains to obtain yeast biomass for the development of new fish feed recipes and represents a preliminary stage for characterizing the microbial communities from barley, necessary for the improvement of biotechnological processes associated with food industry.

MATERIALS AND METHODS

The yeast was isolated from barley samples from Moara Domnească Farm (Belciugatele-Ilfov) with the following substrates: barley seed, bran, and barley cultivated soil. The used culture media are: **DRBC Agar** (agar-15 g/L, dextrose-10 g/L, dichloran - 0,002 g/L, magnesium sulphate -0,5 g/L, monopotassium phosphate-1 g/L and Bengal rose dye, 0,025 g/), **YPD Agar** (dextrose-20 g/L, peptone-20 g/L, yeast extract-10 g/L and agar-20g/L) and **YPG** (glucose-20 g/L, peptone-20 g/L, yeast extract-10 g/L and agar-20g/L).

Using the decimal dilution technique and seeding with the "In the lawn" technique on the DRBC Agar media, the isolated strains have been subjected to specific tests, for the purpose of their taxonomic identification. For each dilution in the interval $10^{-1} \div 10^{-3}$ was obtained 1 ml of suspension (by display) on 3 Petri plates and then a mean was made for the developed colonies. The determinations were performed in three repetitions.

The selected yeast strains were inoculated on liquid and solid media YPD Agar at 28°C for 72 h and identificated based on macroscopic observations (the appearance of a colony) and microscopic observations, according to the Lodder (1974), Barnett, Payne and Yarrow (1990), Pitt and Hocking (2009) classification criteria. For the identification of microorganisms through the microscopic analysis, fresh samples were prepared between slides for the identification of the cellular form of the type of microorganism. The Siemens microscope was used, and the smears were observed with the lens of 100x magnification, in a drop of cedar oil, using immersion oil (Vassu et al., 2001).

Physiological and biochemical characteristics of the yeast isolates were determined according to Kraepelin (1984) and Kurtzman (2011). To determine the physiological properties - assimilation / fermentation of various carbon sources and azote, we used classic lab techniques and quick identification kits - API tests. API tests allow the simultaneous highlighting of several biochemical characters, by associating several individual tests, which lead to the identification of a species with a high probability coefficient. The results obtained were compared with a reference strain of *Saccharomyces cerevisiae* Lalvin EC1118.

The colonies considered representative of a particular species or gender were isolated in pure culture and maintained on culture medium

YPD Agar and cryopreserved in glycerol 20% at -20°C.

RESULTS AND DISCUSSIONS

The shape and size of the cells have the peculiarities of growth depending on their morphological and physiological characteristics and can be observed under a microscope the size and shape of cells from young cultures in full activity in the standard liquid medium (Figure 1). Wet preparations in which the cells are suspended in a 0.1% agar solution are preferably used for the measurement, in order to avoid the movement of the cells. It is also noted the mode of vegetative propagation (budding, possibly splitting), the type of budding (polar, multipolar), the angle that the daughter cell makes with the mother cell giving different tree chains.

Colony characters were used for preliminary identification. Yeast strains produced different types of colonies on DRBCA medium such as raised, creamy white color colonies. After 48 hours of growth on YPGA medium our strains formed white colonies, elevated and convex with slightly different surfaces. Microphotographs of morphological diversity of isolates (Figure 2) and different colonies from different samples are shown in (Figure 3). Strains were observed for *Saccharomyces* characteristic oval cell shape and budding characters. Out of seven isolates, three isolates showed oval cell shape with budding character and classified as *Saccharomyces* yeast strain.

The yeast strains, *Saccharomyces cerevisiae* identificated after the macroscopic aspect, formed convex colonies with a glossy surface, cream-white colored with a diameter of 1-2 mm. Examined under a microscope, yeast cells were spherical or oval in shape, with diameters ranging from 5 to 20µm (Figure 1).

Following the results obtained at the macro and microscopic characterization of the isolated strains (Table 1) it turns out that the isolated yeast strains showed colonies with diameters between 2 and 4 mm, with lenticular or convex profile, having a circular perimeter with a creamy appearance, smooth and matte, and other colonies had a glossy surface, cream-white colored, with a diameter of 1-2 mm.

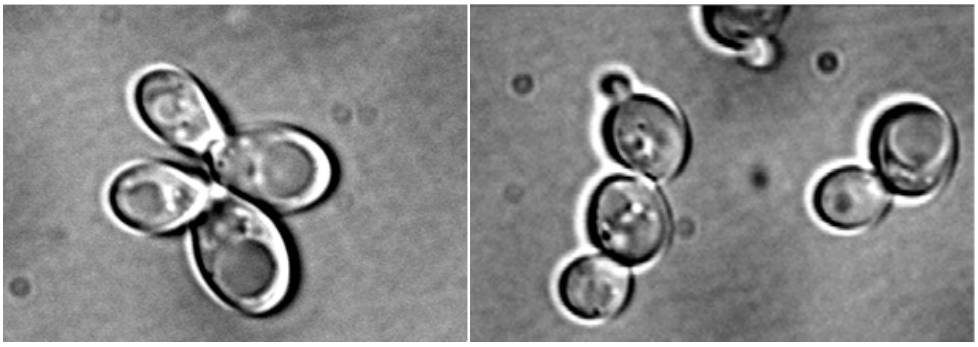

Figure 1. The shape of *Saccharomyces cerevisiae* cells in young cultures

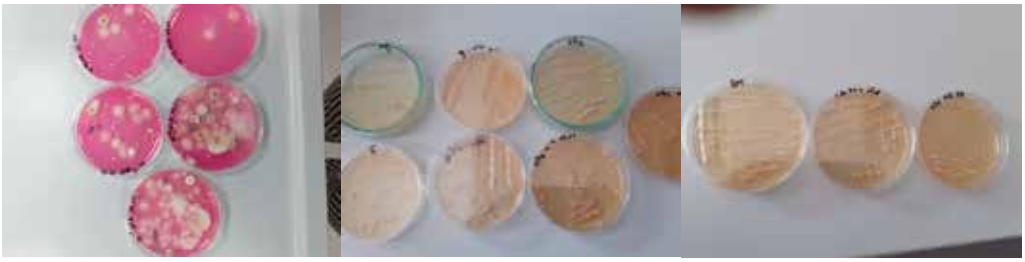

Figure 2. Morphological diversity of isolates

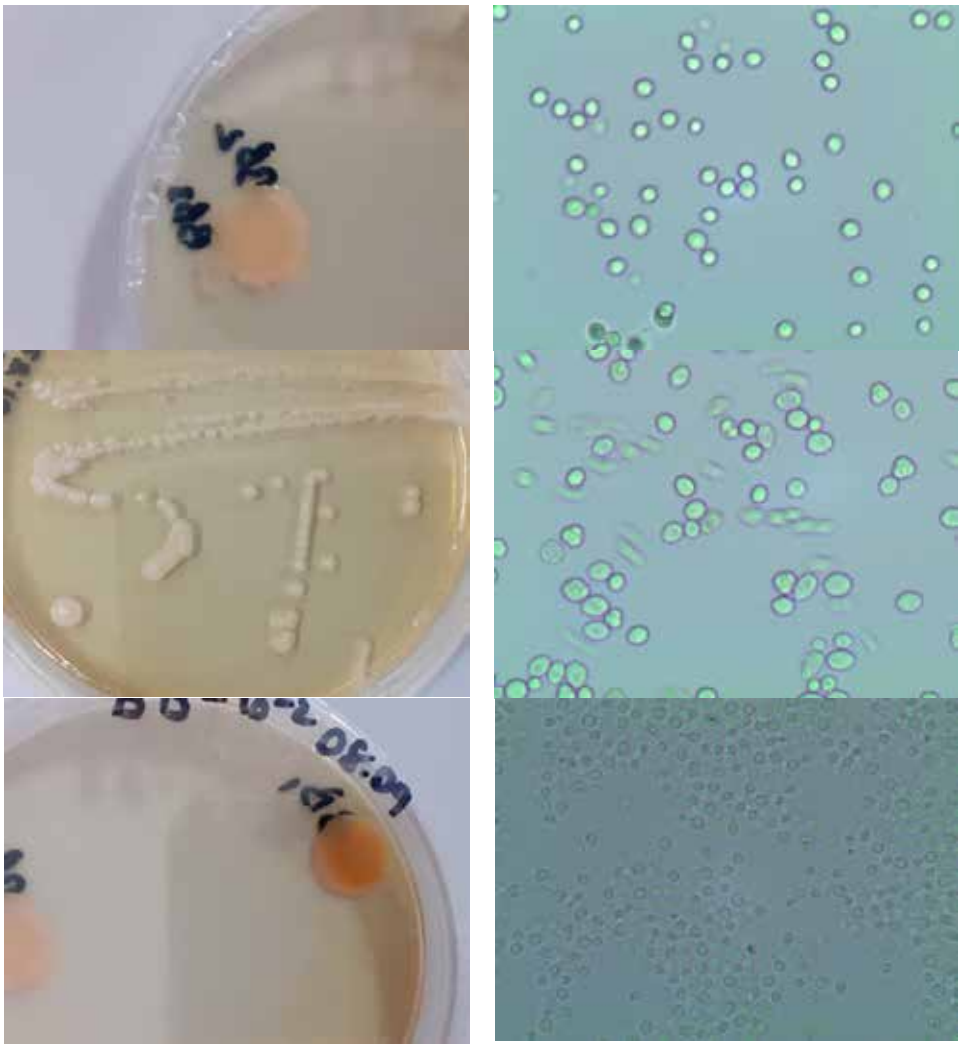

Figure 3. Aspect of the colonies and cells (40x) formed by isolated yeast strains

Table 1. Characteristics macro and microscopic of yeasts isolated from barley

Nr.crt.	Isolated strains (code)	Source	Character of the colony	Microscopic observations
1	OB1	Barley seeds	Smooth, glossy colonies with round edges	Small cells of round shape, isolated or grouped in pairs
2	OB2	Barley seeds	Irregular colonies, light cream-colored, smooth, buttery	Elliptical cells, isolated or grouped in small chains
3	OB3	Barley seeds	Umbonate colonies with scalloped edges, light cream-colored	Oval or elliptical cells, isolated or grouped in pairs
4	OB5	Barley bran	Umbonate colonies with scalloped edges, light cream-colored	Oval or elliptical cells, isolated or grouped in pairs
5	OB6.1	Barley bran	Colonies round, white to cream, glossy, smooth, buttery	Cells round to ovoid, isolated or grouped in pairs
6	OS1.1	Soil	Colonies round, white to cream, glossy, smooth, buttery	Cells round to ovoid, isolated or grouped in pairs
7	OS2	Soil	Smooth, glossy colonies with round edges	Cells round to ovoid, isolated or grouped in pairs

Table 2. Assimilation reactions/fermentation and other characteristics biochemical identification

Carbohydrates	Yeast strain						
	OB1	OB2	OB3	OB5	OB6.1	OS1.1	OS2
Gram reaction	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+
Galactose	+	+	-	+	+	+	+
Sucrose	+	+	+	-	+	+	-
Maltose	+	-	-	-	-	+	-
Lactose	+	-	-	+	v	-	+
Raffinose	+	+	-	-	-	+	+
Trehalose	-	+	+	-	+	+	-
Fructose	+	+	+	+	+	+	+
Arabinose	-	-	-	-	-	-	-
Urease	-	-	+	v	-	-	-

Code in table: +, positive=Present; −, negative=absent; v=variable

The fermentation capacity of the main sugars by the yeast strains isolated represents a stable character of the alcoholic yeasts. Following the tests, we found that there are differences between the yeast strains isolated regarding the nature of fermentable sugars and the ability of yeasts to ferment some monosaccharides, disaccharides and trisaccharide's.

All analyzed strains were found to ferment D-glucose, sucrose and fructose, except for the strains OB5 and OS2 which did not ferment the sucrose. No yeast fermented L- arabinose (the yeasts lack the proper enzymes), and D-galactose was not fermented by the yeast strain OB3. Maltose and raffinose were weakly fermented by isolated yeast strains (Table 2).

The colonies considered representative of a particular species or gender were isolated in pure culture and maintained on culture medium YPD Agar and cryopreserved in glycerol 20% at -20°C (Figure 4).

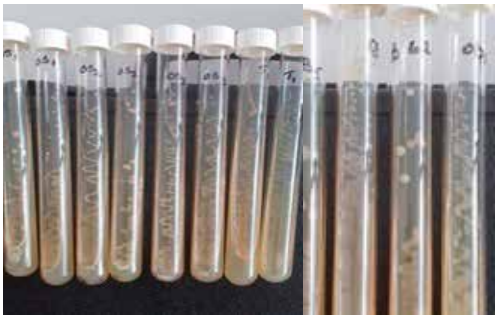

Figure 4. Yeast strains isolated in pure culture

Yeasts have the ability to assimilate urea, which is a good source especially if the culture medium contains biotin in sufficient quantity. Isolated yeast strains do not have the ability to hydrolyze high concentrations of urea in complex environments that contain peptone as the only source of nitrogen because they do not produce urease.

CONCLUSIONS

At this stage, we isolated and identified in the barley samples 7 strains of genera belonging to the classes: *Ascomycetes* (sporogenic yeasts with the species *Aureobasidium pullulans*), *Deuteromycetes* (imperfect fungi- non-sporogenic yeasts) and the genus *Candida* (with the species *Candida mycoderma*). Most of the yeasts identified turned out to be yeasts of the genus *Saccharomyces*, but a number of yeasts belonging to other gene have been recorded non-*Saccharomyces* (*Candida, Cryptococcus, Torulaspora, Metschnikowia pulcherrima, Pichia*), and a number of results were inconclusive. The *Saccharomyces* genus has the highest share in the analyzed microbiota with the species *Saccharomyces cerevisiae*, followed by non-*Saccharomyces* species, like: *Aureobasidium pullulans, Torulaspora delbruckiib* and *Metschnikowia pulcherrima*.

Based on the results obtained, it can be concluded that the highest incidence in the studied microflora is held by sporogenic species. Future research will focus on the molecular identification of selected yeasts and use of isolated strains of *Saccharomyces cerevisiae* mixed with *Metschnikowia pulcherrima* strains and other non-Saccharmomyces strains (like *Torulaspora*

delbruckii) to obtain biomass as a potential source for fish nutrients.

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