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The first description of brewing beer with marine yeasts

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Abstract: Most of the yeast species we encounter daily are terrestrial, but they are also found in the marine environment. Marine yeasts occur in estuaries, coastal and open marine waters, sediments, and as epi- and endobionts of various marine organisms. This study focuses on the fermentation capabilities of marine yeasts isolated from the central Adriatic Sea for use in the brewing process. Sampling was performed at a sea temperature of 12°C to avoid collecting yeasts introduced into the marine environment by human activities. Four nutrient media designed for yeast isolation and growth were used to isolate yeasts from the marine samples: Yeast Malt Agar (YM), Yeast Malt Agar with antibiotics, acidified Yeast Malt Agar, and Sabouraud Dextrose Agar. Sixteen yeast colonies were successfully isolated, and only three were positive in the fermentation test and were further determined to species level. Of the three species identified as capable of fermentation, *Candida famata* completely fermented the wort and produced a beer. *Candida pelliculosa* fermented slowly over an extended period and did not produce a usable beer, while *Candida sake* did not ferment the wort at all. To our knowledge, this is the first time that marine yeast isolates have been used experimentally for beer fermentation, indicating potential for use as non-conventional yeasts in brewing and beer production. Further investigations should include professional standard fermentation, aroma and genetic studies.
***Keywords*:** marine yeast; fermentation; beer; *Candida famata*; non-conventional yeasts; Adriatic Sea

Sažetak: PRVI OPIS PROIZVODNJE PIVA POMOĆU MORSKIH KVASACA. Većina kvasca s kojima se svakodnevno susrećemo su kopnene vrste, ali ih ima i u morskom okolišu. Morski kvasci nalaze se u estuarijima, obalnim i otvorenim morskim vodama, sedimentima te kao epi- i endobionti raznih morskih organizama. Ovaj rad je usmjeren na testiranje fermentacijske sposobnosti morskih kvasaca izoliranih iz uzoraka prikupljenih u srednjem Jadranu za potencijalnu upotrebu u procesu proizvodnje piva. Uzorkovanje je obavljeno pri temperaturi mora od 12°C kako bi se izbjeglo sakupljanje kvasaca unesenih u morski okoliš ljudskim aktivnostima. Za izolaciju kvasaca iz uzoraka mora korištena su četiri hranjiva medija namijenjena za izolaciju i rast kvasca: kvaščev sladni agar (YM), kvaščev sladni agar s antibioticima, zakiseljeni kvaščev sladni agar i dekstrozni agar Sabouraud. Uspješno je izolirano 16 kolonija kvasca, a samo su tri bile pozitivne u testu fermentacije te su dalje određene do razine vrste. Od tri vrste koje su pokazale potencijal za frementaciju, *Candida famata* potpuno je fermentirala sladovinu uz uspješnu proizvodnju testnog piva. *Candida pelliculosa* fermentirala je sporo tijekom duljeg vremenskog razdoblja i nije proizvela upotrebljivo pivo, dok *Candida sake* uopće nije fermentirala sladovinu. Prema našim saznanjima, ovo je prvi put da su izolati morskog kvasca eksperimentalno korišteni za fermentaciju piva, što ukazuje na potencijal za upotrebu nekonvencionalnog kvasca u pivarstvu i proizvodnji piva. Daljnja istraživanja trebala bi se usmjeriti na proces fermentacije u industrijskoj proizvodnji, aromatski profil piva i molekularnu identifikaciju kvasaca.
***Ključne riječi*:** morski kvasci; fermentacija; pivo; *Candida famata*; nekonvencionalni kvasci; Jadransko more

INTRODUCTION

Yeasts are ubiquitous eukaryotic unicellular microorganisms present in the marine environment. A distinction is made between obligate yeasts, i.e. yeasts that occur in the marine environment, and facultative yeasts, i.e. yeasts that can occur in both marine and terrestrial habitats (Kohlmeyer and Kohlmeyer, 1979; Burgaud *et al*., 2010; Kandasamy *et al*., 2012). The ecology of marine yeasts, especially obligate ones, has not been studied in detail, but it is known that they can be saprophytes, commensals, mutualists, or parasites that tolerate high salt concentrations and are capable of fermentation (Kandasamy *et al*., 2012; Kaewkrajay *et al*., 2020). They are found in estuaries, coastal and open sea water, sediments, on algae and plant surfaces, and various marine animals (Kutty and Philip, 2008). Several hundred to several thousand yeast cells may be present in one litre of nutrient-rich coastal seawater, whereas yeast abundance in oligotrophic open sea waters is only a few dozen cells (Fell, 2001; Krstulović and Šolić, 2006). Yeasts isolated from the marine environment belong to the genera *Candida*, *Cryptococcus*, *Debaryomyces*, *Pichia*, *Hansenula*, *Rhodotorula*, *Saccharomyces*, *Trichos-*

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poron, and *Torulopsis* although many species are not obligatory marine inhabitants (Kutty and Philip, 2008; Chen *et al*., 2009). The halotolerant ascomycetous yeast *Debaryomyces hansenii*, often referred to as its anamorph *Candida famata*, is considered ubiquitous in oceanic regions and other aquatic environments. However, in oligotrophic oceanic waters, basidiomycetes often make up the majority of the total yeast population. *Candida* species tend to be more abundant in coastal regions, but can also be found in oligotrophic areas, albeit in lower abundance (Kandasamy *et al*., 2012).

Yeast research has intensified over the past 40 years as its application in various industries has been recognized. To date, the fermentation potential of *Saccharomyces cerevisiae* has been fully explored, and the industry is searching for new yeast species that could enrich the brewing process with new flavours and properties (Geijer *et al*., 2022).

In addition to standard brewing yeasts, the fermentative capabilities of non-conventional yeast (NCY) are being studied as an alternative to impact the aroma and flavour of fermented beverages, and differentiate products with new profiles of composition (Gschaedler, 2017). The yeasts *Wickerhamomyces anomalus* and *Torulaspora delbruecki* are being explored to develop new flavours, especially in the wine and brewing industries (Basso *et al*., 2016). Several non-conventional yeasts such as *Galactomyces geothricium*, *Kazachstania zonata*, *Kluyveromyces lactis*, *Lindnera meyerae*, *Pichia kluyveri*, *Starmera caribaea*, *Yarowia lipolytica* and *Saccharomycodes ludwigii* are studied for their ability to produce pleasant flavours during the fermentation of wine, beer, and cider and have shown the best potential for further application (Gutiérrez *et al*., 2018). Careful use of NCY can significantly improve the visual characteristics and aroma of beer in addition to differences in flavour and alcohol content (Callejo *et al*., 2019a). *Candida famata* isolates from wine have shown lower fermentation abilities compared to conventional wine yeast, but are capable of producing satisfactory amounts of desirable organic acids and a greater amount of glycerol, which contributes to mouth-feel and sweetness (Mančić *et al*., 2021).

The use of NCY has increased lately as they have proven to be good workhorses in the fermentation industry (Geijer *et al*., 2022). The use of NCY can be beneficial for lowering exposure to biogenic amines, such as histamine or tyramine, commonly present in fermented foods. When combined with alcohol, biogenic amines can become a health problem for a consumer, and by using the *Debaryomyces hansenii* yeast, anamorph *C. famata* the biogenic amine formation can be decreased (Bäumlisberger *et al*., 2015). *Candida pelliculosa* is one of the main species found in grapes and can be used for attaining desirable aromas in wines and the toxins the species produces control the population of other microbes growing in wine during fermentation, including those responsible for the deterioration of wine (Padilla *et al*., 2018). Most *Candida* spp. have biotechnological potential and can be applied in agriculture, food, and chemical industries. *C. utilis* and *C. tropicalis* are used for single-celled protein production (Anupama and Ravindra, 2000), while *C. famata* is used for the bioproduction of riboflavin (Stahmann *et al*., 2000).

The brewing industry is dominated by large companies that have perfected the industrial production of beer, mostly based on *S. cerevisiae*. However, the new developments in brewing is mainly a focus of smaller microbreweries and home brewers willing to experiment with NCY to obtain new aromas and flavours (Gutiérrez *et al*., 2018; Callejo *et al*., 2019b). Yeasts isolated from the marine environment could offer a new fermentation potential and possibly lead to the discovery of products with new sensory attributes (Zaky *et al*., 2014; Bessadok *et al*., 2022). Blue biotechnology and the bioeconomy are in search of numerous groups of marine organisms that have often been ignored for commercial use, as their application can generate new knowledge, goods, and services (OECD, 2016). The main objective of this study was to test the concept of the use of marine yeasts in brewing purposes and to determine whether they can be used as NCY as new fermentation potential. In order to do that, marine yeasts have been isolated, identified to species level, and selected for beer production based on their fermentation potential.

MATERIALS AND METHODS

Isolation of marine yeast

Sea samples were collected on 26 January 2021 at coastal sampling station Kaštelet in Split (43° 30' 09.72" N, 16° 24' 51.48" E) at a depth of 30 cm. While sampling, pH, salinity and temperature were measured with YSI Pro 1030 probe (Yellow Springs, OH, USA).

Collected samples were filtered through 0.45 µm cellulose nitrate membrane filters (Sartorius, Germany) upon arrival at the laboratory.

In total, 20 series of 50 mL and 4 series of 100 mL were filtered to ensure maximum harvest of the yeast. Filters were placed on top of the nutrient medium and incubated for six days at 15°C. Four nutrient mediums were used: Yeast-Malt agar (YM) (10% glucose, 0.3% yeast extract, 0.3% malt extract, 0.5% peptone and 2% agar), acidified YM agar with pH adjusted to 5 with 0.1 N HCl (Kurtzman *et al*., 2011), YM agar with gentamicin in concentration of 50 mg/L and Sabouraud Dextrose Agar (SDA) (40% glucose, 5% casein, 5% meat peptone and 1.5% agar). After incubation, the yeast-like colonies were picked out and re-inoculated on YM agar.

Characterisation and identification of the isolates

To determine the morphological characteristics and size of the isolated yeast cells, colony from each culture was examined under a microscope (Olympus BX50, 1000x magnification). Confirmed yeast cultures

were tested for their ability to perform fermentation. This was done in medium composed of 2% glucose, 2% peptone, and 1% yeast extract and using Durham tubes to collect the gas. Fermentation tests were incubated at temperatures of 15°C, 20°C, and 25°C and performed in duplicate. Isolated fermentative species were identified to species level in triplicate using the API® ID 32 C assay (bioMérieux, Marcy l'Etoile, France) according to the manufacturer's instructions.

Two nutrient broths were used to prepare selected fermentative yeast cultures for brewing. The first, consisting of 1% yeast extract, 2% peptone water, and 2% glucose, was used to resuscitate yeast cultures from a slant. The second nutrient broth consisted of 1% yeast extract, 1% peptone water and 10% malt extract and was used to cultivate a large number of yeasts needed for fermentation of beer wort. After cultivation, the yeast was sedimented by centrifugation at 4000 rpm for 5 minutes at 20°C. The supernatant was removed and the sedimented yeast cells were used for the wort fermentation.

Fermentative performance of the isolated yeasts

Selected fermentative marine yeast species were used to ferment four litres of wort alongside two control fermentations with *S. cerevisiae* (Fermoale AY4, AEB Group). Fermentation was performed in duplicate for each yeast in sterilised 5-litre glass fermenters.

The wort for the experiment was brewed using the All Grain Brew in a Bag (BIAB) method, in which the grains are placed in a bag that is submerged during the mashing process. The grain bill for approximately 20 litres consisted of 4 kg of Pale Ale malt IREX and 50 g of Weyermann CaraAroma Malt. The mashing time was 60 minutes at a temperature of 67°C. Following mashing, the bag is simply removed and squeezed to drain the residual wort. The wort was boiled for 60 minutes to sterilize and hop additions. For bittering and flavouring, 30 g of the Styrian Aurora hop strain was added, which yielded 20 international bittering units (IBU). The wort was cooled to room temperature, then the suspension of isolated yeasts was added. The glass fermenters were stored in a dark room at a temperature of 20°C for 14 days. After completion of fermentation, the fermenters were transferred to a cold room for cold crash at 4°C for 3 days. The beer was bottled and stored at room temperature for 14 days.

The blond-style ale fermented with *S. cerevisiae* (Fermoale AY4, AEB Group) was used as a fermentation control. The parameters of the control fermentation served as a reference for comparing the fermentation capabilities of the isolated yeast species. The fermentation of wort was performed twice for all isolates and repeated a third time for the isolate that successfully fermented the wort. The parameters observed were the duration of fermentation and the original and final gravity of the beer. The alcohol volume (ABV) content was estimated as the difference of original gravity (OG) and final gravity (FG) multiplied by factor 131.25 (ABV=(OG-FG)×131.25) (Bamforth, 2016).

Sensory analysis

The sensory analysis of beers fermented with *S. cerevisiae* and *C. famata* was performed after two weeks of ageing. The samples were served in tulip-shaped glasses cooled to 16°C. To wash off the aftertaste of the previous sample between tastings, the assessors drank water and ate unsalted crackers. Experienced technologists from the local brewery (n=5, 3 men, 2 women) evaluated the sensory characteristics of the beer using descriptive analysis. The sensory attributes monitored were colour, aroma (malt, hops, bread, citrus, fruit), taste (sweet, bitter, and sour), and mouthfeel (body, astringency). Each assessor rated the intensity of the descriptors on a five-point scale (0 = imperceptible, 1 = very weak, 3 = weak, 5 = medium, 7 = intense, and 9 = very intense). The averaged results for each beer were plotted on a radar chart.

RESULTS

At sampling point the physical sea parameters were measured as follows: pH was 8.11, salinity 33.30 and the sea temperature 12°C. To avoid accidental collection of potential opportunistic pathogens, the sample was collected at the time when low sea surface temperature was expected.

A volume of 1000 mL was sufficient to isolate several yeast species from the coastal sea by membrane filtration. However, a simple and rapid isolation procedure is hindered by heterotrophic bacteria, which are more abundant in the marine environment and grow faster than yeast cells. To circumvent this problem, a Wickerham's Yeast Malt agar, Yeast Malt agar plus gentamicin, acidified Yeast Malt agar, and Sabouraud Dextrose agar (SDA) were used. Also, the best volume for morphological identification of yeast colonies per 45 µm filter was 50 mL, as larger volumes yielded more bacterial colonies.

To reduce the stress on marine yeasts and inhibit the growth of some potentially pathogenic and opportunistic terrestrial yeast species, incubation was performed close to the *in-situ* temperature. The best medium for isolation of marine yeasts proved to be YM with the addition of gentamicin. Bacterial growth was significantly reduced, which allowed better growth of marine yeasts.

Visual inspection of the morphology of the grown yeast colonies revealed different sizes, shapes, and colours. The colonies that were rounded and elevated with straight edges and whitish were considered for further analyses. Besides the colour, selective criteria were shape of the cells (round or elongated ovals with straight edges) and presence of the visible buds under the microscope (Fig. 1). Out of 24 yeast-like colonies isolated, 16 were determined to be yeasts by further morphological examination of the cells under the microscope.

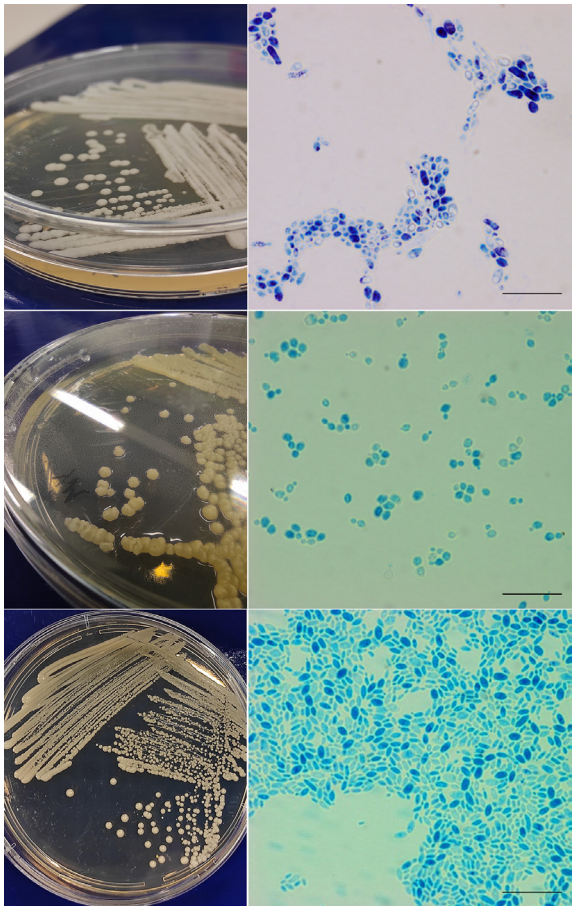

Fig. 1. Photographs of purified colonies on YM agar and microscope images of isolated marine yeasts with the ability to ferment. Upper panel: *Candida pelliculosa*; middle panel: *C. famata;* bottom panel: *C. sake*. Microscope images are magnified x1000 and bars represent 20 µm.

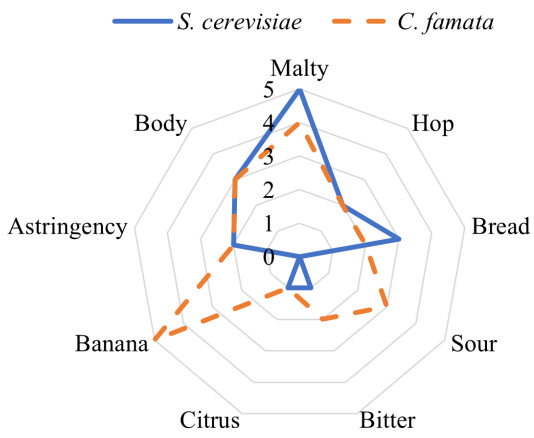

Fig. 2. Quantitative descriptive sensory analysis of beers fermented with *Saccharomyces cerevisiae* and *Candida famata*. The beer scores represent the mean values of the attributes evaluated for each yeast strain (n=5). Data from the sensory analysis performed by the experienced technologists at the local brewery.

The fermentation test was performed with the isolated yeast cultures, using *S. cerevisiae* (Dr. Oetker) as a control. Higher fermentation test temperatures have been used to determine if marine yeasts are capable of fermenting wort in a time period required to produce blond-style beer. Three of sixteen yeast cultures isolated from the marine environment had a positive fermentation result with the gas collected in the Durham tubes.

To determine the species of the isolated yeast colonies, an API 32C assay was performed after 24 and 48 hours, as instructed by manufacturer. The tests were performed in triplicate. The yeasts selected for the fermentative potential were identified as *C. pelliculosa*, *C. famata* and *C. sake*. The test results are shown in Table 1.

The microscope images of the identified marine yeast isolates are shown in Fig. 1. The microscopic measurements showed that *C. sake* had the smallest cell size, while *C. pelliculosa* had the largest. The average cell sizes with standard deviation of the isolated yeasts are shown in Table 2.

The 6.25 g of *C. pelliculosa*, 5.37 g of *C. sake* and 6.05 g of *C. famata* were collected and stored overnight at 4°C prior to the brewing process. The fermentation inoculum of the selected yeasts contained 4.6 x 10^7 cells g^{-1}.

The fermentation was carried out in a closed room where the ambient temperature varied significantly (from 7°C to 21°C). Measurements of parameters such as temperature, pH, and gravity during the fermentation were not performed due to bacterial contamination of the fermenters witnessed in preliminary studies.

The original gravity of the wort used in this experiment was 1.044. The control fermentation lasted 14 days and resulted in a beer with a final gravity of 1.010 and an alcohol content by volume of 4.46% (ABV). Of the three yeasts isolated, *C. famata* fermented the wort in 14

Table 1. Isolated yeast species determined by API ID 32C test after 48 and 72 hours.

Sample	Incubation	ID 32C	Species I	ID%	Species II	ID%
K1	48h	4264351311	*C. pelliculosa*	99.9	*C. utilis*	0.1
K3	48h	5777151313	*C. famata*	99.9	*C. membranifaciens*	0.1
B1	72h	7143350117	*C. sake*	99.6	*C. parapsilosis*	0.2

Table 2. Cell size and width with SD of marine yeast isolates tested positive on the fermentation test

Yeast	*C. pelliculosa*	*C. famata*	*C. sake*
Length (µm)	5.65 ± 1.19	4.06 ± 0.45	3.95 ± 0.33
Width (µm)	2.88 ± 0.27	3.37 ± 0.56	2.22 ± 0.20

days, similar to the control yeast, and produced a beer with a final gravity of 1.010 and 4.46% ABV. Despite constant activity in the fermenter, *C. pelliculosa* failed to fully ferment the wort within the expected time frame. After 52 days of fermentation, the relative density of the wort was 1.035, which excluded this yeast from further study. Due to its insufficient fermentation activity, the *C. sake* isolate was also excluded from further use in beer production.

Beer fermented with *C. famata* was bottled and aged for two weeks at room temperature. During aging, the beer partially carbonated. The beer corresponded to the experimental beer style 34C according to the Beer Judge Certification Program (BJCP), which includes all beers that cannot be classified in any other category already defined (Strong and England, 2015).

Sensory analysis by means of sensory description and sensory panel was not used, since the aim of the study was to proof the concept of using marine yeast for beer fermentation. However, the technologists at the local brewery, who have great experience in beer production, described the sensory characteristics of the beer after two weeks the of aging process (Fig. 2).

They found the aroma, appearance, taste, mouthfeel and overall impression of the beer to be unique. Aromas of clove and fruit were sensed, indicating possible increased phenol and ester production by the yeast. The acidity indicated a higher lactic acid and acetic acid production than the control sample fermented with *S. cerevisiae* (Fermoale AY4, AEB Group). The results of the fermentation experiment with sensory description are summarised in Table 3.

DISCUSSION

There is little information in the recent scientific literature on the potential practical use and application of marine yeast in biotechnological processes, Adriatic Sea yeasts not being an exception. The number of yeast cells per litre in the Adriatic ranges from a few hundred to a few thousand in coastal waters and from a few dozen to a few hundred in oligotrophic open waters (Krstulović and Šolić, 2006), making it a respectable source of unexplored biotechnological potential. Marine yeasts of various genera have been tested for bioethanol production, the production of various pharmaceutical products, and for the production of industrial enzymes (Zaky *et al*., 2014; Chi *et al*., 2016; Zaky *et al*., 2020). To our knowledge, this is the first attempt to describe a new concept of fermenting beer using marine yeasts.

Improvements in isolation and cultivation protocols have allowed the discovery of new yeasts from the marine environment. Media containing antibiotics have been shown to reduce bacterial growth, often making it difficult to isolate yeasts easily and effectively. Although all media used in this study have been previously recommended as suitable for isolation of yeasts from the marine environment (Zaky *et al*., 2014), YM agar containing the antibiotic gentamicin proved to be the most suitable medium for the sample from the Adriatic coastal sea, as bacterial growth was significantly reduced.

The API® ID 32 C test was used to identify the isolated and microscopically inspected yeast colonies to species level as it is a rapid identification method (Szabó *et al*., 2008). To ensure consistency of results, each selected colony was tested in triplicate, and the yeasts with fermentative capabilities were identified as *C. sake*, *C. famata*, and *C. pelliculosa*. The genus *Candida* is often present in the marine environment, and only 12 species have been described to date: *C. aaseri*, *C. albicans*, *C.*

Table 3. The starting (OG) and final gravity (FG) of the wort with fermentation time in days, alcohol volume (ABV) and sensory description of the beer fermented with isolated and control yeasts. N.D. means not determined.

Yeast isolate	OG	FG	Fermentation (days)	ABV* (%)	Sensory description
S. cerevisiae	1.044	1.010	14	4.46	Colour: gold with low white head. Aroma: moderate malty with low hop. Flavour: low malty characters with bready notes; low bitterness and hop flavour. Mouthfeel: light body with low carbonation.
C. famata	1.044	1.010	14	4.46	Colour: gold with low white head Aroma: moderate malty with sour and banana notes. Flavour: low malty characters, sour and banana flavours; low bitterness and hop flavour. Mouthfeel: light body with low carbonation.
C. pelliculosa	1.044	1.035	52	N.D.	N.D.
C. sake	1.044	N.D.	N.D.	N.D.	N.D.

atlantica, *C. haemulonii*, *C. intermedia*, *C. maris*, *C. maritima*, *C. norvegica*, *C. sake*, *C. torresii*, *C. tropicalis*, and *C. zeylanoides* (Kutty and Philip, 2008). Unlike *C. sake*, *C. famata* and *C. pelliculosa* are considered terrestrial yeasts, but it is possible to isolate them from the marine environment (Libkind *et al*., 2017). Furthermore, *C. famata* is regularly found in coastal waters and sediments, confirming its natural occurrence in the marine environment (de Araujo *et al*., 1995; Bahri *et al*., 2010). It is also described as an anamorph of the yeast species *D. hansenii*, a cryotolerant, halophilic, nonpathogenic and one of the most widespread yeast species in the marine environment (Lépingle *et al*., 2000; Voronovsky *et al*., 2004; Yaguchi *et al*., 2017). It cannot be dismissed that this yeast species was introduced from land, since the sample was collected only one meter from the coast (Kutty and Philip, 2008). However, to minimize the possible influence of bathers and accidental contamination, the sample was collected in sterile glass bottles in January, when sea temperature was low and human activity minimal, and the fermentation test was performed at lower temperatures.

Although it is reported that *C. pelliculosa* can tolerate up to 12.5% alcohol volume (Padilla *et al*., 2018), this isolate did not ferment beer in the set-up conditions. The reason for this could be the large variation in room temperature where fermentation took place. However, with better temperature control, the *C. pelliculosa* isolate might also be a good non-conventional yeast for beer production. This concept needs to be further investigated. When compared to commercial brewer yeasts (Turgeon *et al*., 2021), the fermentation capabilities of *C. famata* from this study, required similar time.

Although terrestrial variant of *C. famata* is recognized as the cause of 1.3% of candidiasis in humans (Bahri *et al*., 2010), the incidence is rare and it is often misidentified as a causative agent due to frequent errors in identification (Desnos-Ollivier *et al*., 2008; Beyda *et al*., 2012; Castanheira *et al*., 2013; Kim *et al*., 2015; Kumar *et al*., 2022). Furthermore, *C. famata* obtained from the marine environment has undergone reclassification as *D. hansenii*, characterized as a nonpathogenic marine yeast with optimal growth at 25°C, thus elucidating its limited pathogenic potential (Kurtzman and Robnett, 2013; Al-Qaysi *et al*., 2017; Yaguchi *et al*., 2017). However, commercial application of this yeast isolate requires further analysis to specify potential toxicity and health effects, fermentation performance and by-product composition.

Nevertheless, *C. famata* has been previously studied for riboflavin synthesis and ethanol production, although some genetic modifications were required (Voronovsky *et al*., 2004; Senthilraja *et al*., 2011; Zaky *et al*., 2014). The fermentative potential of various strains of *C. famata* isolated from wild and cultivated blueberries for winemaking was extensively studied by Mančić *et al*. (2021). According to their study, this yeast had good fermentation potential at low alcohol levels. Resistance of *C. famata* to 5% ABV ranged from 80% to 90% at, while at 9% ABV the resistance decreased to below 37%. The stress caused by high alcohol could lead to weaker yeast growth, fermentation or formation of undesirable by-products. *C. famata* isolate used in our study was not subjected to much stress during fermentation of beer since the final alcohol volume was 4.46% ABV.

Preliminary sensory evaluation of the beer produced with *C. famata* showed that the sour aroma was present, although the precise acid concentrations was not determined. The similar aromas were not detected in the control blond-ale style beer fermented with *S. cerevisiae* (Fermoale AY4, AEB Group). Mančić *et al.* (2021) reported higher concentrations of lactic and acetic acids in wine fermented with a pure *C. famata* culture than in a control wine sample fermented with *S. cerevisiae*. It should be noted, however, that the results of fermentation of wine and beer cannot be compared, since the production of wine with added yeast also involves microorganisms living on the grapes, whereas the production of beer usually relies on a pure yeast cultures without the influence of other microorganisms (Gutiérrez *et al*., 2018).

Our results suggest that marine yeast isolates have potential for use in wort fermentation and beer production. However, this study is just the beginning of a biotechnological potential of marine yeast in the fermentation process that needs to be explored more thoroughly. Further improvements in the fermentation process and other technological and safety features, as well as genetic identification, should be investigated.

CONCLUSIONS

Marine yeasts from the Adriatic coastal sea were successfully isolated and cultured on YM agar with gentamicin. Following the fermentation test, three isolates - *C. famata*, *C. pelliculosa*, and *C. sake* - were identified. *Candida famata* demonstrated complete fermentation of the wort while *C. pelliculosa* exhibited partial fermentation. *C. sake* did not ferment the wort under the given conditions. Notably, beer fermented with *C. famata* met sensory expectations within the parameters defined by the BJCP, indicating the potential commercial utility of marine yeast in the brewing industry.

This study marks the pioneering use of marine yeast isolates in experimental beer fermentation. The results of this study contribute to a broader understanding of the potential application of marine yeasts in blue biotechnology. Further studies should adopt a more comprehensive approach, including analysis to specify potential toxicity and health effects, genetic profiling of the isolates, investigation of physico-chemical properties of the fermented beer, and more detailed sensory analysis. In addition, the technical features of fermentation should be further investigated to realize the full brewing potential of marine yeast isolates.

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