Petri dish method to select yeasts able to produce more pigmented table olives

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ABSTRACT. The study of pigment adsorption of yeasts used for table olive fermentation may allow the protection of olive colour, by excluding those strains adsorbing phenolic compounds responsible for the colour. Fifty-one table olive yeasts were grown on Petri dishes using two olive-based screening media - 'olive pulp agar' and 'olive seed agar'; the red, green, and blue colour components of the yeast’s biomass were measured. Wide and significant differences among the yeasts were observed. Based on the statistical analysis, ten yeasts were selected, excluding all the strains exhibiting a too high pigment adsorption. The research proposes a simple analytical method to characterize yeasts for their pigment adsorption, thus allowing the enhancement of the table olive colour. The two media may be prepared using any olive cultivar, thus allowing a specific screening of the yeasts. The selection of those yeasts unable to adsorb olive pigments may allow the production of more pigmented table olives.

Keywords: fermentation; pigment adsorption; screening media; selection; table olives; yeasts.

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Introduction

Table olives have been a component of the Mediterranean diet for centuries, and their consumption is currently increasing worldwide; they are rich in bioactive molecules with nutritional, antioxidant, anti-inflammatory or hormone-like properties (Durante et al., 2018).

In order to improve this fermented product, different approaches were carried out.

One approach considered olive cultivars and their chemical characteristics, in order to implement antioxidant content, fatty acid and sugar profiles that are influenced by cultivar and processing (Issaoui et al., 2011).

Another approach considered autochthonous microflora; this was oriented towards the knowledge of the evolution of the microorganisms during table olive fermentation, according to the production technology used (Valenčič et al., 2010). Thus, lactic acid bacteria were selected to identify starter cultures able to control table olive production giving microbiological stability and prolonged shelf life (Alfonzo et al., 2018).

More recently, many studies on yeasts associated with table olives were carried out to identify adjunct cultures able to positively interact with lactic acid bacteria (Tufariello et al., 2019). Thus, the identification of the yeasts associated with table olives was carried out (Muccilli, Caggia, Randazzo, & Restuccia, 2011; Tofalo, Schirone, Perpetuini, Suzzi, & Corsetti, 2012).

A zymogram screening for certain technological characteristics, such as cellulase, polygalacturonase, β-glucosidase, peroxidase, lipase/esterase, glucanase, protease, polysaccharolytic (pectolytic and xylanolytic) activities can aid yeast selection (Bevilacqua, Beneduce, Sinigaglia, & Corbo, 2013; Tofalo, Perpetuini, Schirone, Suzzi, & Corsetti, 2013; Bonatsou, Benítez, Rodríguez-Gómez, Panagou, & Arroyo-López, 2015).

Obviously, in all the selection protocols it is essential to exclude all the harmful yeasts (Arroyo-López et al., 2012): a) the fermentative strains performing a vigorous production of gas (CO2) that may penetrate olives and damage the fruits, producing 'fish-eye' spoilage; b) the polysaccharolytic (pectolytic and xylanolytic) strains, that cause the degradation of the polysaccharides of the olive fruit cell wall; c) the strains possessing polygalacturonase activity, that can grow and form pellicles in olive brines, thus causing a softening of olives kept in storage; d) the strains affecting sensory attributes of table olives.

Recently, the colour shelf life of table olives was studied (Sánchez, López- López, Beato, Castro, & Montaño, 2017); at present no author has proposed the screening of olive yeasts for their ability to interact - in negative or positive way - with olive colour.
The aim of this research was to propose a new approach to select yeasts for table olive fermentation by studying their pigment adsorption activity by: a) growing yeasts in Petri dishes on two olive-based media, b) photographing the yeast biomass, c) measuring its red, green, and blue colour components, and d) performing the statistical analysis of the data. This approach would identify yeast strains able to produce more pigmented table olives.

Material and methods

A total of 51 different yeast strains - isolated from 18 samples of olive pulps and brines during spontaneous fermentation of Calabrian table olives - were used. Sample number, cultivar, kind - brine or olive pulp - and pH, and strain number, morphology, film forming ability and gas production are reported in Table 1.

The table olive varieties used were the following six: Carolea, Geracese, Nocellara, Ottobratica, Roggianella, and Sinopolese.

The proposed screening media ‘olive pulp agar’ and ‘olive seed agar’ were respectively based on homogenized olive pulps and homogenized olive seeds; they are designed to study the yeast parietal adsorption activity, similar to an existing chromogenic grape-skin-based medium (Caridi, 2013). To prepare the two media, 200 g of homogenized olive pulp or olive seed were suspended in 1 litre of distilled water, treated at 110°C for 5 min. to extract olive pigments, and filtered through gauze. The volume of the filtered extract was measured using a graduate cylinder and the corresponding amount of the following ingredients was added: citric acid monohydrate 100, disodium hydrogen phosphate 50, dextrose 40, casein peptone 15, and yeast extract 9 g L⁻¹. The solution was divided into test tubes (5 mL per tube) and heated at 110°C for 5 min. Agar 40 g L⁻¹ was dissolved in distilled water, divided into test tubes (5 mL per tube) and sterilized by autoclaving at 121°C for 15 min. Then, one test tube containing the medium and one containing the agar solution, both maintained at 50°C in a water bath, were poured together in Petri dishes (60×15 mm). After careful mixing with a sterile L-shaped plastic spreader, the medium was allowed to solidify. The yeast strains - pre-cultured in YPD agar for two days at 25°C - were inoculated in the Petri dishes containing the two media by spreading over the surface using a sterile L-shaped plastic spreader. After 10 days of incubation at 25°C, the biomass was carefully mixed and spread on a sterile loop to prepare a flat surface to be photographed.

The colour assessment was performed on the photographs of the yeast biomass spread on the loop, measuring their red, green, and blue components by Adobe Photoshop CS for Windows XP (Adobe Systems, Inc., San Jose, CA, USA). The region of interest of the photo was set to 5×5 pixels taking four replicates for each strain. Photoshop’s red-green-blue colour mode assigns an intensity value to each region. In a colour image, the intensity values ranges from zero (black) to 255 (white) for each of the red, green, and blue components. Accordingly, low olive pigment adsorption matched higher values of the red, green, and blue components; conversely, high olive pigment adsorption matched lower values. This is because strains with high adsorption activity have a more coloured biomass than strains with low adsorption activity.

All the analyses were performed in duplicate; data were subjected to statistical analysis using Stat Graphics Centurion XVI for Windows XP (Stat Point Technologies, Inc., Warrenton, VA, USA) according to Fisher’s LSD (Least Significant Difference) (p < 0.05).

Results and discussion

Table 2-4 report the strain and sample number, the biomass colour for the red (Table 2), green (Table 3), and blue (Table 4) components of the yeast biomass grown on olive pulp agar, as measured using Photoshop, and the distribution in homogeneous groups (p < 0.05) given by statistical analysis.

Regarding the red component (Table 2), the yeasts were distributed in 20 homogeneous groups showing a mean value of 138, with a minimum of 105 and a maximum of 164. The behaviour of 28 out the 51 strains is judged to be negative since they exhibit values inferior or equal to the mean; this indicates that their pigment adsorption activity is higher than the average level. However, since the red pigment is not always considered essential by producers or consumers, these strains have not been excluded.

Regarding the green component (Table 3), the yeasts were distributed in 23 homogeneous groups showing a mean value of 121, with a minimum of 85 and a maximum of 163. The behaviour of 28 out the 51
strains is judged to be extremely negative, since they exhibit values inferior or equal to the mean. This characteristic severely excludes their use as adjunct culture, particularly to produce green olives.

Regarding the blue component (Table 4), the yeasts were distributed in 28 homogeneous groups showing a mean value of 104, with a minimum of 70 and a maximum of 155. The behaviour of 29 out the 51 strains is judged to be negative. However, similar to the red pigment, blue pigment is not always considered essential so these strains have not been excluded.

Table 5-7 report the strain and sample number, the biomass colour for the red (Table 5), green (Table 6), and blue (Table 7) components of the yeast biomass grown on olive seed agar, as measured using Photoshop, and the distribution in homogeneous groups (p < 0.05) given by statistical analysis.

**Table 1.** Sample number, cultivar, kind - brine or olive pulp - and pH of all 18 samples; strain number, morphology, film forming ability and gas production of all 51 yeast strains.

| Sample | Strain |
|--------|--------|
| Number | Cultivar | Kind | pH | Number | Morphology | Film | Gas |
| I      | Geracee | Brine | 3.76 | L852 | Elliptic | + | + |
| I      | Geracee | olive pulp | 4.26 | L844 | Elliptic | + | + |
| II     | Geracee | Brine | 3.82 | L845 | Elliptic | - | + |
| II     | Geracee | olive pulp | 4.27 | L835 | Filamentous | + | - |
| II     | Geracee | olive pulp | 4.27 | L834 | Elliptic | - | + |
| III    | Carolea | Brine | 3.98 | L854 | Elliptic | + | + |
| III    | Carolea | olive pulp | 4.42 | L835 | Filamentous | + | - |
| III    | Carolea | olive pulp | 4.42 | L836 | Elliptic | + | + |
| IV     | Geracee | Brine | 3.66 | L861 | Filamentous | + | - |
| IV     | Geracee | olive pulp | 4.19 | L877 | Elliptic | + | - |
| IV     | Geracee | olive pulp | 4.19 | L880 | Filamentous | + | - |
| V      | Carolea | Brine | 3.86 | L859 | Filamentous | + | - |
| V      | Carolea | olive pulp | 4.43 | L881 | Elliptic | + | - |
| V      | Carolea | olive pulp | 4.43 | L885 | Filamentous | + | - |
| VI     | Geracee | Brine | 3.81 | L864 | Filamentous | + | - |
| VI     | Geracee | olive pulp | 4.25 | L886 | Filamentous | + | - |
| VII    | Nocellara | Brine | 4.18 | L865 | Filamentous | + | - |
| VII    | Nocellara | olive pulp | 4.73 | L888 | Filamentous | + | - |
| VII    | Nocellara | olive pulp | 4.73 | L891 | Filamentous | + | - |
| VIII   | Geracee | Brine | 3.87 | L857 | Elliptic | + | + |
| VIII   | Geracee | olive pulp | 4.38 | L892 | Elliptic | + | + |
| VIII   | Geracee | olive pulp | 4.38 | L895 | Elliptic | + | + |
| IX     | Carolea | Brine | 3.47 | L867 | Filamentous | + | - |
| IX     | Carolea | olive pulp | 4.00 | L894 | Filamentous | + | - |
| IX     | Carolea | olive pulp | 4.00 | L895 | Filamentous | + | - |
| X      | Carolea | Brine | 3.76 | L870 | Filamentous | + | - |
| X      | Carolea | olive pulp | 4.34 | L898 | Filamentous | + | - |
| XI     | Carolea | Brine | 3.71 | L915 | Filamentous | + | - |
| XI     | Carolea | Brine | 3.71 | L916 | Filamentous | + | - |
| XI     | Carolea | olive pulp | 4.28 | L914 | Filamentous | + | - |
| XII    | Carolea | Brine | 3.66 | L871 | Filamentous | + | - |
| XII    | Carolea | Brine | 3.66 | L871 | Filamentous | + | - |
| XII    | Carolea | olive pulp | 4.09 | L900 | Elliptic | + | - |
| XII    | Carolea | olive pulp | 4.09 | L902 | Filamentous | + | - |
| XIII   | Carolea | Brine | 3.57 | L873 | Filamentous | + | - |
| XIII   | Carolea | Brine | 3.57 | L874 | Filamentous | + | - |
| XIII   | Carolea | olive pulp | 4.19 | L904 | Elliptic | + | - |
| XIII   | Carolea | olive pulp | 4.19 | L905 | Elliptic | + | - |
| XIV    | Nocellara | Brine | 4.48 | L875 | Filamentous | + | - |
| XIV    | Nocellara | olive pulp | 4.97 | L908 | Filamentous | + | - |
| XIV    | Nocellara | olive pulp | 4.97 | L909 | Filamentous | + | - |
| XV     | Ottobratica | Brine | 4.23 | L913 | Filamentous | + | - |
| XVI    | Roggiannella | Brine | 5.77 | L839 | Filamentous | + | + |
| XVI    | Roggiannella | olive pulp | 6.22 | L837 | Filamentous | + | + |
| XVI    | Roggiannella | olive pulp | 6.22 | L847 | Filamentous | + | + |
| XVII   | Sinopolese | Brine | 4.34 | L856 | Filamentous | + | + |
| XVII   | Sinopolese | olive pulp | 4.80 | L840 | Elliptic | - | + |
| XVII   | Sinopolese | olive pulp | 4.80 | L841 | Elliptic | + | - |
| XVII   | Sinopolese | olive pulp | 4.80 | L848 | Elliptic | + | - |
| XVIII  | Carolea | Brine | 5.21 | L850 | Filamentous | + | - |
| XVIII  | Carolea | olive pulp | 5.75 | L842 | Filamentous | + | + |
| XVIII  | Carolea | olive pulp | 5.75 | L849 | Filamentous | + | - |
Table 2. Strain and sample number, value of the red component of the biomass colour on olive pulp agar, as measured using Photoshop, and distribution in homogeneous groups (p < 0.05) given by statistical analysis; the values inferior or equal to the mean are in italics.

| Strain and sample | Red component | Homogeneous groups |
|-------------------|---------------|-------------------|
| L841-XVII        | 105.50        | a                 |
| L888-VII         | 110.25        | a                 |
| L849-XVIII       | 122.75        | b                 |
| L848-XVII        | 125.25        | b                 |
| L850-XVIII       | 124.75        | bc                |
| L891-VII         | 127.00        | bcd               |
| L916-XI          | 127.00        | bcd               |
| L832-I           | 127.75        | bcd               |
| L842-XVIII       | 127.75        | bcde              |
| L885-V           | 128.50        | bcde              |
| L859-V           | 128.75        | bcde              |
| L881-V           | 129.25        | bcde              |
| L839-XVI         | 129.25        | bcde              |
| L833-II          | 130.00        | bcdef             |
| L836-III         | 130.25        | bcdef             |
| L835-III         | 130.50        | bcdef             |
| L905-XIII        | 131.25        | bcdefgh           |
| L864-VI          | 132.00        | bcdefgh           |
| L877-IV          | 132.25        | bcdefghi          |
| L847-XVI         | 135.75        | cdefghij          |
| L886-VI          | 134.00        | cdefghij          |
| L844-I           | 154.00        | cdefghij          |
| L908-XIV         | 154.50        | cdefghijk         |
| L893-VIII        | 154.75        | defghijk          |
| L892-VIII        | 157.00        | efghijk           |
| L856-XVII        | 157.75        | fghijkl           |
| L865-VII         | 158.00        | fghijkl           |
| L871-XII         | 158.25        | fghijkl           |
| L857-VIII        | 159.25        | ghijklm           |
| L854-III         | 141.00        | hijklmn           |
| L861-IV          | 141.25        | ijklnn            |
| L894-IX          | 142.50        | jklmno            |
| L875-XIV         | 142.50        | jklmno            |
| L857-XVI         | 143.50        | jklmnop           |
| L867-IX          | 144.00        | klmnop            |
| L902-XII         | 144.25        | klmnop            |
| L880-IV          | 145.75        | lmnpq             |
| L914-XI          | 146.75        | lmnpq             |
| L898-X           | 148.25        | mnpq              |
| L904-XIII        | 148.25        | mnpq              |
| L895-IX          | 149.00        | mnpqr             |
| L854-II          | 149.00        | mnpqr             |
| L874-XIII        | 149.30        | nopqr             |
| L909-XIV         | 150.25        | nopqrs            |
| L900-XII         | 150.75        | nopqrs            |
| L870-X           | 152.00        | opqrs             |
| L913-XV          | 153.25        | pqrqs             |
| L915-XI          | 154.75        | qrst              |
| L840-XVII        | 158.25        | rst               |
| L845-II          | 160.00        | st                |
| L875-XIII        | 164.00        | t                 |

Regarding the red component (Table 5), the yeasts were distributed in 30 homogeneous groups showing a mean value of 135, with a minimum of 101 and a maximum of 169. The behaviour of 27 out the 51 strains is judged to be negative.

Regarding the green component (Table 6), the yeasts were distributed in 21 homogeneous groups showing a mean value of 133, with a minimum of 90 and a maximum of 164. The behaviour of 24 out the 51 strains is judged to be negative.

Regarding the blue component (Table 7), the yeasts were distributed in 20 homogeneous groups showing a mean value of 130, with a minimum of 86 and a maximum of 159. The behaviour of 28 out the 51 strains is judged to be negative.
The main purpose of this research was to demonstrate that is possible to select yeasts for table olive fermentation according to their pigment adsorption activity; Table 8 summarizes the main characteristics of the 10 yeast strains selected on the base of their adsorption activity.

It is important to note that strains exhibiting the aptitude to highly adsorb the green component from olive pulp agar have obviously been excluded. The remaining strains have been examined based on the number of negative results.

In general, the tested yeasts showed wide and significant differences in their colour components in both the media. Statistical distribution of the yeasts in many homogeneous groups clearly stresses the presence of

| Strain and sample | Green component | Homogeneous groups |
|-------------------|-----------------|--------------------|
| L841-XVII         | 85.25           | a                  |
| L847-XVI          | 96.50           | b                  |
| L859-XVI          | 97.75           | b                  |
| L888-VII          | 102.75          | bc                 |
| L916-XI           | 105.25          | bcd                |
| L895-IX           | 110.00          | cde                |
| L848-XVII         | 111.50          | cdef               |
| L902-XII          | 111.75          | cdefg              |
| L904-XIII         | 111.75          | cdefg              |
| L849-XVIII        | 111.75          | cdefg              |
| L837-XVI          | 112.25          | def                |
| L905-XIII         | 112.50          | def                |
| L842-XVIII        | 113.00          | def                |
| L891-VII          | 115.25          | efgh               |
| L885-V            | 115.75          | efghij             |
| L867-IX           | 116.00          | efghij             |
| L864-VI           | 116.00          | efghij             |
| L893-VIII         | 116.50          | efghijk            |
| L881-V            | 117.25          | efghijk            |
| L908-XIV          | 117.50          | efghijk            |
| L854-III          | 118.75          | efghijkkm          |
| L850-XVIII        | 119.25          | egfhijkkm          |
| L900-XII          | 119.25          | egfhijkkm          |
| L892-VIII         | 119.50          | fghijklmn          |
| L857-VIII         | 119.50          | fghijklmn          |
| L835-III          | 119.75          | fghijklmn          |
| L886-VI           | 120.00          | fghijklmn          |
| L859-V            | 121.00          | ghijklmnno         |
| L894-IX           | 122.25          | hijklmnno          |
| L871-XII          | 122.25          | hijklmnno          |
| L909-XIV          | 124.00          | ijklnopq           |
| L877-IV           | 124.00          | ijklnopq           |
| L856-XVII         | 125.00          | jklmnopq           |
| L832-I            | 125.50          | klnopq             |
| L833-II           | 125.75          | klnopq             |
| L865-VII          | 125.75          | klnopq             |
| L874-XIII         | 125.75          | klnopq             |
| L836-III          | 126.00          | lmnopq             |
| L844-I            | 127.00          | mnopq              |
| L915-XI           | 127.00          | mnopqr             |
| L875-XIV          | 127.75          | mnopqr             |
| L914-XI           | 128.75          | nopqrs             |
| L861-IV           | 129.75          | opqrst             |
| L898-X            | 132.00          | pqrstu              |
| L870-X            | 133.25          | qrstu               |
| L840-XVII         | 136.50          | rstu               |
| L873-XIII         | 137.75          | stu                 |
| L880-IV           | 139.00          | tu                  |
| L915-XV           | 140.00          | uv                  |
| L834-II           | 148.75          | v                   |
| L845-II           | 162.75          | w                   |
significant differences in their ability to adsorb olive pigments. The present work proposes a new approach, based on microbial culturing techniques, to perform the study of the adsorption phenomena in olive yeasts.

One important implication is that the chromogenic media can be tailored to each olive cultivar by preparing the media using the individual cultivar with its specific pigments.

An enhanced knowledge of the effects that yeasts have on olive processing may allow the protection of olive colour, excluding the more adsorbing strains or those which degrade the phenolic compounds responsible for the colour. Although these strains may not be suitable for table olive production, they may find a use in the decolouration of olive wastewater. For example, *Geotrichum candidum* was identified in alpeorujo (Giannoutsou, Meintanis, & Karagouni, 2004), a residue of olive oil production, and its ability to discolour black olive mill wastewater has been reported (Assas, Ayed, Marouani, & Hamdi, 2002).

Table olives may be subjected to a progressive decrease in their greenish appearance (Gallardo-Guerrero et al., 2013); the fading of the green colour may occur mainly during the first months of storage (Romero-Gil et al., 2019).

Table 4. Strain and sample number, value of the blue component of the biomass colour on olive pulp agar, as measured using Photoshop, and distribution in homogeneous groups (p < 0.05) given by statistical analysis; the values inferior or equal to the mean are in italics.

| Strain and sample | Blue component | Homogeneous groups |
|-------------------|----------------|--------------------|
| L841-XVII        | 69.75          | a                  |
| L847-XVI         | 75.75          | ab                 |
| L902-XII         | 80.50          | bc                 |
| L839-XVI         | 82.75          | bed                |
| L904-XIII        | 86.00          | cade               |
| L900-XII         | 86.75          | cde                |
| L837-XVI         | 88.75          | cdef               |
| L916-XI          | 92.00          | deef               |
| L915-XI          | 92.50          | efgh               |
| L903-XIII        | 93.25          | efghi              |
| L895-IX          | 93.25          | efghi              |
| L842-XVIII       | 93.25          | efghi              |
| L874-XIII        | 93.50          | efghi              |
| L909-XIV         | 95.25          | efghi              |
| L888-VII         | 97.50          | ghijkl             |
| L886-XVII        | 97.75          | ghijkl             |
| L835-III         | 99.00          | ghijkl             |
| L908-XIV         | 100.00         | ghijkl             |
| L894-IX          | 100.50         | ghijkl             |
| L886-VI          | 101.50         | ghijkl             |
| L848-XVII        | 101.75         | ghijkl             |
| L871-XII         | 101.75         | ghijkl             |
| L891-VI          | 102.00         | ghijkl             |
| L881-V           | 102.75         | ijklmnop            |
| L864-VI          | 103.25         | jklmnopq            |
| L914-XI          | 103.50         | jklmnopq            |
| L834-III         | 104.00         | jklmnopq            |
| L840-XVIII       | 104.25         | jklmnopq            |
| L873-XIII        | 104.25         | jklmnopq            |
| L875-XIV         | 104.75         | jklmnopq            |
| L885-V           | 105.25         | jklmnopq            |
| L892-VIII        | 106.00         | jklmnopq            |
| L857-VIII        | 107.25         | jklmnopq            |
| L859-V           | 107.50         | mnopqrstuvwxyz      |
| L836-III         | 107.75         | mnopqrstuvwxyz      |
| L865-VII         | 108.50         | mnopqrstuvwxyz      |
| L867-IX          | 110.00         | mnopqrstuvwxyz      |
| L844-I           | 111.25         | mnopqrstuvwxyz      |
| L861-IV          | 112.00         | mnopqrstuvwxyz      |
| L913-XV          | 112.50         | mnopqrstuvwxyz      |
| L832-I           | 115.00         | mnopqrstuvwxyz      |
| L898-X           | 116.75         | mnopqrstuvwxyz      |
| L893-VIII        | 117.00         | mnopqrstuvwxyz      |
| L877-IV          | 117.25         | mnopqrstuvwxyz      |
| L833-II          | 117.75         | mnopqrstuvwxyz      |
| L870-X           | 118.25         | mnopqrstuvwxyz      |
| L849-XVIII       | 119.25         | mnopqrstuvwxyz      |
| L850-XVIII       | 120.25         | mnopqrstuvwxyz      |
| L880-IV          | 121.00         | mnopqrstuvwxyz      |
| L834-II          | 144.25         | mnopqrstuvwxyz      |
| L845-II          | 155.50         | mnopqrstuvwxyz      |
Table 5. Strain and sample number, value of the red component of the biomass colour on olive seed agar, as measured using Photoshop, and distribution in homogeneous groups (p < 0.05) given by statistical analysis; the values inferior or equal to the mean are in italics.

| Strain and sample | Red component | Homogeneous groups |
|-------------------|---------------|--------------------|
| L835-III          | 101.25        | a                  |
| L836-III          | 114.00        | b                  |
| L880-IV           | 115.00        | b                  |
| L861-IV           | 115.25        | bc                 |
| L877-IV           | 116.75        | bcde               |
| L840-XII          | 121.00        | bcde               |
| L881-V            | 121.25        | bcde               |
| L859-V            | 122.50        | bcde               |
| L886-VI           | 123.75        | bcdeghi            |
| L892-VIII         | 124.25        | bcdeghi            |
| L865-VI           | 124.25        | bcdeghi            |
| L877-VIII         | 124.75        | bcdeghi            |
| L880-V            | 125.00        | bcdeghi            |
| L861-V            | 125.25        | bcdeghi            |
| L886-VI           | 126.75        | bcdeghij           |
| L833-II           | 126.75        | bcdeghijkl         |
| L888-VII          | 127.00        | bcdeghijklm        |
| L832-I            | 127.50        | bcdeghijklm        |
| L854-III          | 128.00        | bcdeghijklm        |
| L848-XII          | 128.75        | bcdeghijklm        |
| L850-XVIII        | 130.25        | bcdeghijklm        |
| L844-I            | 131.00        | bcdeghijklmnop      |
| L834-II           | 131.25        | bcdeghijklmnop      |
| L890-XIV          | 132.25        | bcdeghijklmnop      |
| L891-VII          | 133.50        | bcdeghijklmnop      |
| L914-XI           | 133.75        | bcdeghijklmnop      |
| L849-XVIII        | 134.25        | bcdeghijklmnop      |
| L842-XIII         | 135.50        | bcdeghijklmnop      |
| L867-IX           | 136.75        | bcdeghijklmnop      |
| L839-XVI          | 137.00        | bcdeghijklmnop      |
| L845-II           | 137.50        | bcdeghijklmnop      |
| L856-XVII         | 138.50        | bcdeghijklmnop      |
| L837-XVI          | 139.25        | bcdeghijklmnop      |
| L900-XII          | 141.30        | bcdeghijklmnop      |
| L916-XI           | 141.50        | bcdeghijklmnop      |
| L893-VIII         | 142.25        | bcdeghijklmnop      |
| L874-XIII         | 142.50        | bcdeghijklmnop      |
| L896-X            | 143.75        | bcdeghijklmnop      |
| L847-XVI          | 144.25        | bcdeghijklmnop      |
| L913-XV           | 144.75        | bcdeghijklmnop      |
| L905-XIII         | 145.25        | bcdeghijklmnop      |
| L841-XVII         | 145.50        | bcdeghijklmnop      |
| L909-XIV          | 147.00        | bcdeghijklmnop      |
| L915-XI           | 147.75        | bcdeghijklmnop      |
| L870-X            | 148.00        | bcdeghijklmnop      |
| L871-XII          | 149.25        | bcdeghijklmnop      |
| L873-XIII         | 150.75        | bcdeghijklmnop      |
| L894-IX           | 153.25        | bcdeghijklmnop      |
| L904-XIII         | 154.00        | bcdeghijklmnop      |
| L895-IX           | 155.00        | bcdeghijklmnop      |
| L902-XII          | 167.75        | bcdeghijklmnop      |
| L875-XIV          | 169.50        | bcdeghijklmnop      |

Table 6. Strain and sample number, value of the green component of the biomass colour on olive seed agar, as measured using Photoshop, and distribution in homogeneous groups (p < 0.05) given by statistical analysis; the values inferior or equal to the mean are in italics.

| Strain and sample | Green component | Homogeneous groups |
|-------------------|-----------------|--------------------|
| L855-III          | 90.25           | a                  |
| L836-III          | 109.25          | b                  |
| L880-IV           | 114.50          | bcd                |
| L877-IV           | 114.75          | bcd                |
| L861-IV           | 115.25          | bcd                |
| L881-V            | 119.25          | cde                |
| L859-V            | 121.25          | cdefg              |
| L865-VI           | 121.50          | cdefg              |
| L888-VII          | 122.00          | cdefg              |
Table 7. Strain and sample number, value of the blue component of the biomass colour on olive seed agar, as measured using Photoshop, and distribution in homogeneous groups (p < 0.05) given by statistical analysis; the values inferior or equal to the mean are in italics.

| Strain and sample | Green component | Homogeneous groups |
|-------------------|-----------------|--------------------|
| L908-XIV          | 122.50          | cdefg              |
| L886-VI           | 122.75          | cdefg              |
| L840-XVII         | 123.75          | defgh              |
| L864-VI           | 124.75          | efgh               |
| L885-V            | 125.25          | efghi              |
| L849-XVIII        | 126.25          | efghij             |
| L854-III          | 127.50          | efghijk            |
| L892-VIII         | 128.50          | efghijkl           |
| L832-I            | 128.50          | efghijkl           |
| L833-II           | 128.75          | fgijklk            |
| L857-VIII         | 129.00          | fgijklm            |
| L850-XVIII        | 129.50          | ghijklmn           |
| L844-I            | 129.75          | ghijklm            |
| L848-XVII         | 130.00          | ghijklmn           |
| L834-III          | 133.00          | hijklmn            |
| L914-XI           | 134.25          | ijklmno            |
| L891-VII          | 134.75          | jklmnop             |
| L842-XVIII        | 135.00          | jklmnop             |
| L839-XVI          | 135.25          | jklmnop             |
| L898-X            | 136.50          | klmnop              |
| L837-XVI          | 136.75          | klmnop              |
| L900-XII          | 136.75          | klmnop              |
| L845-II           | 137.75          | lnmnop              |
| L867-IX           | 138.25          | mnopq               |
| L874-XIII         | 138.25          | mnopq               |
| L856-XVII         | 139.50          | no pq               |
| L841-XVII         | 140.00          | no pq               |
| L871-XII          | 140.25          | nopq                |
| L870-X            | 140.50          | nopq                |
| L847-XVI          | 140.50          | nopq                |
| L916-XI           | 140.75          | nopq                |
| L893-VIII         | 142.50          | op qr               |
| L913-XV           | 145.50          | opqrs               |
| L905-XIII         | 145.75          | p qrs               |
| L909-XIV          | 144.00          | p qrs               |
| L895-IX           | 146.25          | qr st               |
| L915-XI           | 146.75          | qr st               |
| L902-XII          | 150.75          | rst                 |
| L894-IX           | 151.00          | rst                 |
| L873-XIII         | 152.25          | st                  |
| L904-XIII         | 154.00          | t                   |
| L875-XIV          | 165.75          | u                   |

| Strain and sample | Blue component | Homogeneous groups |
|-------------------|----------------|--------------------|
| L835-III          | 86.25          | a                  |
| L908-XIV          | 111.75         | b                  |
| L836-III          | 112.75         | b                  |
| L840-XVII         | 115.50         | b                  |
| L880-IV           | 116.25         | bc                 |
| L861-IV           | 119.25         | bcd                |
| L850-XVIII        | 120.50         | b c d e f           |
| L877-IV           | 121.00         | b c d e f           |
| L914-XI           | 121.25         | b c d e f           |
| L881-V            | 123.50         | cdef               |
| L865-VII          | 123.50         | cdef               |
| L900-XII          | 123.50         | cdef               |
| L888-VII          | 123.75         | cdef               |
| L898-X            | 124.00         | cdefg              |
| L892-VIII         | 124.75         | cdefghi             |
| L854-III          | 125.50         | cdefghij           |
| L847-XVI          | 126.25         | cdefghijkl          |
| L886-VI           | 126.75         | cdefghijkl          |
Many different technological characteristics may be studied in order to characterize and select olive yeasts as adjunct culture.

Considering the yeast’s ability to adsorb olive pigments, the results confirm that the proposed approach is easy, cheap, fast, and allows an efficacious selection of yeasts for potential use as adjunct cultures in table olive fermentation.

After this initial screening, only the strains remaining at the end of this selection will be further studied, with a great saving of time and money.

The two olive-based media can be prepared using any olive cultivar, thus allowing the specific selection of the most suitable strain of yeast for each olive variety.

Table 8. Summary of the main characteristics of the 10 pre-selected olive yeasts.

| Sample | Strain | Colour of the biomass on olive pulp agar | Colour of the biomass on olive seed agar |
|--------|--------|------------------------------------------|-----------------------------------------|
|        |        | Blue component                           | Homogeneous groups                       |
| Number | Cultivar | Kind | pH | Number | Morphology | Film | Gas | Red | Green | Blue | Red | Green | Blue |
| IX     | Carolea | olive pulp | 4.00 | L894 | filamentous | + | - | 142.50 | 122.25 | 100.50 | 153.25 | 151.00 | 146.00 |
| X      | Carolea | brine | 3.76 | L870 | filamentous | + | - | 152.00 | 133.25 | 118.25 | 148.00 | 140.50 | 133.25 |
| X      | Carolea | olive pulp | 4.54 | L898 | filamentous | + | - | 148.25 | 132.00 | 116.75 | 143.75 | 136.50 | 124.00 |
| XI     | Carolea | brine | 3.71 | L915 | filamentous | + | - | 154.75 | 127.00 | 92.50 | 147.75 | 146.75 | 146.75 |
| XII    | Carolea | brine | 3.66 | L871 | filamentous | + | - | 138.25 | 122.25 | 101.75 | 149.25 | 140.25 | 134.00 |
| XIII   | Carolea | brine | 5.57 | L873 | filamentous | + | - | 164.00 | 137.75 | 104.25 | 150.75 | 152.25 | 147.50 |
| XIV    | Nocellara | brine | 5.79 | L874 | filamentous | + | - | 149.50 | 125.75 | 93.50 | 142.50 | 138.25 | 128.25 |
| XIV    | Nocellara | olive pulp | 4.97 | L909 | filamentous | + | - | 142.50 | 127.75 | 104.75 | 169.50 | 163.75 | 159.00 |
| XV     | Ottobratica | brine | 4.23 | L913 | filamentous | + | - | 153.25 | 140.00 | 112.50 | 144.75 | 145.50 | 130.00 |

## Conclusion

...
The research provides a useful tool to characterize olive yeasts in relation to pigment adsorption, allowing the improvement of olive colour.

Further studies will be carried out using the best yeast strains as adjunct cultures for the production of more pigmented table olives.

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