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BIOTECHNOLOGY

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 (CO_2) 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	*Geracese*	Brine	3.76	L832	Elliptic	+	+
I	*Geracese*	olive pulp	4.26	L844	Elliptic	+	+
II	*Geracese*	Brine	3.82	L845	Elliptic	-	+
II	*Geracese*	olive pulp	4.27	L833	Filamentous	+	-
II	*Geracese*	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	*Geracese*	Brine	3.66	L861	Filamentous	+	-
IV	*Geracese*	olive pulp	4.19	L877	Elliptic	+	-
IV	*Geracese*	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	*Geracese*	Brine	3.81	L864	Filamentous	+	-
VI	*Geracese*	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	*Geracese*	Brine	3.87	L857	Elliptic	+	+
VIII	*Geracese*	olive pulp	4.38	L892	Elliptic	+	+
VIII	*Geracese*	olive pulp	4.38	L893	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*	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	*Roggianella*	Brine	5.77	L839	Filamentous	+	+
XVI	*Roggianella*	olive pulp	6.22	L837	Filamentous	+	+
XVI	*Roggianella*	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	*123.25*	*b*
L850-XVIII	*124.75*	*bc*
L891-VII	*127.00*	*bcd*
L916-XI	*127.00*	*bcd*
L832-I	*127.75*	*bcde*
L842-XVIII	*127.75*	*bcde*
L885-V	*128.50*	*bcdef*
L859-V	*128.75*	*bcdef*
L881-V	*129.25*	*bcdef*
L839-XVI	*129.25*	*bcdef*
L833-II	*130.00*	*bcdefg*
L836-III	*130.25*	*bcdefg*
L835-III	*130.50*	*bcdefg*
L905-XIII	*131.25*	*bcdefgh*
L864-VI	*132.00*	*bcdefghi*
L877-IV	*132.25*	*bcdefghi*
L847-XVI	*133.75*	*cdefghij*
L886-VI	*134.00*	*cdefghij*
L844-I	*134.00*	*cdefghij*
L908-XIV	*134.50*	*cdefghijk*
L893-VIII	*134.75*	*defghijk*
L892-VIII	*137.00*	*efghijkl*
L856-XVII	*137.75*	*fghijkl*
L865-VII	*138.00*	*fghijkl*
L871-XII	*138.25*	*fghijkl*
L857-VIII	139.25	ghijklm
L854-III	141.00	hijklmn
L861-IV	141.25	ijklmn
L894-IX	142.50	jklmno
L875-XIV	142.50	jklmno
L837-XVI	143.50	jklmnop
L867-IX	144.00	klmnop
L902-XII	144.25	klmnop
L880-IV	145.75	lmnopq
L914-XI	146.75	lmnopq
L898-X	148.25	mnopq
L904-XIII	148.25	mnopq
L895-IX	149.00	mnopqr
L834-II	149.00	mnopqr
L874-XIII	149.50	nopqr
L909-XIV	150.25	nopqrs
L900-XII	150.75	nopqrs
L870-X	152.00	opqrs
L913-XV	153.25	pqrs
L915-XI	154.75	qrst
L840-XVII	158.25	rst
L845-II	160.00	st
L873-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.

Table 3. Strain and sample number, value of the green 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	Green component	Homogeneous groups
L841-XVII	*85.25*	*a*
L847-XVI	*96.50*	*b*
L839-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*	*defg*
L905-XIII	*112.50*	*defg*
L842-XVIII	*113.00*	*defgh*
L891-VII	*115.25*	*efghi*
L885-V	*115.75*	*efghij*
L867-IX	*116.00*	*efghij*
L864-VI	*116.00*	*efghij*
L893-VIII	*116.50*	*efghijk*
L881-V	*117.25*	*efghijkl*
L908-XIV	*117.50*	*efghijkl*
L854-III	*118.75*	*efghijklm*
L850-XVIII	*119.25*	*efghijklm*
L900-XII	*119.25*	*efghijklm*
L892-VIII	*119.50*	*fghijklmn*
L857-VIII	*119.50*	*fghijklmn*
L835-III	*119.75*	*fghijklmn*
L886-VI	*120.00*	*fghijklmn*
L859-V	*121.00*	*ghijklmno*
L894-IX	122.25	hijklmno
L871-XII	122.25	hijklmno
L909-XIV	124.00	ijklmnopq
L877-IV	124.00	ijklmnopq
L856-XVII	125.00	jklmnopq
L832-I	125.50	klmnopq
L833-II	125.75	klmnopq
L865-VII	125.75	klmnopq
L874-XIII	125.75	klmnopq
L836-III	126.00	lmnopq
L844-I	127.00	mnopq
L915-XI	127.00	mnopq
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
L913-XV	140.00	uv
L834-II	148.75	v
L845-II	162.75	w

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 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*	*bcd*
L904-XIII	*86.00*	*cde*
L900-XII	*86.75*	*cde*
L837-XVI	*88.75*	*cdef*
L916-XI	*92.00*	*defg*
L915-XI	*92.50*	*efgh*
L905-XIII	*93.25*	*efghi*
L895-IX	*93.25*	*efghi*
L842-XVIII	*93.25*	*efghi*
L874-XIII	*93.50*	*efghi*
L909-XIV	*95.25*	*efghij*
L888-VII	*97.50*	*fghijk*
L856-XVII	*97.75*	*fghijkl*
L835-III	*99.00*	*ghijklm*
L908-XIV	*100.00*	*ghijklm*
L894-IX	*100.50*	*ghijklmn*
L886-VI	*101.50*	*ghijklmn*
L848-XVII	*101.75*	*hijklmno*
L871-XII	*101.75*	*hijklmno*
L891-VII	*102.00*	*hijklmno*
L881-V	*102.75*	*ijklmnop*
L864-VI	*103.25*	*jklmnopq*
L914-XI	*103.50*	*jklmnopq*
L854-III	*104.00*	*jklmnopq*
L840-XVII	*104.25*	*jklmnopq*
L873-XIII	*104.25*	*jklmnopq*
L875-XIV	104.75	jklmnopq
L885-V	105.25	klmnopq
L892-VIII	106.00	klmnopqr
L857-VIII	107.25	lmnopqrs
L859-V	107.50	mnopqrst
L836-III	107.75	mnopqrstu
L865-VII	108.50	mnopqrstuv
L867-IX	110.00	nopqrstuvw
L844-I	111.25	opqrstuvwx
L861-IV	112.00	pqrstuvwxy
L913-XV	112.50	qrstuvwxy
L832-I	115.00	rstuvwxyz
L898-X	116.75	stuvwxyz
L893-VIII	117.00	tuvwxyz
L877-IV	117.25	uvwxyz
L833-II	117.75	vwxyz
L870-X	118.25	wxyz
L849-XVIII	119.25	wxyz
L850-XVIII	120.25	xyz
L880-IV	121.00	yz
L834-II	144.25	A
L845-II	155.50	B

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-XVII	*121.00*	*bcdefg*
L881-V	*121.25*	*bcdefg*
L859-V	*122.50*	*bcdefgh*
L886-VI	*123.75*	*cdefghi*
L892-VIII	*124.25*	*defghi*
L865-VII	*124.25*	*defghi*
L857-VIII	*124.75*	*efghi*
L885-V	*125.00*	*efghij*
L864-VI	*125.50*	*fghijk*
L833-II	*125.75*	*fghijkl*
L888-VII	*127.00*	*fghijklm*
L832-I	*127.50*	*fghijklm*
L854-III	*128.00*	*fghijklm*
L848-XVII	*128.75*	*ghijklmn*
L850-XVIII	*130.25*	*hijklmno*
L844-I	*131.00*	*hijklmnop*
L834-II	*131.25*	*ijklmnop*
L908-XIV	*132.25*	*ijklmnop*
L891-VII	*133.50*	*jklmnopq*
L914-XI	*133.75*	*klmnopqr*
L849-XVIII	*134.25*	*lmnopqrs*
L842-XVIII	*135.50*	*mnopqrst*
L867-IX	136.75	nopqrstu
L839-XVI	137.00	nopqrstuv
L845-II	137.50	opqrstuv
L856-XVII	138.50	opqrstuvw
L837-XVI	138.50	opqrstuvw
L900-XII	139.25	pqrstuvwx
L916-XI	141.50	qrstuvwxy
L893-VIII	142.25	rstuvwxyz
L874-XIII	142.50	stuvwxyz
L898-X	143.75	tuvwxyz
L847-XVI	144.25	uvwxyz
L913-XV	144.75	uvwxyzA
L905-XIII	145.25	uvwxyzA
L841-XVII	145.50	vwxyzAB
L909-XIV	147.00	wxyzABC
L915-XI	147.75	xyzABC
L870-X	148.00	yzABC
L871-XII	149.25	yzABC
L873-XIII	150.75	zABC
L894-IX	153.25	ABC
L904-XIII	154.00	BC
L895-IX	155.00	C
L902-XII	167.75	D
L875-XIV	169.50	D

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
L835-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-VII	*121.50*	*cdefg*
L888-VII	*122.00*	*cdefg*

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*	*fghijkl*
L857-VIII	*129.00*	*fghijklm*
L850-XVIII	*129.50*	*ghijklm*
L844-I	*129.75*	*ghijklm*
L848-XVII	*130.00*	*ghijklm*
L834-II	*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	lmnopq
L867-IX	138.25	mnopq
L874-XIII	138.25	mnopq
L856-XVII	139.50	nopq
L841-XVII	140.00	nopq
L871-XII	140.25	nopq
L870-X	140.50	nopq
L847-XVI	140.50	nopq
L916-XI	140.75	nopq
L893-VIII	142.50	opqr
L913-XV	143.50	opqrs
L905-XIII	143.75	pqrs
L909-XIV	144.00	pqrs
L895-IX	146.25	qrst
L915-XI	146.75	qrst
L902-XII	150.75	rst
L894-IX	151.00	rst
L873-XIII	152.25	st
L904-XIII	154.00	t
L875-XIV	163.75	u

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	Blue component	Homogeneous groups
L835-III	*86.25*	*a*
L908-XIV	*111.75*	*b*
L836-III	*112.75*	*b*
L840-XVII	*113.50*	*b*
L880-IV	*116.25*	*bc*
L861-IV	*119.25*	*bcd*
L850-XVIII	*120.50*	*bcdef*
L877-IV	*121.00*	*bcdef*
L914-XI	*121.25*	*bcdef*
L881-V	*123.50*	*cdefg*
L865-VII	*123.50*	*cdefg*
L900-XII	*123.50*	*cdefg*
L888-VII	*123.75*	*cdefg*
L898-X	*124.00*	*cdefgh*
L892-VIII	*124.75*	*cdefghi*
L854-III	*125.50*	*cdefghij*
L847-XVI	*126.25*	*defghijkl*
L886-VI	*126.75*	*defghijkl*

Strain and sample	Blue component	Homogeneous groups
L864-VI	*127.25*	*defghijkl*
L849-XVIII	*127.50*	*defghijklm*
L874-XIII	*128.25*	*defghijklm*
L885-V	*128.50*	*defghijklm*
L859-V	*128.75*	*defghijklm*
L839-XVI	*129.50*	*efghijklmn*
L913-XV	*130.00*	*efghijklmno*
L848-XVII	*130.25*	*fghijklmnop*
L841-XVII	*130.25*	*fghijklmnop*
L909-XIV	*130.25*	*fghijklmnop*
L857-VIII	131.75	ghijklmnopq
L902-XII	131.75	ghijklmnopq
L833-II	132.00	ghijklmnopq
L867-IX	132.50	ghijklmnopq
L870-X	133.25	ghijklmnopq
L842-XVIII	133.75	hijklmnopq
L837-XVI	133.75	hijklmnopq
L871-XII	134.00	ijklmnopq
L832-I	134.50	ijklmnopq
L856-XVII	135.25	jklmnopq
L895-IX	135.50	klmnopq
L844-I	135.75	lmnopq
L891-VII	137.25	mnopqr
L916-XI	138.75	nopqrs
L905-XIII	139.75	opqrs
L834-II	140.00	pqrs
L893-VIII	141.25	qrs
L894-IX	146.00	rs
L845-II	146.75	rs
L915-XI	146.75	rs
L873-XIII	147.50	s
L904-XIII	148.00	s
L875-XIV	159.00	t

Table 8. Summary of the main characteristics of the 10 pre-selected yeast strains.

Sample				Strain				Colour of the biomass on olive pulp agar			Colour of the biomass on olive seed agar		
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	4.34	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
Number	Cultivar	Kind	pH	Number	Morphology	Film	Gas	Red	Green	Blue	Red	Green	Blue
XIII	*Carolea*	brine	3.57	L873	filamentous	+	-	164.00	137.75	104.25	150.75	152.25	147.50
XIII	*Carolea*	brine	3.57	L874	filamentous	+	-	149.50	125.75	93.50	142.50	138.25	128.25
XIV	*Nocellara*	brine	4.48	L875	filamentous	+	-	142.50	127.75	104.75	169.50	163.75	159.00
XIV	*Nocellara*	olive pulp	4.97	L909	filamentous	+	-	150.25	124.00	95.25	147.00	144.00	130.25
XV	*Ottobratica*	brine	4.23	L913	filamentous	+	-	153.25	140.00	112.50	144.75	143.50	130.00

Conclusion

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.

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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References

Alfonzo, A., Martorana, A., Settanni, L., Matraxia, M., Corona, O., Vagnoli, P., ... Francesca, N. (2018). Approaches to improve the growth of starter lactic acid bacterium OM13 during the early stages of green Spanish-style table olive production. *Grasas y Aceites,69*(3), e265. doi: 10.3989/gya.0103181

Arroyo-López, F. N., Romero-Gil, V., Bautista-Gallego, J., Rodríguez-Gómez, F., Jiménez-Díaz, R., García-García, P., ... Garrido-Fernández, A. (2012). Yeasts in table olive processing: desirable or spoilage microorganisms? *International Journal of Food Microbiology, 160*(1), 42-49. doi: 10.1016/j.ijfoodmicro.2012.08.003

Assas, N., Ayed, L., Marouani, L., & Hamdi, M. (2002). Decolorization of fresh and stored-black olive mill wastewaters by *Geotrichum candidum*. *Process Biochemistry, 38*(3), 361-365. doi: 10.1016/S0032-9592(02)00091-2

Bevilacqua, A., Beneduce, L., Sinigaglia, M., & Corbo, M. R. (2013). Selection of yeasts as starter cultures for table olives. *Journal of Food Science, 78*(5), M742-M751. doi: 10.1111/1750-3841.12117

Bonatsou, S., Benítez, A., Rodríguez-Gómez, F., Panagou, E. Z., & Arroyo-López, F. N. (2015). Selection of yeasts with multifunctional features for application as starters in natural black table olive processing. *Food Microbiology, 46*, 66-73. doi: 10.1016/j.fm.2014.07.011

Caridi, A. (2013). Improved screening method for the selection of wine yeasts based on their pigment adsorption activity. *Food Technology and Biotechnology, 51*(1), 137-144.

Durante, M., Tufariello, M., Tommasi, L., Lenucci, M. S., Bleve, G., & Mita, G. (2018). Evaluation of bioactive compounds in black table olives fermented with selected microbial starters. *Journal of the Science of Food and Agriculture, 98*(1), 96-103. doi: 10.1002/jsfa.8443

Gallardo-Guerrero, L., Gandul-Rojas, B., Moreno-Baquero, J., López-López, A., Bautista-Gallego, J., & Garrido-Fernández, A. (2013). Pigment, physicochemical, and microbiological changes related to the freshness of cracked table olives. *Journal of Agricultural and Food Chemistry, 61*(15), 3737-3747. doi: 10.1021/jf400240e

Giannoutsou, E. P., Meintanis, C., & Karagouni, A. D. (2004). Identification of yeast strains isolated from a two-phase decanter system olive oil waste and investigation of their ability for its fermentation. *Bioresource Technology, 93*(3), 301-306. doi: 10.1016/j.biortech.2003.10.023

Issaoui, M., Dabbou, S., Mechri, B., Nakbi, A., Chehab, H., & Hammami, M. (2011). Fatty acid profile, sugar composition, and antioxidant compounds of table olives as affected by different treatments. *European Food Research and Technology, 232*(5), 867-876. doi: 10.1007/s00217-011-1455-3

Muccilli, S., Caggia, C., Randazzo, C. L., & Restuccia, C. (2011). Yeast dynamics during the fermentation of brined green olives treated in the field with kaolin and Bordeaux mixture to control the olive fruit fly. *International Journal of Food Microbiology, 148*(1), 15-22. doi: 10.1016/j.ijfoodmicro.2011.04.019

Romero-Gil, V., Rodríguez-Gómez, F., Ruiz-Bellido, M. A., Cabello, A. B., Garrido-Fernández, A., & Arroyo-López, F. N. (2019). Shelf-life of traditionally-seasoned *Aloreña de Málaga* table olives based on package appearance and fruit characteristics. *GrasasY Aceites, 70*(2), e306. doi: 10.3989/gya.0708182

Sánchez, A. H., López- López, A., Beato, V. M., Castro, A., & Montaño, A. (2017). Stability of color in Spanish-style green table olives pasteurized and stored in plastic containers. *Journal of the Science of Food and Agriculture, 97*(11), 3631-3641. doi: 10.1002/jsfa.8222

Tofalo, R., Perpetuini, G., Schirone, M., Suzzi, G., & Corsetti, A. (2013). Yeast biota associated to naturally fermented table olives from different Italian cultivars. *International Journal of Food Microbiology, 161*(3), 203-208. doi: 10.1016/j.ijfoodmicro.2012.12.011

Tofalo, R., Schirone, M., Perpetuini, G., Suzzi, G., & Corsetti, A. (2012). Development and application of a real-time PCR-based assay to enumerate total yeasts and Pichia anomala, Pichia guillermondii and Pichia kluyveri in fermented table olives. *Food Control, 23*(2), 356-362. doi: 10.1016/j.foodcont.2011.07.032

Tufariello, M., Anglana, C., Crupi, P., Virtuosi, I., Fiume, P., Di Terlizzi, B., ... Bleve, G. (2019). Efficacy of yeast starters to drive and improve Picual, Manzanilla and Kalamàta table olive fermentation. *Journal of the Science of Food and Agriculture, 99*(5), 2504-2512. doi: 10.1002/jsfa.9460

Valenčič, V., Mavsar, D. B., Bučar-Miklavčič, M., Butinar, B., Čadež, N., Golob, T., ... Možina, S. S. (2010). The impact of production technology on the growth of indigenous microflora and quality of table olives from Slovenian Istria. *Food Technology and Biotechnology, 48*(3), 404-410.