Caridi A, 2021. Selection of Calabrian strains of Saccharomyces sensu stricto for red wines. Acta Alimentaria, Volume 50(4), Pages 565-573, ISSN 0139-3006

which has been published in final doi https://doi.org/10.1556/066.2021.00119

Selection of Calabrian strains of *Saccharomyces sensu stricto* for red wines

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ABSTRACT

Phenolic compounds provide important quality attributes to red wines interacting with the organoleptic impact of wines. Yeast mannoproteins can interact with grape phenolic compounds, responsible for colour and antioxidant activity of wines. The aim of this work was to perform oenological characterization and specific selection of Calabrian strains of *Saccharomyces sensu stricto*. Among the considered traits, the yeast aptitude to preserve grape pigments and colour intensity was included. Among the best six yeast strains - Sc2731, Sc2742, Sc2756, Sc2773, Sc2774, and Sc2823 - strain Sc2742 exhibits the highest Folin-Ciocalteu's index and strain Sc2774 the highest colour intensity. These two selected yeasts may be used as starter for the production of red wines in order to preserve grape pigments and colour intensity.

KEYWORDS

phenolic compounds; red wines; *Saccharomyces sensu stricto*; yeast mannoproteins.

1. INTRODUCTION

Natural *Saccharomyces cerevisiae* yeast strains exhibit very large diversity; breeding programs that take advantage of this characteristic are widely used for selecting starters for wine industry (Vion et al., 2021). The number of proposed traits is constantly increasing, producing the goal of selecting yeasts for specific characteristics, such as low production of toxic compounds, high ability to bind

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them, increasing wine colour and phenolic content, and improving wine healthiness (Caridi et al., 2004; 2007).

Yeast interaction with the environment relies mainly on the external cell wall layer composed of mannoproteins (Lozančić et al., 2021); yeast mannoproteins can interact both with grape phenolic compounds, responsible for colour and antioxidant activity of wines, and with toxic compounds, such as ochratoxin A (Caridi et al., 2020).

Phenolic compounds provide important quality attributes to red wines (Osete-Alcaraz et al., 2019) interacting with the organoleptic impact of wines (Delgado Cuzmar et al., 2018). The different yeast adsorption aptitude determines behaviour differences that are detectable during winemaking; in fact, significant differences for colour, phenolic compounds, and anthocyanin content are described in wines produced with different selected yeasts (Caridi et al., 2004). Yeast influences the formation of stable pigments in red winemaking (Morata et al., 2016)

The polygenic basis and the inheritability of the oenological trait 'wine colour adsorption' in yeasts was demonstrated (Caridi et al., 2007). This observation is of great practical interest since allows to carry out genetic improvement programs on the descendants of the best wine strains, leading towards an ideal strain that exhibits all the desired characteristics.

The *Saccharomyces sensu stricto* group encompasses species ranging from the industrially ubiquitous yeast *Saccharomyces cerevisiae* to those that are confined to geographically limited environmental niches (Borneman and Pretorius, 2015).

The aim of this work was to perform oenological characterization and specific selection of Calabrian strains of *Saccharomyces sensu stricto* able to control red winemaking. The strains were tested for conventional oenological characteristics; the best strains were tested for the following specific characteristics: (a) protection of the grape phenolic content; (b) preservation of the colour intensity.

2. MATERIALS AND METHODS

2.1. Pre-selection trials

A total of 89 autochthonous strains of *Saccharomyces sensu stricto* were isolated from grape must of the Calabrian cultivars *Magliocco, Nerello, and Greco bianco* or from various black or white grape cultivars, mixed at pressing, produced by different wineries in the area of Lamezia Terme (Catanzaro, South Italy). The grape musts were inoculated in YPD agar with the following composition: yeast extract 10 g L^{-1}, peptone from casein 10 g L^{-1}, dextrose 20 g L^{-1}, and agar 15 g L^{-1}. After 3-5 days of incubation at 25 °C, the colonies were observed under the microscope, inoculated and grown in nutrient broth, re-isolated and then identified by their spore production. Purified strains were stored at -80 °C using a cryo-preservative bead storage system (Microbank TM, Pro-Lab Diagnostics, Canada). The strains were pre-selected by studying type of growth, sporulation, acetic acid production, and H_2S production. The type of growth, dispersed or flocculent, was evaluated after incubation at 25 °C for 3 days in nutrient broth with the following composition: yeast extract 10 g L^{-1}, peptone from casein 10 g L^{-1}, and dextrose 20 g L^{-1}. The sporulation was evaluated by observation at the microscope after incubation at 25 °C for 7 days in Petri plates using a nutrient medium with the following composition: sodium acetate anhydrous 1 g L^{-1}, and agar 20 g L^{-1} (Fowell, 1952). All the yeast that produced spores of the genus *Saccharomyces* passed the test and were considered *Saccharomyces sensu stricto*. The intensity of acetic acid production was evaluated in Petri plates after incubation at 25 °C for 3 days using a nutrient medium with the following composition: yeast extract 3 g L^{-1}, dextrose 10 g L^{-1}, calcium carbonate 3 g L^{-1}, and agar 15 g L^{-1}. The intensity of H_2S production was evaluated in Petri plates after incubation at 25 °C for 3 days using the Candida Elective Agar medium according to Nickerson (1953).

2.2. Selection trials

After the pre-selection, the best strains were chosen to study their aptitude to adsorb grape pigments in Grape Skin Agar (Caridi et al., 2007; Caridi, 2013). On this medium, the yeast biomass takes a wide range of colours, from white to dark brown. Strains with white biomass have low aptitude to

adsorb, while strains with dark brown biomass have medium-high adsorption aptitude. After 10 days of anaerobic incubation by AnaeroGen gas-pack catalyst (Oxoid LTD, Hampshire, England) at 28 °C, yeast biomass was subjected to the computer-assisted evaluation of the red, green, and blue components using Photoshop CS for Windows XP from Adobe (Caridi et al., 2007; Caridi, 2013). According to biomass colour adsorption on the Grape Skin Agar, the best strains were chosen. The yeasts were further characterised by micro-winemaking trials to confirm their low ability to adsorb grape pigments and phenolics during fermentation. Black grapes of Calabrian *Gaglioppo* cultivar were given pre-fermentative maceration to extract pigments from skins and seeds. They were destemmed, crushed and cold soaked at 4°C for three days, performing a punch down twice per day. The must obtained after pressing was divided in aliquots of 100 mL, pasteurised (110 °C for 10 min), inoculated at 5% in triplicate with the wine yeasts, and incubated at 20°C. The weight loss caused by CO_2 production after three days of fermentation was determined according to Caridi (2003); so, the fermentation vigour was expressed as g of CO_2 100 mL^{-1} of must. In the same way, the end of fermentation was determined by weighing the bottles containing the 100 ml of inoculated must twice, at a distance of 24 hours; if the difference in weight was less than 0.01 g, the fermentation was considered finished. So, at the end of fermentation, wines were centrifuged at 4,500 rpm for 5 min and diluted 1:5 (v/v) with a pH 3.5 buffer (citric acid monohydrate 0.1 M, Na_2HPO_4 0.2 M). The wine absorbance at 420, 520, and 620 nm was read using an Anadeo1 spectrophotometer (Bibby Sterilin Ltd); the intensity (I) and the tint (T) were calculated with the following formulas: $I = A_{420} + A_{520} + A_{620}$ and $T = A_{420} : A_{520}$. The total phenolic content was determined using the Folin-Ciocalteu index according to Singleton and Rossi (1965).

2.3. Statistical procedures

All the analyses were performed in triplicate and data were subjected to statistical evaluation by StatGraphics Centurion XVI for Windows XP from StatPoint.

3. RESULTS AND DISCUSSION

Of the 89 yeast strains isolated, 11% originate from *Magliocco* grapes, 11% from *Nerello* grapes, 9% from *Greco bianco* grapes, 6% from *Gaglioppo* grapes, 6% from *Guardavalle* grapes, 6% from *Pecorello* grapes, 33% from mixed black grapes, and 18% from mixed white grapes (Fig. 1A). Only one strain is flocculent, all the other strains exhibiting dispersed growth in liquid medium (Fig. 1B). Only 25% of the strains show medium or high spore production, 19% show low spore production while 56% do not produce spores of the genus *Saccharomyces* (Fig. 1C). Only 10% of the strains show medium or high acetic acid production, 1% show low acetic acid production, while 89% do not significantly produce it in the used medium (Fig. 1D). Only 14% of the strains show high H_2S production, 82% show medium H_2S production, while 4% show low H_2S production (Fig. 1E).

Based on these results, the 89 strains were reduced to 20, all exhibiting dispersed growth in liquid medium, good capacity to produce spores attributable to the *Saccharomyces sensu stricto* group; moreover, they do not significantly produce acetic acid in the medium used and exhibit low or medium H_2S production.

Table 1 reports the results obtained after testing the 20 strains on the Grape Skin Agar.

← *approximate position of Table 1*

The red component values vary from a min of 72.75 (strain Sc2744) to a max of 141.25 (strain Sc2823). The green component values vary from a min of 35.50 (strain Sc2744) to a max of 104.50 (strain Sc2823). The blue component values vary from a min of 37.75 (strain Sc2744) to a max of 90.75 (strain Sc2823). The strains are respectively distributed in 16, 13, and 14 homogeneous groups (hg). The five strains showing the lowest values of the red component - Sc2744, Sc2753, Sc2770, Sc2786, and Sc2815 - were excluded because they adsorb too much grape pigments.

Table 2 reports mean and standard deviation of the fermentation vigour after 3 days for the 15 strains selected on the basis of the biomass colour adsorption. The values vary from a min of 6.52 g 100 mL^{-1} for strain Sc2814 to a max of 9.07 g 100 mL^{-1} for strain Sc2803. The strains are distributed in nine hg. No strain was excluded for this parameter.

← *approximate position of Table 2*

Table 3 reports three colour components of the wines produced by micro-winemaking trials using the 15 strains.

← *approximate position of Table 3*

The yellow component values range from a min of 1.118 (strain Sc2762) to a max of 3.140 (strains Sc2774). The red component values range from 0.703 (strain Sc2762) to 1.918 (strain Sc2774). The blue component values range from 0.243 (strain Sc2762) to 0.913 (strain Sc2774). The higher values give rise to wines with more intense colours, showing low adsorption ability of the yeasts, and vice versa. The chromatic characteristics of the wines obtained with the 15 strains allow them to be placed in 9, 12 and 11 hg. The six strains showing red component values lower than the mean value - Sc2734, Sc2750, Sc2754, Sc2760, Sc2762, and Sc2803 - were excluded because they adsorb too much grape pigments.

Table 4 reports mean and standard deviation of colour intensity, tint, and Folin-Ciocalteu index determined in the wines produced by micro-winemaking trials using the 15 strains.

← *approximate position of Table 4*

The intensity values range from a min of 2.065 (strain Sc2762) to a max of 5.971 (strain Sc2774). The strains are distributed in 10 hg. Considering the tint parameter, the values range from 1.590 (strain Sc2762) to 1.717 (strain Sc2814). The strains are distributed in 12 hg. The values of Folin-Ciocalteu index range from 11.32 mg L^{-1} (strain Sc2754) to 17.30 mg L^{-1} (strain Sc2742). The strains are distributed in nine hg. The higher values mean wines with high phenolic content indicating, therefore, a low adsorption ability of the yeasts. The six strains showing colour intensity values lower than the mean value - Sc2734, Sc2750, Sc2754, Sc2760, Sc2762, and Sc2803 - were excluded because they adsorb too much grape pigments. The seven strains showing Folin-Ciocalteu index values lower than the mean value - Sc2734, Sc2751, Sc2754, Sc2760, Sc2762, Sc2776, and Sc2814 - were excluded because they adsorb too much phenolic compounds.

It is possible to note (Tables 1 and 3) that there is not a full correlation between biomass colour and wine colour; this is mainly due to the fact that the wine colour is affected by different factors, other than by yeast adsorption activity (Caridi et al. 2007); consequently, it is possible to observe yeast strains with a similar parietal adsorption aptitude but with different chromatic characteristics of the produced wines.

4. CONCLUSIONS

Among the best six yeast strains - Sc2731, Sc2742, Sc2756, Sc2773, Sc2774, and Sc2823 - strain Sc2742 exhibits the highest Folin-Ciocalteu's index and strain Sc2774 the highest colour intensity. These two selected yeasts may be used as starter for the production of red wines in order to preserve grape pigments and colour intensity.

ACKNOWLEDGMENT

The research was carried out in collaboration with ***ARSSA Calabria***, now ***ARSAC*** - Azienda Regionale per lo Sviluppo dell'Agricoltura Calabrese (Viale Trieste 93, I-87100 Cosenza, Italy), acting as financial Organization through dr. Enrico Maria Cristiano and dr. Benito Scazziota. All the yeast strains tested in the present research belong to ARSSA and they are kept in the collection of the laboratory of Microbiology at the *Mediterranea* University of Reggio Calabria.

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Table 1. Mean, standard deviation, and homogeneous group distribution of 20 yeast strains based on red, green and blue component of the microbial biomass grown on Grape Skin Agar elaborated by StatGraphics using *Least Significant Difference* method with 95% of significance

	Red component		Green component		Blue component	
Strain	Mean ± SD	hg	Mean ± SD	hg	Mean ± SD	hg
Sc2731	116.50 ± 1.29	ghi	71.75 ± 0.96	g	66.25 ± 0.96	hi
Sc2734	120.75 ± 2.63	l	78.50 ± 1.29	hi	67.5.0 ± 1.91	i
Sc2742	116.25 ± 3.59	gh	70.00 ± 3.16	g	64.00 ± 3.16	gh
Sc2744	72.75 ± 2.50	a	35.50 ± 1.91	a	37.75 ± 1.50	a
Sc2750	120.00 ± 5.35	hil	75.75 ± 3.86	h	68.50 ± 3.11	il
Sc2751	108.00 ± 4.97	f	66.00 ± 2.58	f	61.5.0 ± 2.89	fg
Sc2753	80.25 ± 2.75	b	42.00 ± 1.41	b	41.50 ± 1.00	b
Sc2754	100.75 ± 0.50	de	59.00 ± 1.63	de	58.50 ± 2.38	ef
Sc2756	120.25 ± 2.36	il	80.25 ± 3.77	i	71.00 ± 3.37	l
Sc2760	113.75 ± 2.06	g	69.25 ± 1.71	g	64.25 ± 1.71	gh
Sc2762	107.00 ± 2.45	f	61.25 ± 1.26	e	56.25 ± 2.06	e
Sc2770	89.50 ± 1.29	c	48.00 ± 0.82	c	50.00 ± 1.41	d
Sc2773	100.75 ± 3.40	de	58.00 ± 1.63	d	55.5.0 ± 0.58	e
Sc2774	130.00 ± 1.41	m	86.00 ± 1.41	l	76.75 ± 2.75	m
Sc2776	104.25 ± 1.71	ef	62.00 ± 1.41	e	57.00 ± 1.63	e
Sc2786	92.25 ± 1.50	c	48.25 ± 1.71	c	46.50 ± 1.91	c
Sc2803	128.00 ± 2.00	m	84.00 ± 2.00	l	75.50 ± 3.00	m
Sc2814	117.25 ± 2.06	ghil	76.00 ± 1.15	h	66.00 ± 2.58	hi
Sc2815	99.75 ± 4.11	d	57.25 ± 2.63	d	51.75 ± 2.87	d
Sc2823	141.25 ± 2.22	n	104.50 ± 2.52	m	90.75 ± 1.71	n
Minimum	72.75		35.50		37.75	
Maximum	141.25		104.50		90.75	
Mean	108.96		66.66		61.34	

SD: standard deviation; hg: homogeneous groups; values followed by different small letters in the same column are significantly different ($p < 0.05$).

Table 2. Mean, standard deviation, and homogeneous group distribution of the 15 yeast strains based on fermentation vigour (g 100 mL^{-1}) after 3 days of fermentation in pasteurised must from black grapes elaborated by StatGraphics using *Least Significant Difference* method with 95% of significance

Strain	Mean ± SD	hg
Sc2731	8.17 ± 0.37	cdef
Sc2734	7.08 ± 0.13	ab
Sc2742	8.50 ± 0.44	def
Sc2750	7.33 ± 0.53	abc
Sc2751	8.50 ± 0.56	def
Sc2754	6.63 ± 1.25	a
Sc2756	8.82 ± 0.24	ef
Sc2760	8.73 ± 0.33	ef
Sc2762	7.75 ± 0.22	bcd
Sc2773	6.92 ± 0.15	ab
Sc2774	8.50 ± 0.17	def
Sc2776	6.68 ± 0.34	a
Sc2803	9.07 ± 0.24	f
Sc2814	6.52 ± 1.17	a
Sc2823	8.13 ± 0.35	cde
Minimum	6.52	
Maximum	9.07	
Mean	7.82	

SD: standard deviation; hg: homogeneous groups; values followed by different small letters in the same column are significantly different ($p < 0.05$).

Table 3. Mean, standard deviation, and homogeneous group distribution of the 15 yeast strains based on yellow (420 nm), red (520 nm) and blue (620 nm) components of the experimental wines elaborated by StatGraphics using *Least Significant Difference* method with 95% of significance

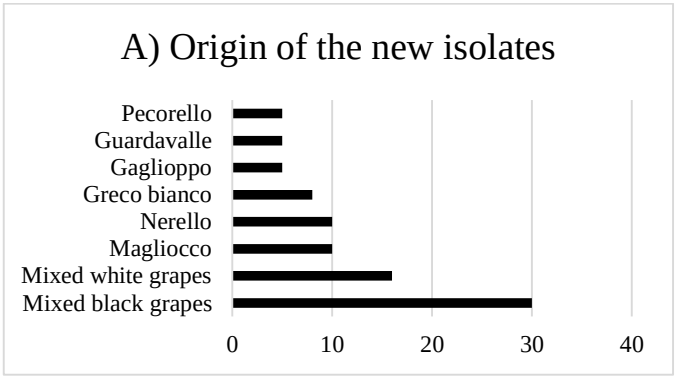
	Wine colour parameters					
Strain	420 nm	hg	520 nm	hg	620 nm	hg
Sc2731	2.977 ± 0.02	gh	1.772 ± 0.01	gh	0.787 ± 0.01	g
Sc2734	2.162 ± 0.11	c	1.280 ± 0.07	c	0.527 ± 0.04	d
Sc2742	2.957 ± 0.13	gh	1.735 ± 0.11	fgh	0.768 ± 0.05	fg
Sc2750	2.400 ± 0.06	d	1.430 ± 0.03	d	0.643 ± 0.01	e
Sc2751	2.740 ± 0.05	ef	1.703 ± 0.02	efg	0.778 ± 0.00	g
Sc2754	1.260 ± 0.19	a	0.782 ± 0.14	a	0.330 ± 0.06	b
Sc2756	3.078 ± 0.15	h	1.853 ± 0.04	hi	0.825 ± 0.04	gh
Sc2760	1.648 ± 0.16	b	0.995 ± 0.11	b	0.428 ± 0.06	c
Sc2762	1.118 ± 0.05	a	0.703 ± 0.03	a	0.243 ± 0.03	a
Sc2773	2.960 ± 0.03	gh	1.813 ± 0.03	ghi	0.808 ± 0.03	g
Sc2774	3.140 ± 0.18	h	1.918 ± 0.10	i	0.913 ± 0.05	i
Sc2776	3.060 ± 0.20	h	1.813 ± 0.11	ghi	0.893 ± 0.07	hi
Sc2803	2.128 ± 0.10	c	1.278 ± 0.03	c	0.553 ± 0.04	d
Sc2814	2.793 ± 0.23	fg	1.627 ± 0.13	ef	0.760 ± 0.08	fg
Sc2823	2.547 ± 0.03	de	1.573 ± 0.00	e	0.693 ± 0.01	ef
Minimum	1.118		0.703		0.243	
Maximum	3.140		1.918		0.913	
Mean	2.465		1.485		0.663	

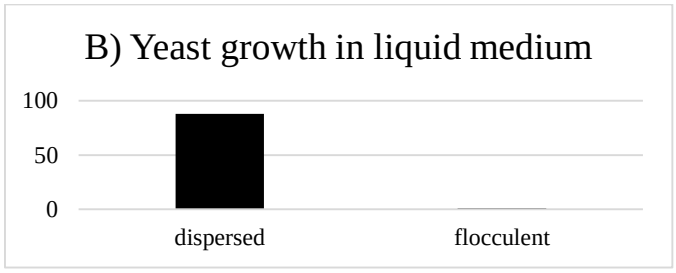
SD: standard deviation; hg: homogeneous groups; values followed by different small letters in the same column are significantly different ($p < 0.05$).

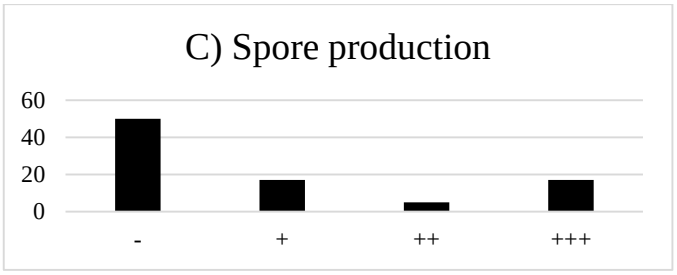
Table 4. Mean, standard deviation, and homogeneous group distribution of the 15 yeast strains based on colour intensity and tint and on total phenolic content, evaluated by Folin-Ciocalteu index, of the experimental wines elaborated by StatGraphics using *Least Significant Difference* method with 95% of significance

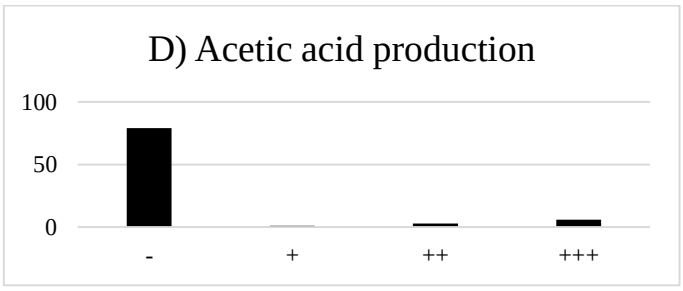
Wine colour parameters						
Strain	Intensity	hg	Tint	hg	Folin-Ciocalteu index	hg
Sc2731	5.535 ± 0.04	fg	1.680 ± 0.00	efgh	15.63 ± 0.91	de
Sc2734	3.968 ± 0.21	c	1.689 ± 0.01	fgh	14.48 ± 0.79	bcd
Sc2742	5.460 ± 0.29	fg	1.706 ± 0.05	gh	17.30 ± 0.96	f
Sc2750	4.473 ± 0.09	d	1.678 ± 0.00	efgh	15.32 ± 1.00	cde
Sc2751	5.221 ± 0.07	ef	1.608 ± 0.01	ab	13.72 ± 0.61	b
Sc2754	2.372 ± 0.39	a	1.618 ± 0.05	abc	11.32 ± 0.70	a
Sc2756	5.757 ± 0.23	gh	1.660 ± 0.04	cdefg	16.45 ± 0.61	ef
Sc2760	3.071 ± 0.34	b	1.659 ± 0.03	cdef	14.40 ± 0.73	bcd
Sc2762	2.065 ± 0.09	a	1.590 ± 0.03	a	13.78 ± 0.71	b
Sc2773	5.581 ± 0.08	fgh	1.632 ± 0.01	abcd	15.87 ± 1.07	e
Sc2774	5.971 ± 0.33	h	1.637 ± 0.00	bcde	15.67 ± 0.83	de
Sc2776	5.767 ± 0.38	gh	1.687 ± 0.01	fgh	13.30 ± 0.61	b
Sc2803	3.958 ± 0.17	c	1.664 ± 0.04	defg	15.50 ± 0.15	cde
Sc2814	5.180 ± 0.44	ef	1.717 ± 0.003	h	14.15 ± 1.05	bc
Sc2823	4.812 ± 0.04	de	1.619 ± 0.02	abc	15.57 ± 0.95	de
Minimum	2.065		1.590		11.32	
Maximum	5.971		1.717		17.30	
Mean	4.613		1.656		14.83	

SD: standard deviation; hg: homogeneous groups; values followed by different small letters in the same column are significantly different ($p < 0.05$).









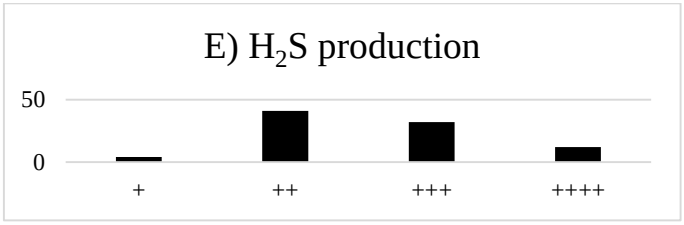


Fig. 1. Frequency groups of the 89 new isolates studied for: A) Origin of the new isolates; B) Yeast growth in liquid medium; C) Spore production; D) Acetic acid production; E) H_2S production.