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Exploring the Biodiversity of Red Yeasts for In Vitro and In Vivo Phenotypes Relevant to Agri-Food-Related Processes

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Abstract: Red yeasts grow on food wastes, show antagonistic activity against food-spoilage microorganisms, produce food supplements, and may be utilized as feed-supplements themselves to positively modulate the quali-quantitative composition of intestinal microbiota. Therefore, they show a variety of possible biotechnological applications in agri-food-related processes. Here, to further explore the biotechnological potential of red yeasts, eleven strains ascribed to different species of the genera *Rhodotorula* and *Sporobolomyces*, differing for biomass and carotenoids production, were characterized in vitro for biofilm formation, invasive growth, and growth at the temperature range of 20–40 °C and in vivo for their antagonistic activity against the fungal pathogen and patulin producer *Penicillium expansum*. Most of them formed cellular MAT and showed invasive growth as well as adhesion to plastic materials. Four strains determined a significant reduction of fruit decay caused by *P. expansum* on apple fruit while the remaining seven showed different degrees of biocontrol activity. Finally, none of them grew at body temperature (>37 °C). Statistical analyses of both qualitative and quantitative phenotypic data, including biomass and carotenoids production, gathered further information on the most interesting strains for the biotechnological exploitation of red yeasts in agri-food-related process.

Keywords: biofilm formation; biocontrol; *Rhodotorula*; *Sporobolomyces*; MAT

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1. Introduction

Red yeasts, mainly ascribed to the genera *Rhodotorula* and *Sporobolomyces* within Pucciniomycotina, are well known producers of microbial oils, surfactants, and enzymes of interest for chemical and pharmaceutical industries and carotenoids [1–3]. In the food sector, the growing interest in the biotechnological exploitation of red yeasts is due to their production of carotenoids. These compounds have important biological properties such as antioxidant, photoprotective [4,5], and putative antitumor activities [6]. Accordingly, the demand for carotenoid-containing food products, such as red soy cheese, meat, alcoholic beverages, and by-products of meat and fish, is globally increasing (https://www.factmr.com/report/1196/carotenoids-market, accessed on 1 December, 2020).

Even though carotenoids may be chemically synthesized, rising health concerns against artificial synthetic pigments render their production through microbial-based technologies particularly attractive. Of particular interest is the possibility to obtain these metabolites from by-products and wastes, thus increasing the sustainability of their production. In this respect, some of the red yeasts grow at high cell density on a plethora of different carbon and nitrogen sources and they are generally characterized by tolerance to inhibitory compounds present in growth substrates obtained from wastes of the food industry [7]. Moreover, red yeasts could be efficiently used for the bio-preservation of foods during storage. *Rhodotorula mucilaginosa* counteracts *Penicillium expansum* and *Botrytis cinerea* on apple [8] and *Rhizopus* sp. on strawberries [9]. *Rhodotorula glutinis* presents

biocontrol activity against *B. cinerea* [10–12], *P. expansum* [13], *Alternaria alternata* [14], and *Rhizopus stolonifer* [15]. Similarly, *Sporobolomyces roseus* and *Sporobolomyces kratochvilovae* were described as possible biocontrol agents against *B. cinerea* and *P. expansum*, respectively, on apple fruit [16,17]. The mechanisms involved in yeast biocontrol activity have been elucidated, at least in part in a number of different species [18,19]. With respect to red yeasts, *S. roseus* was shown to antagonize *B. cinerea* by competing for nutrients [16], while *Rhodosporidium toruloides* and *R. glutinis* biocontrol activity is mediated by their attachment to *B. cinerea* through the involvement of cell wall glycoproteins [12,20]. Pandin et al. [21] indicated biofilm forming ability as one of the characters to be evaluated for a rapid screening of biocontrol agents. On the other hand, transition to a filamentous form of growth, which is required for both biofilm formation and invasive growth, has been correlated to yeast pathogenesis on immune-compromised individuals [22]. Thus, the evaluation of yeast ability to grow at the body temperature and to switch to an invasive form of growth is of particular importance given the fact that red yeast species have been proposed directly as feed supplements [23,24].

Due to their vast biotechnological potential, the molecular tools for heterologous genes expression in red yeasts were developed and, recently, an extensive study of the codon bias in these yeasts was carried out in view of the optimization of the heterologous gene sequences for transgene expression [25].

Here, in order to further explore the biotechnological potential of red yeasts, eleven *Rhodotorula* and *Sporobolomyces* strains already characterized for biomass and carotenoids production [26] were further characterized in vitro for biofilm forming ability, invasive growth and growth temperature interval, and in vivo for their antagonistic activity against *P. expansum* on apple.

2. Materials and Methods

2.1. Microorganisms and Culture Media

The *Rhodotorula* and *Sporobolomyces* strains utilized and their origins are reported in Table 1. Strain DiSVAC2.5t1 is deposited at the Industrial Yeast Collection of the University of Perugia, with accession number DBVPG 10619. DiSVA C71t0 belongs to the Yeast Collection of Dipartimento di Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche. The remaining strains were purchased by Centraalbureau voor Schimmelcultures and the Industrial Yeast Collection of the University of Perugia. All strains were previously characterized for the production of biomass and carotenoids [26] and strain DiSVAC2.5t1 was subject to genomic [27,28] and proteomic analyses [29].

Table 1. Yeast strains utilized.

Strain	Species
DBVPG6091	*Rhodotorula mucilaginosa*
DBVPG6094	*Rhodotorula mucilaginosa*
DiSVAC2.5t1	*Rhodotorula mucilaginosa*
DBVPG7019	*Rhodotorula mucilaginosa*
DiSVAC71t0	*Rhodotorula mucilaginosa*
CBS2366	*Rhodotorula glutinis*
CBS2367	*Rhodotorula glutinis*
CBS6016	*Rhodotorula toruloides*
CBS315	*Rhodotorula toruloides*
CBS7228	*Sporobolomyces oryzicola*
CBS7998	*Rhodotorula cresolica*

DBVPG: Industrial Yeast Collection, University of Perugia, Perugia, Italy; DiSVA: Yeast Collection of Dipartimento di Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche, Ancona, Italy; CBS: Centraalbureau voor Schimmelcultures (CBS) Utrecht, The Netherlands.

Media used were YEPD: 1% yeast extract, 2% peptone, 2% glucose; YEPDagar: as YEPD added with agar at the percentage indicated in subscript; $SCD_{2\%}$: 0.67% Yeast

Nitrogen Base w/o amino acids, 2% glucose; $SCD_{0.1\%}$: 0.67% Yeast Nitrogen Base w/o amino acids, 0.1% glucose; SCGly: 0.67% Yeast Nitrogen Base w/o amino acids, 2% glycerol.

2.2. Adhesion to Plastic Materials

Adhesion to plastic material was evaluated as described by [30]. Briefly, yeast strains were precultured in 50 mL $SCD_{2\%}$ within 250 mL baffled flasks at 30 °C under shacking conditions (180 rpm). After 12 h cells were centrifuged (3000 rpm), washed and resuspended in sterile distilled water, and inoculated in $SCD_{0.1\%}$ to an OD_{600} of 1.0. Three replicates of 100 µL aliquots of cell suspension were deposited in 96 wells microtiter polystyrene, polypropylene and polyvinyl chloride plates and incubated in static at 30 °C. After 12 h, 100 µL of 1% crystal violet was added to each well and after 20 min wells were rinsed repeatedly with sterile distilled water and dabbed with absorbent paper. A total of 100 µL 0.1 SDS was added to each well and, after 25 min at 25 °C, all samples were transferred to a new microtiter plate to evaluate absorbance at 570 nm (Abs_{570}) and 590 nm (Abs_{590}). *Saccharomyces cerevisiae* strains UNISSM25 (MATa/MATa HO/HO HMRa/HMRa HMLa/HMLa) and UNISS3238-32Δ*flo11* (MATα *leu2-1 lys2-801 flo11Δ::URA3 ura3-52*), whose adhesion to plastic materials had already been described [31,32], were utilized as positive and negative controls of adhesion, respectively. Both strains belong to the Microbial Culture Collection of the University of Sassari, Sassari, Italy.

2.3. Cell MAT Formation

Cell MAT formation was evaluated by inoculating 5 µL of cell suspensions (OD_{600} 0.5) in the center of Petri dishes containing $YEPD_{0.3\%}$, $YEPD_{2\%}$, $YEPD_{4\%}$. At least three replicates for each plate were prepared. The plates were incubated with yeast inoculum side up for 15 days at 25 °C after which cell MATs were photographed with a digital camera.

2.4. Invasive Growth

One-mL aliquots of yeast cultures inoculated in $SCD_{2\%}$ and incubated for 12 h at 180 rpm at 30 °C were centrifuged at 14,000× *g* 1 min a 20 °C. The cell pellet was resuspended in 1 mL sterile distilled water to OD_{600} of 0.5 and 10 µL aliquots were spotted on $SCD_{0.1\%}$ and incubated at 25 °C. After 10 days, each plate was photographed with a digital camera before and after washing with sterile distilled water.

2.5. Growth at Different Temperatures

Growth at different temperatures was evaluated by means of spot assay on $YEPD_{2\%}$, incubated at 20, 25, 30, 35, and 37 °C. Briefly, yeast strains were precultured in $YEPD_{2\%}$ and a small aliquot of cell biomass was diluted in sterile distilled water to OD_{600} of 5. Cell suspensions were serially diluted (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}) and 4 µL of each dilution were seeded on agar medium. Yeast growth was evaluated after 48 h.

2.6. Biocontrol In Vivo

Apple fruits (*Malus domestica* Borkh. cvs Golden Delicious) were washed with sodium hypochlorite (0.8% as chlorine), rinsed with tap water, and air dried. Three wounds (3 mm depth) were engraved with a sterile micropipette tip at the equatorial region of each fruit and 1×10^6 yeast cells were inoculated into each wound. After 2 h at 26 °C, each wound was inoculated with by pipetting 10 µL of a conidial suspension of *P. expansum* (1×10^6 conidia/mL). A negative control of infection was set by pipetting 20 µL of sterile Ringer solution into each wound. To confirm the pathogenicity of the fungal strain, a positive control was obtained by pipetting 10 µL of Ringer solution and after 2 h 10 µL of a conidial suspension of *P. expansum*. Upon air drying, the fruits were placed in plastic holders (60 cm × 40 cm × 15 cm, one layer), wrapped in transparent polyethylene bags to prevent evaporation, and stored in the dark at 26 °C and 85 ± 5% relative humidity. The diameter of decayed tissue around each wound was measured at 48 h intervals to evaluate the incidence of *P. expansum* infection. There were 9 apples per treatment, the

treatments were arranged in a completely randomized block design, and each experiment was repeated three times. Results are expressed as percentage of decay reduction (means ± standard deviation of three independent replicates of three biological samples) with respect to the inoculated control after 7 days of incubation.

2.7. Statistical Analysis

The significance of differences among quantitative phenotypic data was assessed using one-way ANOVA, followed by multiple comparison by Dunnett's test ($p < 0.05$), using Minitab® for Windows release 12.1 (Minitab LLC, State College, Pennsylvania, USA). For each yeast strain, the significance of difference in the diameter of decayed tissue of the inoculated sample with respect to the control, was assessed by *t*-test ($p < 0.05$). To analyze the similarity between the tested yeast strains, a factor analysis of mixed data (FAMD) was carried out by taking into account both qualitative and quantitative phenotypic variables. To get further insights into the similarities between observations, the factors derived out of FAMD where used to conduct a k-means cluster analysis. All the calculations were carried out using the R software ver. 3.6.3 (The R Foundation for Statistical Computing, Vienna, Austria) and the R-packages "FactoMineR" [33] and "factoextra" [34].

3. Results and Discussion

3.1. Biofilm Forming Ability

The ability of yeast to form biofilm on biotic and abiotic surfaces is a phenotypic character with both negative and positive implications. It is undesired within the clinical realm [35–37] but also in food processing and drinking water distribution systems [38–40]. In contrast, biofilm formation is required in some food processes including the maturation of cheeses and sausages [41–43] and the biological aging of wine [44]. In addition, biofilm forming ability is required for the cleaning up of hazardous waste sites [45] and for heavy metal removal [46].

Since in *S. cerevisiae* biofilm forming ability is investigated through the evaluation of cellular MAT formation and adhesion to plastic materials [30], these phenotypes were analyzed in 11 red yeasts of different origins (Table 1). Cellular MAT formation, which refers to the production of large colonies on the surface of low density agar [47], was evaluated onto $YEPD_{0.3\%}$, $YEPD_{2\%}$, and $YEPD_{4\%}$ [48]. According to [48], on $YEPD_{0.3\%}$, it is possible to observe colony spreading and the formation of the radial spoke pattern. On $YEPD_{2\%}$, the architecture of MAT is revealed while on $YEPD_{4\%}$, dense and complex MAT with ruffling morphology may appear. With the exception of *R. glutinis* CBS2367, all strains produced cellular MAT on $YEPD_{0.3\%}$ (Figure 1) with variable colony expansion and architecture. In particular, *R. mucilaginosa* DBVPG6091, DiSVAC71t0, and DiSVAC2.5t1, *S. oryzicola* CBS7228 and *R. cresolica* CBS7998 formed wide MATs (diameter $\geq$ 4 cm) with a smooth hub and a peripheral smooth ring. *R. mucilaginosa* DBVPG7019 and *R. toruloides* CBS315 produced smaller MATs (diameter 3.71 ± 0.43 and 3.24 ± 0.34 cm, respectively) but these were characterized by a more complex architecture. That of DBVPG7019 showed a ruffed central hub surrounded by irregular radial spoke pattern and peripheral ring. That of *R. toruloides* CBS315 was characterized by a small elevated central hub, an evident radial spoke pattern and an irregular peripheral ring. *R. mucilaginosa* DBVPG6094 and *R. toruloides* CBS6016 produced MATs with diameter $\leq$ 3 cm with ruffled hub, radial spoke patterns, and jagged peripheral ring. *R. glutinis* CBS2366 MAT measured about 2 cm and was smooth. At higher agar percentages in YEPD, MAT size decreased while their density increased [48]. Thus, similar to *S. cerevisiae*, moisture and strength of the substrate affected MAT size and density.

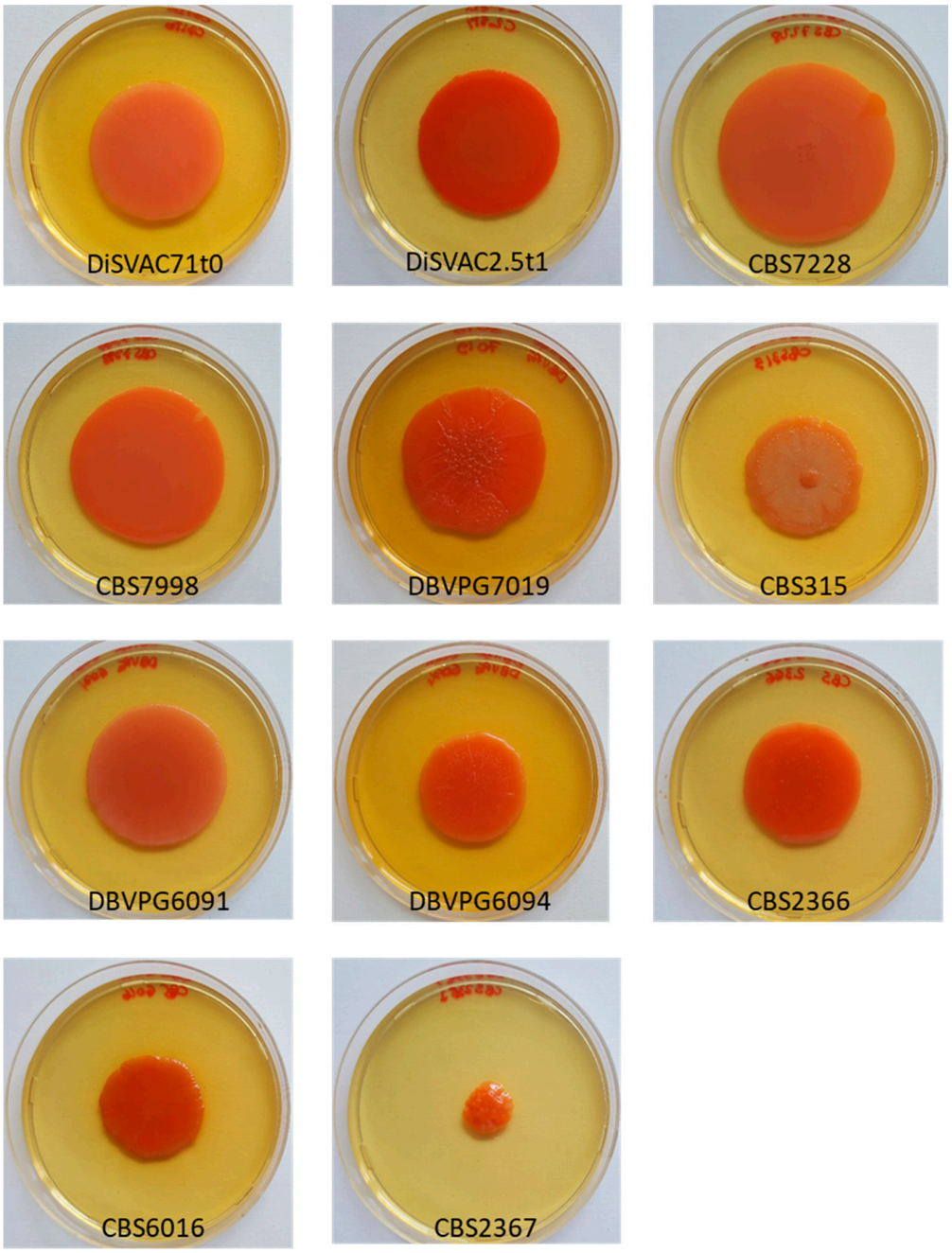

Figure 1. MAT morphology on $YEPD_{0.3\%}$. Cells growing in YEPD were washed, resuspended in sterile distilled water, and 5 µL of each cell suspension were spotted on $YEPD_{0.3\%}$. After 15 days of incubation at 25 °C, plates were photographed with a digital camera. Results are representative of three independent replicates.

Cellular MAT formation involves cell–cell adhesion and adhesion to the surface of the growth medium. In *S. cerevisiae*, Flo11p is among the factors responsible for MATs formation [49] and architecture [50,51] but it is involved also in the adhesion to plastic surfaces [49]. The mechanisms implicated in adhesion to solid surfaces are scarcely characterized in *Rhodotorula* and *Sporobolomyces*. Buck and Andrews [20] reported that adhesion to polystyrene (PS) is mediated by the production of a thick layer of mucilage positively charged and localized over developing buds in *R. toruloides*.

Here, to gather further information on red yeast biodiversity in terms of biofilm forming ability, adhesion was evaluated on: polystyrene (PS), polypropylene (PP), and

polyvinyl chloride (PVC). These three plastic materials are utilized for different purposes. PS is used mainly for foodservice containers; PP, which is characterized by high mechanical strength, is utilized in the production of laboratory (test tubes, beaker, centrifuge tubes) and medical devices (cannulas, syringes, oxygenators, hoses); and PVC is used for medical devices (catheters, prostheses, etc.) and for plumbing water systems. Thus, the 11 yeasts were grown in low glucose containing medium and adherence was tested on PS, PP, and PVC microplates. For this experiment, two strains of *S. cerevisiae*, namely UNISSM25 and UNISS3238-32Δ*flo11*, were utilized as positive and negative controls of adhesion, respectively (Table 2). UNISSM25 is a wild flor strain, widely characterized for its biofilm forming ability [31], while UNISS3238-32Δ*flo11* is a non-biofilm-forming mutant [32,52].

Table 2. Adhesion to plastic materials.

Strain	Abs_{570} (Mean ± Std)		
	PS	PP	PVC
DBVPG6091	2.67 ± 0.32	3.43 ± 0.10	0.82 ± 0.13
DBVPG6094	1.21 ± 0.05	1.32 ± 0.07	1.64 ± 0.21
DBVPG7019	2.10 ± 0.47	0.88 ± 0.13	2.30 ± 0.56
DiSVAC2.5t1	2.75 ± 0.13	2.93 ± 0.32	2.56 ± 0.12
DiSVAC71t0	3.16 ± 0.13	3.17 ± 0.06	3.08 ± 0.28
CBS2366	1.26 ± 0.12	1.47 ± 0.15	1.57 ± 0.14
CBS2367	1.50 ± 0.63	2.55 ± 0.30	1.77 ± 0.15
CBS6016	3.44 ± 0.04	3.40 ± 0.04	3.50 ± 0.00
CBS315	2.18 ± 0.33	2.05 ± 0.18	1.85 ± 0.13
CBS7228	2.11 ± 0.14	2.23 ± 0.40	2.14 ± 0.32
CBS7998	0.88 ± 0.32	1.21 ± 0.09	1.70 ± 0.35
UNISSM25	2.68 ± 0.06	2.71 ± 0.12	3.21 ± 0.21
UNISS3238-32 Δ*flo11*	0.48 ± 0.03	1.02 ± 0.12	1.11 ± 0.19

Notes: Data are mean ± standard deviation of three independent experiments. UNISSM25 and UNISS3238-32Δ*flo11* were utilized as positive and negative controls of adhesion, respectively.

Results, reported in Table 2, show that some of the strains, although differing in the degree of adhesion, presented a consistent phenotype on the three plastic materials. Among these, *R. mucilaginosa* DiSVAC71t0 and *R. toruloides* CBS6016 showed the overall strongest adhesion with $Abs_{570} > 3$. This was higher than that of the positive control *S. cerevisiae* M25. *R. mucilaginosa* DiSVAC2.5t1 and *S. oryzicola* CBS7228, which displayed adhesion values in the range of those expressed by M25 ($Abs_{570} > 2$), were followed by *R. mucilaginosa* DBVPG6094 and *R. glutinis* CBS2366 ($Abs_{570} < 2$). The remaining strains presented a variable phenotype, depending on the plastic material. In particular, *R. mucilaginosa* DBVPG6091 proved strongly adhesive to PP, but it showed lower adhesion to PS and very weak adhesion to PVC. *R. glutinis* CBS2367 showed stronger adhesion to PP than to PS and PVC and *R. glutinis* CBS7998 showed very weak adhesion to PS. *R. mucilaginosa* DBVPG7019 showed $Abs_{570} > 2$ on PS and PVC and very low adhesion to PP while *R. toruloides* CBS315 was characterized by Abs_{570} around 2 on the three plastic materials.

3.2. Invasive Growth and Temperature Growth Interval

Invasive growth is described as the production of filaments that penetrate the surface of agar plates and resist to washing of the agar medium surface. This phenotype was evaluated in vitro on low glucose containing medium ($SCD_{0.1\%}$) as suggested by [48]. On this medium, all strains, with the exception of CBS7228 and DiSVAC71t0, showed invasive growth with CBS6016 showing the strongest phenotype (Figure 2). Invasive growth has been associated with fruit decay in *Pichia fermentans*, thus raising the problem of the biological risk associated with the use of dimorphic yeasts as biocontrol agents [53]. Moreover, it is considered a putative virulence character in clinically relevant yeast [54,55]. Thus, while evaluating the growth temperature interval of these red yeasts, also temperatures compatible with human body infections were considered and the 11 yeast strains were

tested for growth at 20, 25, 30, 37, and 40 °C. *R. mucilaginosa* DBVPG7019, DiSVAC71t0, DiSVAC2.5t1, *R. toruloides* CBS6016, CBS315, *S. oryzicola* CBS7228 grew at 20 °C and all strains grew easily at 25 °C. CBS2366 and CBS2367 failed to grow at 30 °C and none of them grew at 37 and 40 °C (Table 3), thus suggesting the incapability of these strains to infect humans [56].

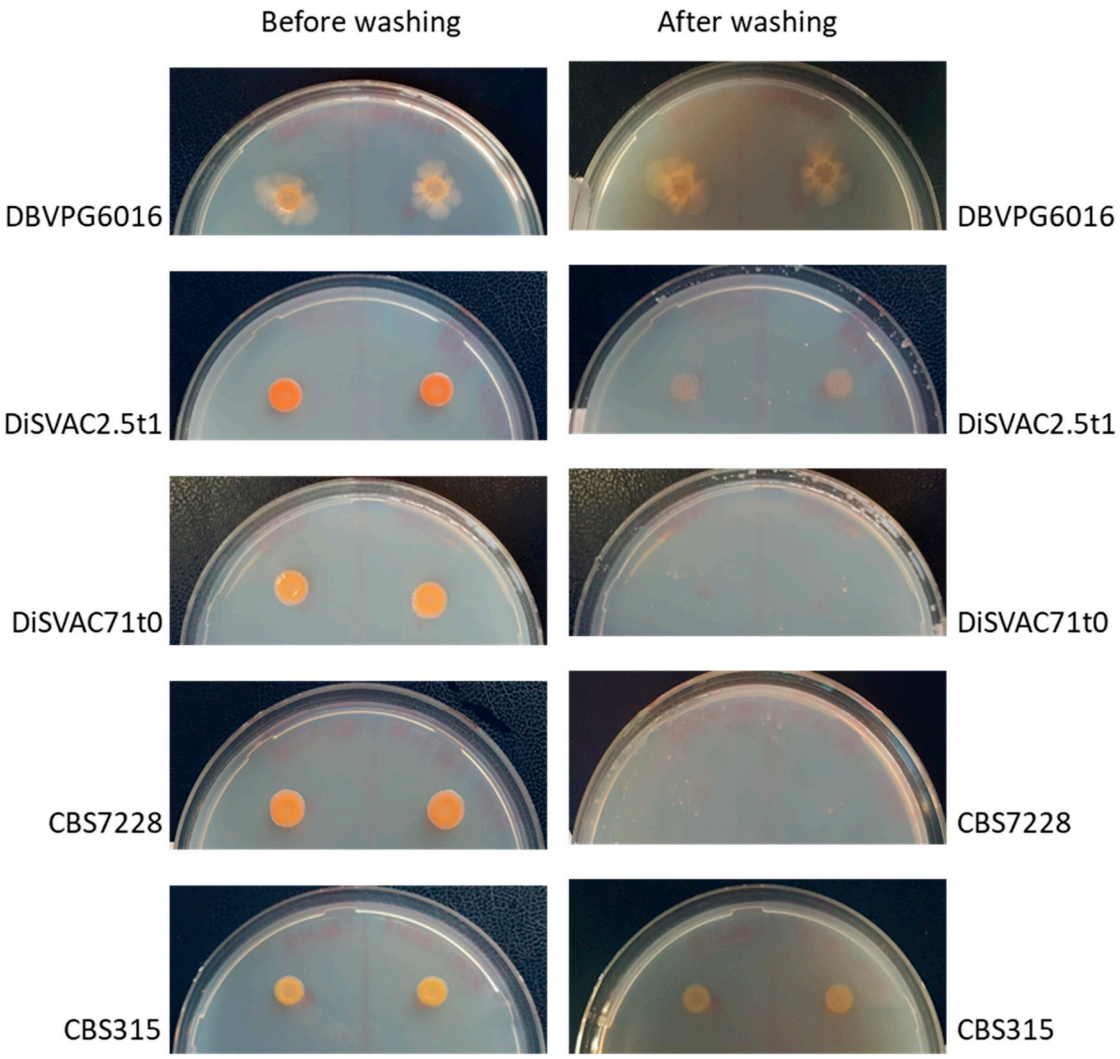

Figure 2. Invasive growth of strains representative of different phenotypes. Cells exponentially growing in $SCD_{2\%}$ were washed, resuspended in sterile distilled water, spotted on $SCD_{0.1\%}$, and incubated at 25 °C per 10 days. Petri dishes were photographed with a digital camera before and after washing with sterile distilled water. Results presented are representative of three independent replicates.

Table 3. Temperature growth interval.

Strain	Temperature (°C)				
	20	**25**	**30**	**37**	**40**
DBVPG7019	+	+	+	-	-
CBS7228	+	+	+	-	-
CBS315	+	+	+	-	-
CBS2366	-	+	-	-	-
DBVPG6094	-	+	w	-	-

Table 3. *Cont.*

Strain	Temperature (°C)				
	20	**25**	**30**	**37**	**40**
DBVPG6091	-	+	+	-	-
CBS7899	-	+	+	-	-
CBS2367	-	+	-	-	-
CBS6016	+	+	w	-	-
DiSVAC71t0	+	+	+	-	-
DiSVAC2.5t1	+	+	+	-	-

Notes: +, growth; -, no growth; w, weak growth.

3.3. Biocontrol Activity on Apple Fruit

Once inoculated into wounds artificially inflicted to apple fruit and infected with *P. expansum*, the 11 strains proved highly diverse in terms of biocontrol activity. With the only exception of CBS2366, all the other strains presented significant reductions of the diameter of the lesions in respect to the control ($p < 0.05$). The most promising were *R. mucilaginosa* DiSVAC71t0 and *S. oryzicola* CBS7228 which determined more than >50% reduction of *P. expansum* decay on apple. These were followed by *R. mucilaginosa* DBVPG6094 and DiSVAC2.5t1 which caused a decay reduction ≥ 40%. *R. mucilaginosa* DBVPG6091 and DBVPG7019 and *R. toruloides* CBS6016 and CBS315 showed a percentage of decay reduction >20 <40 and the remaining strains showed less than 20% reduction of *P. expansum* lesions on apple fruit (Figure 3). Other red yeasts reduced significantly the incidence of plant pathogenic fungi. For example, *R. glutinis* proved effective against *Botrytis cinerea* on strawberries and apples (US patent US5525132A) [57,58] possibly due to the attachment capability of the antagonistic *R. glutinis* to *B. cinerea* [59]. *R. glutinis* was also applied in combination with rhamnolipids to be more efficacious against *A. alternata* infection in cherry tomato fruit than either agent alone [60].

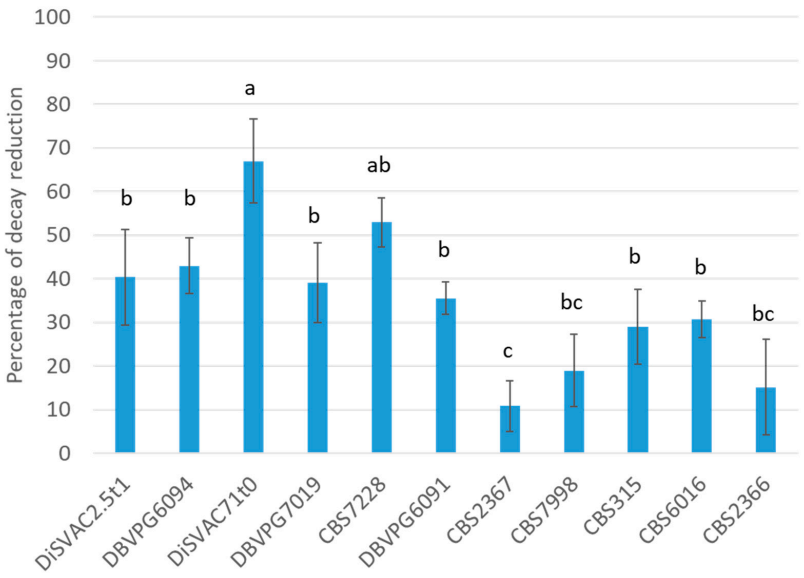

Figure 3. Biocontrol activity on apple fruit. Apple fruits were wounded (three wounds/fruit) along the equatorial region, inoculated with yeast cells and *P. expansum*, and stored in the dark at 26 °C and 85 ± 5% relative humidity for up to 7 days. The diameter of decayed tissue around each wound was measured at 48 h intervals to evaluate the incidence of *P. expansum* infection. Results are expressed as percentage of decay reduction (means ± standard deviation of three independent replicates of three biological samples) with respect to the inoculated control. Same superscript letters indicate results not significantly different among strains ($p < 0.05$).

3.4. FAMD and Cluster Analysis of Phenotypic Data

To summarize the information about the phenotypes studied and to allow the identification of red yeast strains and/or phenotypes for future works aimed at red yeasts exploitation in food biotechnological processes, FAMD followed by k-means cluster analyses were carried out (Figure 4A). Variables analyzed also included biomass and carotenoids production [26]. The average silhouette approach estimated the presence of 5 optimal clusters. Of these, cluster 4 aggregates most of the strains, while clusters 2 and 3 are each constituted by only one strain. In accordance with the contribution of the phenotypic variables to the first and second dimension (Figure 4B), strains grouped in cluster 5 (*S. oryzicola* CBS7228 and *R. mucilaginosa* DiSVAC2.5t1 and DiSVAC71t0) displayed the highest biocontrol activity (≥40% blue mold reduction) and among the strongest adhesion phenotypes on plastic materials (particularly on PVC), hence suggesting a possible means of high throughput in vitro selection for potentially effective biocontrol agents.

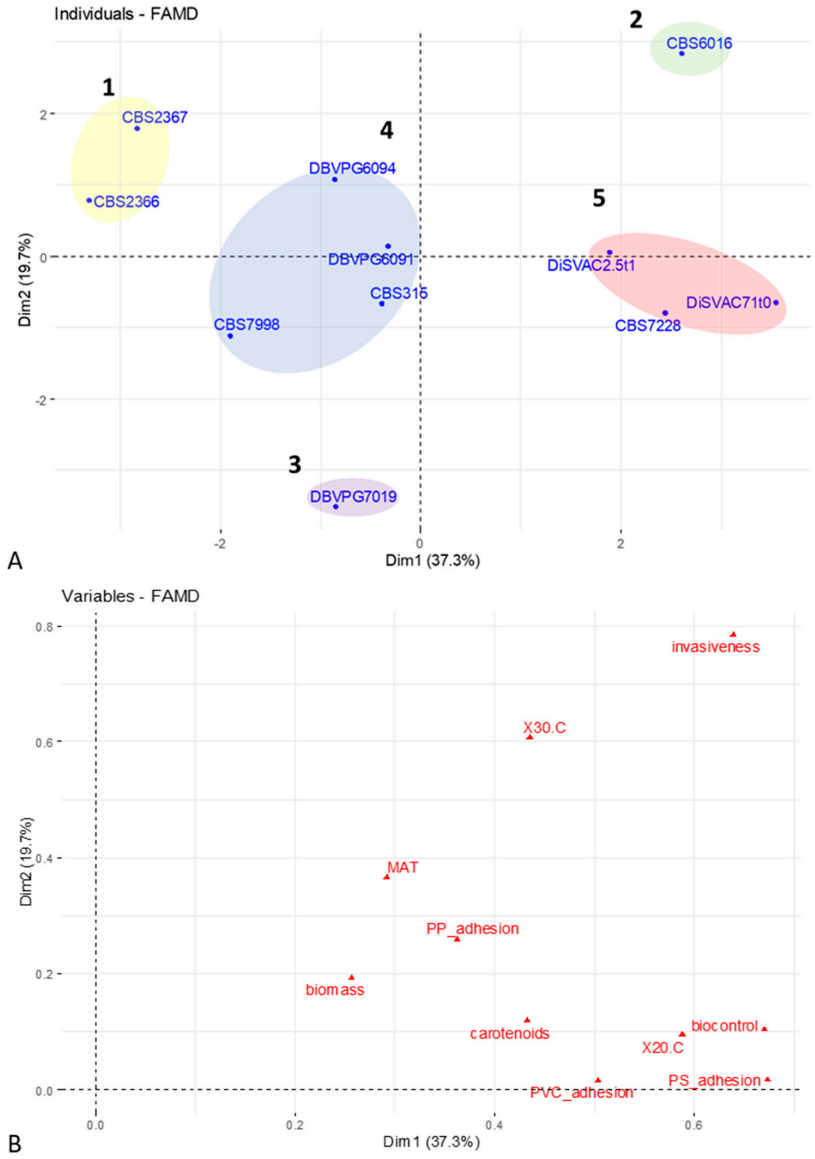

Figure 4. FAMD and cluster analysis of phenotypic data. (**A**): FAMD of the first two dimensions (total explained variance equal to 57%). Individuals with similar profiles are close to each other on the factor map and are enclosed in ellipses of the same color, as determined after k-means clustering. (**B**): Plot of variables on the first two dimension (total explained variance equal to 57%).

As recently reviewed by [40], yeast biofilm formation in food and food processing plants could be considered as a positive or negative phenotype. Here, the high biofilm forming ability coupled with the high carotenoid content of the strains in cluster 5 suggests their possible utilization for the continuous production of carotenoids in bioreactors where yeast cells are immobilized on inert surfaces [61]. In addition, the high biomass obtained by growing these strains on glycerol suggests the use of these strains for the valorization of such by-products, thus increasing the sustainability of carotenoid production [26]. In this respect, the lack of invasiveness of CBS7228, together with its biocontrol activity and adhesion to plastic materials, makes this strain the most interesting for future analyses. Indeed, as already reported, invasiveness has been thought to contribute to yeast virulence and fruit decay [19]. Strain CBS6016 (cluster 2), while sharing many of the properties of the yeasts included in cluster 5, shows a higher invasiveness and is, thus, less suitable for the food sector. In cases such as those in which biocontrol activity should be associated to low biofilm forming ability and scarce invasiveness, strain DBVPG7019 (cluster 3) should be preferred for further characterizations. Finally, strains included in cluster 1 appear as the less interesting for biotechnological applications, given their inability to grow at 20 and 30 °C, and their reduced production of carotenoids and scarce biocontrol activity. Similarly, strains in cluster 4 seem to have a limited biotechnological potential as they show average values for what concerns MAT formation, adhesion on PVC, invasiveness, biocontrol activity, and carotenoid production.

4. Conclusions

Phenotypic analyses of eleven red yeasts of different origins suggested that a comprehensive characterization of these yeasts is mandatory to assess their potential use in food processes. Besides carotenoids production and biocontrol activities, also phenotypes not previously evaluated on red yeast are indeed essential for the identification of the most suitable biotechnological process for their exploitation. Particularly, biofilm formation and invasiveness should be carefully evaluated before selecting strains for continuous production of carotenoids or as biocontrol agents and feed supplements, respectively. In addition, the inability of strains to grow at a temperature compatible with human infection should be carefully determined. Finally, statistical analyses allowed the selection of yeast strains with interesting phenotypes for their further characterization and application in agri-food-related processes.

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