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Fruit Microbial Communities of the *Bisucciu* Sardinian Apricot Cultivar (*Prunus armeniaca* L.) as a Reservoir of New Brewing Starter Strains

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Abstract: Local fruit cultivars may improve the originality of specialty beers both directly, by conferring peculiar tastes and flavors, and indirectly, as a reservoir of new starter strains. Accordingly, the fungal and bacterial communities of *Bisucciu* fruit, a Sardinian apricot cultivar used to produce a local fruit beer, were here investigated by culture-dependent methods. From the 16S rDNA and ITS sequence analyses of 68 epiphytic isolates, 5 bacterial species and 19 fungal species were identified. *Aureobasidium pullulans* and *Rhodotorula glutinis* were the dominant fungal species, while *Enterococcus mundtii* (Firmicutes) and *Frigoribacterium faeni* (Actinobacteria) were the most represented species among bacterial isolates. Enrichment cultures of fresh apricot puree, followed by fermentation trials in beer wort and beer wort added with apricot puree, allowed the selection of four isolates of *Pichia kudriavzevii*, *Hanseniaspora uvarum*, *H. pseudoguilliermondii*, and *H. clermontiae* able to dominate over the *Bisucciu* native microbiota and to produce from 0.57% to 0.74% (vol/vol) of ethanol. HS-SPME-GC/MS analysis highlighted a significant increase in the ester and alcohol fractions as well as a reduction in terpenes after fermentation with the selected yeasts. Results obtained suggest that the yeast isolates may contribute to the definition of the taste and flavor of beers when used in mixed fermentations with *Saccharomyces*.

Keywords: microbiota; fruit beer; biodiversity; apricot

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1. Introduction

Nowadays, the market trend of beer is progressively shifting from mass-produced lager brands to the richer flavors, aromas, and taste of craft beers due to their originality and geographical typicity [1]. In this respect, fruit beers have long captured the attention of brewers, as fruit addition results in peculiar characteristics [2]. A study by McGovern et al. [3] presents 9000-year-old evidence that Neolithic Chinese villagers created a beverage that contained honey, rice, and hawthorn fruits and/or grapes. Another early reference comes from Egypt where both dates and pomegranates were used in brewing [4]. For centuries till nowadays, fruits have been used as beer ingredients in Belgian cherry Lambic ('Kriek') or raspberry Lambic ('Framboise') beers. The main aspect that characterizes fruit beer quality is the variety, the quantity, and the type of addition of fruits [5]. In this respect, the use of local fruit cultivars in brew making introduces the concept of authentical *terroir*, promoting the native identity of craft beers and setting a further split with large multinational beers [6].

Pre-fermentation addition of fruit (as fresh fruit, concentrated juice, or puree) to cold wort maximizes the flavor and aromatic potential while reducing the risk of contamination after bottling, due to the complete consumption of fruit sugars during the primary/secondary fermentation. However, the addition of the potential microbial contaminants in the fruit microbiome to a rich substrate (unfermented wort) may lead to undesired fermentations. Thus, the identification of the native microbiota of fruits to be added to wort is mandatory to successfully manage the production of fruit beers. On the one hand, the presence of potential spoiling/pathogenic microorganisms could affect the overall quality of the beer [7]. Thus, fruits are usually added after physio-chemical treatments suited to reduce their microbial load [2]. On the other hand, microbial epiphytes isolated from above-ground plant tissues showed high biotechnological potential and could represent a source of new strains for beer production [8–10]. Particularly, the levels of esters and alcohols in beer can be increased by the metabolisms of lactic acid bacteria (LAB) and yeasts [11–13]. Indeed, non-*Saccharomyces* yeasts produce diverse flavor profiles in alcoholic beverages [12,14]. Non-*Saccharomyces* yeasts are also used to produce low-alcohol beers in which the fermentation is limited by the low fermentative capacity of these microorganisms [13]. In addition, microbial fermentation is a well-known method to increase the stability of perishable food products. Particularly, LAB fermentation reduces the pH, thus preventing the spoilage of fermented products [15]. Some LABs produce antimicrobial metabolites, such as bacteriocin or bacteriocin-like inhibitory substances, which can enhance the microbial robustness of wort and beer [16].

In this context, the present study provides insight into the taxonomic diversity of the cultivable microorganisms on ripe fruits of the *Bisucciu* Sardinian apricot cultivar, with a particular emphasis on the identification of yeast isolates that could be used as starters to produce fruit beers. Indeed, although several starters for beer production have already been identified, the selection of new strains with valuable biotechnological properties remains a topic of interest [17–19].

2. Materials and Methods

2.1. Fruit Sampling, Apricot Puree, and Malt Wort Preparation

Ripened fruits of the *Bisucciu* Sardinian cultivar of apricot (*Prunus armeniaca* L.) were collected from five trees randomly selected near a standard dry-grown commercial orchard in the north of Sardinia (40°81′60.2″ N; 8°66′69.2″ E). The fruits were harvested aseptically, using gloves and sterile bags, subdivided into three batches, and stored at 5 °C for less than 12 h. Fresh puree (FP) was obtained by cutting, deseeding, mashing, and homogenization of 30 kg of fruits using a mechanical blender (Waring Commercial Heavy-Duty Blender, Kansas, MI, USA). FP was thermally processed at 80 °C for 15 min to obtain a pasteurized puree (PP). The total soluble solids of PP were lowered from 15 to 12° Brix, and the acidity was increased to 3.74 ± 0.1 pH, by adding the required volume of sterile distilled water. For the technological characterization of the yeast isolates used in this study, a malt wort (W) was produced in Porto Conte Ricerche Srl (Alghero, Italy). As grist, 17.5 Kg of Pilsner malt (Thomas Fawcett & Sons, Castlefors, UK), and 2,5 Kg of Carapils malt (Weyermaan, Bamberg, Germany) were used. Mashing and bittering (11 International Bittering Units (IBU)) were done as reported by Fanari et al. [2]. A wort specific for fruit beer brewing (WPP) was prepared by adding 5% of pasteurized puree to malt wort prepared as previously described. pH and Brix degrees of PP, W and WPP were determined by pH-meter (Professional Bench Meter, XS Instruments, Modena, Italy) and refractometer (hand refractometer ATC1-E, ATAGO CO., Tokyo, Japan), respectively.

2.2. Isolation of Fungal and Bacterial Communities of Apricot Fruits

Epiphytic yeasts and bacteria were isolated from 15 fruits randomly chosen from each of three batches (5 fruit per batch). From each fruit, 25 g of the skin were cut with a sterile knife, put in a stomacher bag containing 225 mL of peptone water, and homogenized for 3 min in a stomacher blender (Stomacher Seward 400-Laboratory Blender, Seward, Wor-

thing, West Sussex, UK). To isolate the endophytic microbiota, 15 fruits chosen, as previously described, were washed in sterile distilled water, soaked for three minutes in a 3% sodium hypochlorite solution, rinsed with sterile water, immersed for 1 min in a 70% ethyl alcohol solution, and washed 3 times with sterile distilled water. Twenty-five grams from each sterilized fruit were aseptically mashed and homogenized with a stomacher blender in 225 mL peptone water.

Homogenized samples were serially diluted in peptone water and plated onto Plate Count Agar (PCA, VWR International, BDH Chemicals, Leuven, Belgium) to determine the total mesophilic count, Rose Bengal Agar (RBA, VWR International, BDH chemicals, Leuven, Belgium) to determine the fungal count, Man Rogosa Agar (MRS, VWR International, BDH chemicals, Leuven, Belgium) to determine the lactic acid bacteria (LAB) count, FLAB medium to determine the fructophilic LAB count [20]. PCA and FLAB plates were incubated in aerobiosis for 7 days at 30 °C, MRS plates were incubated in anaerobic conditions (Anaerocult® A, Mercks, Darmstadt, Germany) for 7 days at 30 °C, and RBA plates were incubated for 7 days at 25 °C.

To specifically stimulate the growth of fermentative yeast species in *Bisucciu* fruit, two enrichment culture protocols were carried out by using fresh apricot puree (FP). In the "aerobic growth enrichment" (AGE) protocol, 300 mL flasks filled with 50 mL of FP, to secure avoid headspace with a high level of air/oxygen, were incubated in shaking conditions (150 rpm) at 30 °C for 3 days. In the "fermentation growth enrichment" (FGE) protocol, 300 mL flasks were filled with 300 mL of FP, to avoid any residual headspace, and incubated in static conditions at 25 °C for 3 days. Subsequently, 10 g of puree resulting from AGE and FGE protocols were put in sterile stomacher bags containing 90 mL of buffered peptone water (VWR International, BDH chemicals, Leuven, Belgium) and homogenized for 3 min in a stomacher blender. Then, decimal serial dilutions were set up and 100 µL of each solution were spread in YEPD (yeast extract 1%, peptone 2%, D-glucose 2%) plates and incubated at 30 °C for 7 days.

2.3. Identification of Fungi and Bacteria Isolated from Apricot Fruits

Fungal and bacterial colonies obtained on agar plates after direct isolation and enrichment cultures were classified based on macroscopic (colour, size, shape of the colony, etc.) and microscopic observations, according to Masi et al. [21]. Ten isolates from each of the different colony/cell morphologies identified were re-streaked to obtain pure cultures and stored in YEPD and MRS broth added with 20% of glycerol at − 80 °C until subsequent analyses.

The molecular identification of the isolates was carried out by PCR from purified genomic DNA [22] using the primers W001 (5′-AGAGTTTGATCMTGGCTC-3′) and W002 (5′-GNTACCTTGTTACGACTT-3′) for bacteria [23], and ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) for yeasts [24]. The amplicons obtained were analysed using 1.4% agarose gels, at 100 V for 4.5 h. A 100 bp DNA ladder (Promega Italia S.r.l, Milano, Italy) was used as the molecular weight marker. The gels were examined using Chemi Doc XRS imaging system (Bio-Rad, Hercules, CA, USA). Amplicons were then purified with QIAquick PCR purification kits (Qiagen GmbH, Hilden, Germany) and sequenced at Macrogen (Meibergdreef 57 1105 BA, Amsterdam, the Netherlands). The resulting sequences were compared with those available on the GenBank database using the BLAST program (http://www.ncbi.nih.gov/BLAST/ (accessed on 25 July 2022)). Sequences with ≥97% identity were considered to belong to the same species.

2.4. Fermentation Trials

The fermentative ability of the yeasts isolated after the enrichment cultures of apricot puree was assessed on malt wort (W) and malt wort added with 5% of PP (WPP). Yeasts were precultured in YEPD broth at 25 °C for 24 h in shaking conditions. At the end of the precultures, yeast strains were inoculated at the final concentration of 10^6 cells/mL in 250 mL flasks stopped with airlocks and containing 75 mL of the fermentable substrates (W

and WPP). Fermentations were carried out at 25 °C and monitored daily by determining the weight loss [25]. Final gravity, pH and Brix degrees of the fermented substrates were determined by hydrometer, pH-meter and refractometer, respectively. Alcohol produced by yeast was derived from specific gravity values (SG) and Brix degrees of the fermented substrates by applying the formula: alcohol (%vol/vol) = Brix × SG × 10 × 0.9982/16.83. Apparent attenuation was calculated according to the following equation: Attenuation (%) = (Original Gravity − Final Gravity)/(Original Gravity-1) × 100.

Pasteurized apricot puree (PP) was fermented by using 5 LAB strains with GRAS status isolated from dairy products and belonging to the UNISS-MBDS culture collection, namely *Lacticaseibacillus paracasei* S13, *Lactiplantibacillus plantarum* PB, *Lacticaseibacillus casei* S14, *Lacticaseibacillus rhamnosus* R7, and *Pediococcus acidilactici* MA3. After 24 h preculture in MRS broth at 30 °C, bacteria were inoculated in 250 mL flasks, stopped with airlocks, and filled with 75 mL of PP at a final concentration of 1×10^6 cells/mL. Fermentations were carried out in static at 25 °C.

2.5. Volatiles Profile of Fermented Substrates

The volatile analysis of malt wort with (WPP) and without (W) apricot puree, before and after yeast fermentation, as well as that of pasteurized puree (PP) before and after LAB fermentation, was carried out with some modifications to the method reported by [26]. In brief, 5 mL of samples were transferred in a 10 mL headspace vial with 1 g of NaCl and 0.05 mL of 1-Butanol as internal standard (5 g/L in ultrapure water with 5% *v/v* of absolute ethanol), then sealed with PTFE/silicone septa. The volatile compounds were analyzed by means of a HS-SPME-GC/MS technique using DVB-CAR-PDMS fiber (Supelco, Bellefonte, PA, USA). The samples were incubated for 10 min at 60 °C; then, extraction was carried out exposing the fiber to the headspace for 30 min. Both incubation and extraction were performed in agitation. Fibre desorption was done in the injector for 10 min at 250 °C with a split flow of 5 mL/min. The fiber was activated each day following the manufacturer's instructions. The chromatographic analysis was carried out using TRACE GC coupled with an ISQ single quadrupole (Thermo Scientific, Hudson, MA, USA). The analytes were separated on a SLB-5ms capillary column (60 m × 0.25 mm × 0.25 µm film thickness) (Supelco, Bellefonte, PA, USA) using helium as carrier gas at 1 mL/min constant flow rate. The oven temperature program started at 35 °C, held at this temperature for 7 min, then increased to 200 °C at 3 °C/min and held for 7 min, then increased to 250 °C at 5 °C/min, and finally held for 10 min. Transfer line and ion source were both set at 200 °C. A quadrupole scan range was 30–250 amu, and ionization energy was set at 70 eV. Chromatographic data were acquired by means of Trace Finder (Thermo Scientific, Hudson, MA, USA), and identification was carried out by comparison between the mass spectra with those of the data system library (NIST, 2005 software, Mass Spectral Search Program V.2.0d, Washington, DC, USA version 2.2, June 2014). All identity spectrum match factors above 850 resulting from the NIST identity spectrum search algorithm (NISTMS Search 2.2) were considered acceptable for identification. All analyses were conducted in duplicate, the gas chromatographic signals were manually integrated, and the resulting peak areas (A_s) were compared with the peak area of the internal standard (A_{IS}) and expressed in percentage of the total sum area of the (A_s/A_{IS}) ± (SD).

2.6. Statistical Analysis

Unless otherwise stated, experiments were carried out in triplicate from independent pre-cultures. The statistical analyses of the data were performed with one-way ANOVA (Bonferroni adjusted *p*-value = 0.05) followed by a Tukey–Kramer HSD test (all pair comparison) using the R statistical environment [27].

3. Results

3.1. Bacterial and Fungal Communities of Bisucciu Apricot Fruits

Table 1 shows the population densities of culturable bacteria and fungi on the surfaces of apricot fruit. The standard deviation values highlighted that the microbial count of epiphytic microorganisms was extremely variable, suggesting a strong effect of the source of isolation (fruit). No endophytic microorganisms were isolated from the fruits analyzed.

Table 1. Culturable bacterial and fungal populations on the surface of "Bisucciu" apricot fruits.

Microbial Groups	Isolation Media	Log $_{10}$ CFU/g
Total mesophilic count	PCA	4.75 ± 4.58
Fungal count	RBA	4.44 ± 4.07
Lactic acid bacteria	MRS	3.63 ± 3.52
Fructophilic lactic acid bacteria	FLAB	2.37 ± 2.40

Data are means ± standard deviations of three to five independent measurements.

From the 16S rDNA (bacteria) and ITS (fungi) sequence analyses, 3 bacterial genera and 13 fungal genera were detected on apricot fruits, with sixty-eight epiphytic isolates assigned to a species at the 97% threshold classification level. *Aureobasidium pullulans* and *Rhodotorula glutinis* were the dominant fungal species, while the LAB *Enterococcus mundtii* (Firmicutes) and *Frigoribacterium faeni* (Actinobacteria) were the most represented among the bacterial species (Figure 1).

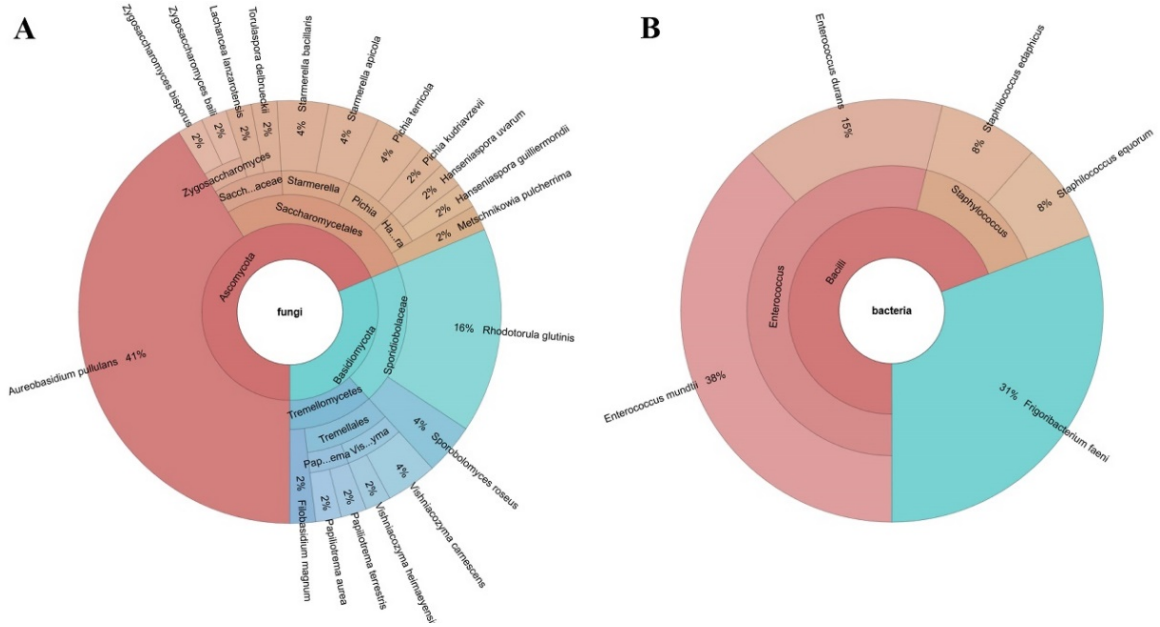

Figure 1. Relative abundances of epiphytic fungal (**A**) and bacterial (**B**) species identified on the fruits of *Bisucciu* apricot cultivar.

Enrichment cultures in fresh apricot puree were carried out to specifically stimulate the growth of yeast strains of the *Bisucciu* microbiota potentially selectable as brewing starters, due to their fermentative abilities and dominance over the apricot microbial populations. After two enrichment protocols, 29 isolates were obtained. These were classified as belonging to *Pichia kudriavzevii* (6 isolates), *Hanseniaspora uvarum* (16 isolates), *H. pseudoguilliermondii* (6 isolates), and *H. clermontiae* (1 isolate) species. *H. clermontiae* was specifically enriched in apricot puree, as this species was not identified among the main epiphytic and endophytic yeasts of apricot fruits.

3.2. Fermentative Ability of Yeast Isolated from the Apricot Microbiota

The fermentative abilities of four yeast isolates after enrichment cultures of apricot puree, namely, *P. kudriavzevii* L1, *H. uvarum* L2, *H. pseudoguilliermondii* L3, and *H. clermontiae* L4 were assessed on malt wort (W) and malt wort added with 5% of pasteurized apricot puree (WPP) (Figures 2 and 3).

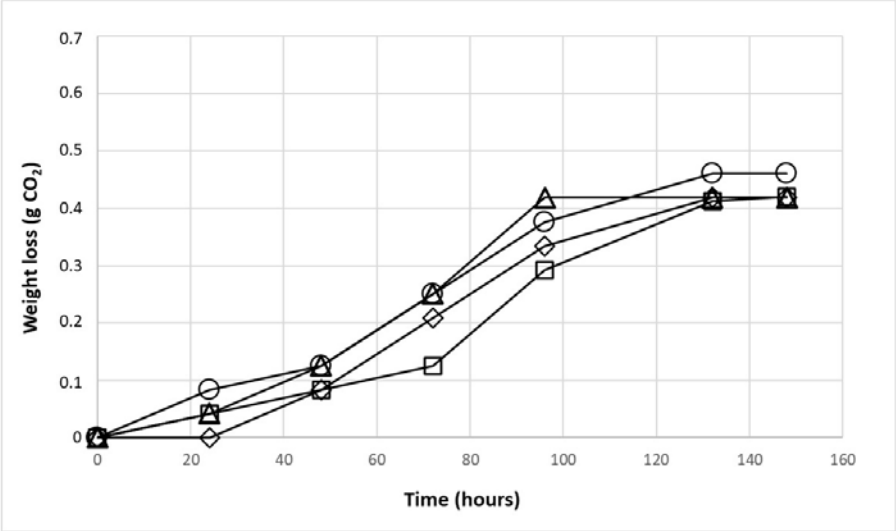

Figure 2. Fermentation kinetics (depicted as cumulative weight loss) of yeasts isolated from apricot fruits growing in malt wort (W). Circle: *Pichia kudriavzevii* L1; diamond: *Hanseniaspora uvarum* L2; triangle: *H. pseudoguilliermondii* L3; square: *H. clermontiae* L4. Data are means ± SD of three independent replicates. Where not visible, error bars lie below the symbols.

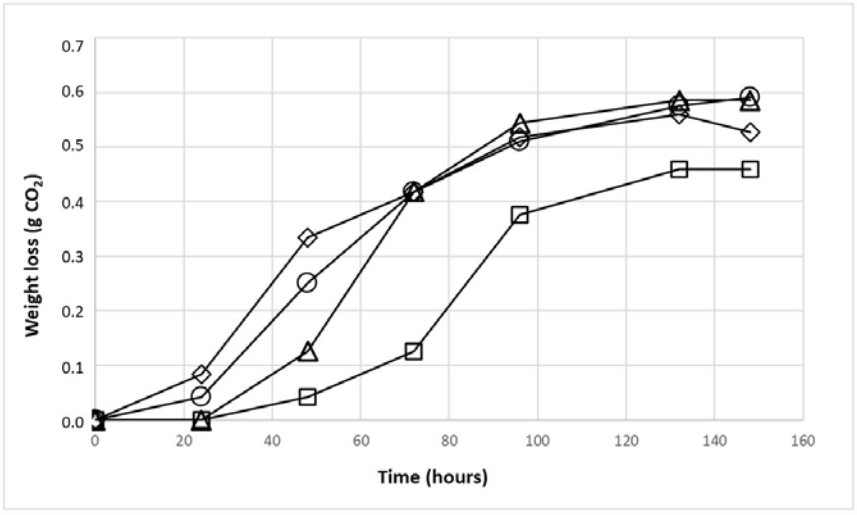

Figure 3. Fermentation kinetics (depicted as cumulative weight loss) of yeasts isolated from apricot fruits growing in malt wort + apricot puree (WPP). Circle: *Pichia kudriavzevii* L1; diamond: *Hanseniaspora uvarum* L2; triangle: *H. pseudoguilliermondii* L3; square: *H. clermontiae* L4. Data are means ± SD of three independent replicates. Where not visible, error bars lie below the symbols.

In malt wort, yeast isolates showed similar fermentation kinetics, lowering the original gravity of wort (1.057± 0.001) to an average final gravity of 1.0529 ± 0.001, which corresponds to an attenuation of 7.221% ± 0.448 and an ethanol concentration of 0.540 ± 0.04 (% vol/vol) (Table 2). In WPP, the increase in the concentration of easily fermentable sugars (glucose and fructose), due to apricot addition, resulted in higher original gravity (1.058 ± 0.001), the reduction of the lag phase, and an increase in ethanol production.

Particularly, the average final gravity was 1.052 ± 0.01 with an attenuation of 9.04% ± 1.02, and an average ethanol concentration of 0.676% ± 0.08 (vol/vol). *H. clermontiae* L4 in WPP showed the lower fermentative power, producing 0.575 ± 0.032% (vol/vol) of ethanol, while the other yeast species attained up to 0.741 ± 0.02% (vol/vol) of ethanol.

Table 2. Technological parameters determined on malt wort (W) and malt wort added with apricot puree (WPP) before yeast fermentation (C) and after six days of fermentation with *Pichia kudriavzevii* (L1), *Hanseniaspora uvarum* (L2), *H. pseudoguilliermondii* (L3), and *H. clermontiae* (L4).

	W					WPP				
	C	***L1***	***L2***	***L3***	***L4***	***C***	***L1***	***L2***	***L3***	***L4***
pH	$5.22^{a} \pm 0.01$	$4.73^{b} \pm 0.02$	$4.48^{c} \pm 0.03$	$4.67^{b} \pm 0.03$	$4.53^{c} \pm 0.02$	$4.59^{a} \pm 0.02$	$4.58^{a} \pm 0.01$	$4.42^{b} \pm 0.01$	$4.44^{b} \pm 0.04$	$4.42^{b} \pm 0.06$
°Brix	$13.25^{a} \pm 0.12$	$12.37^{b} \pm 0.06$	$12.48^{b} \pm 0.51$	$12.49^{b} \pm 0.42$	$12.49^{b} \pm 0.19$	$13.55^{a} \pm 0.27$	$12.21^{c} \pm 0.12$	$12.47^{bc} \pm 0.42$	$12.35^{c} \pm 0.12$	$12.79^{b} \pm 0.27$
Specific Gravity	$1.057^{a} \pm 0.01$	$1.053^{b} \pm 0.01$	$1.053^{b} \pm 0.01$	$1.053^{b} \pm 0.02$	$1.053^{b} \pm 0.01$	$1.058^{a} \pm 0.01$	$1.052^{c} \pm 0.01$	$1.053^{bc} \pm 0.01$	$1.052^{c} \pm 0.00$	$1.054^{b} \pm 0.01$
Ethanol (% vol/vol)	-	$0.59^{a} \pm 0.01$	$0.52^{ab} \pm 0.04$	$0.52^{b} \pm 0.05$	$0.52^{b} \pm 0.02$	-	$0.74^{a} \pm 0.02$	$0.66^{a} \pm 0.06$	$0.73^{a} \pm 0.03$	$0.57^{b} \pm 0.03$
Attenuation (%)	-	$7.9^{a} \pm 0.1$	$6.9^{b} \pm 0.2$	$6.9^{b} \pm 0.1$	$7.0^{b} \pm 0.1$	-	$9.9^{a} \pm 0.2$	$8.8^{b} \pm 0.2$	$9.8^{a} \pm 0.1$	$7.7^{c} \pm 0.1$

Data are means ± standard deviations of three to five independent measurements. The same superscript letters in the same row indicate not significant differences as determined by ANOVA followed by Tukey HSD post-hoc test (adjusted *p*-value = 0.05).

To better evaluate the suitability of yeast isolates as brewing strains, volatile compounds were analyzed in the fermented substrates W and WPP (Figure 4)

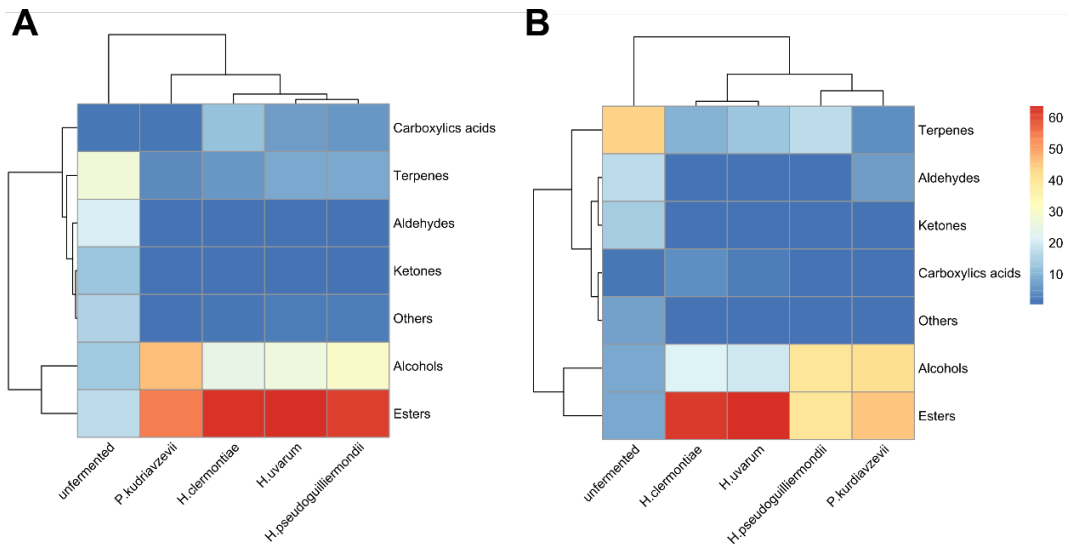

Figure 4. Heatmaps of the relative abundance (%) of volatiles organic compounds in malt wort (**A**) and malt wort added with pasteurized apricot puree (**B**) fermented by *Hanseniaspora clermontiae L4*, *Hanseniaspora uvarum L2*, *Hanseniaspora pseudoguilliermondii L3*, and *Pichia kudriavzevii L1*. Data are mean percentage of total sum area of the integrated peak area (As) compared to the area of the internal standard (AIS).

In the two substrates, yeast fermentation resulted in an increase in the ester and alcohol contents, with the reduction of aldehydes, ketones, and particularly terpenes, in comparison with the unfermented samples. Specific chemical profiles were observed based on the yeast species used as a starter. In the malt wort, the three isolates of the genus *Hanseniaspora* behaved similarly, leading to the higher ester production, while *Pichia kudriavzevii* produced the highest levels of alcohol. In malt wort added with apricot puree, *Hanseniaspora clermontiae* L4 and *Hanseniaspora uvarum* L2 confirmed their fermentative profiles, characterized by the higher and lower content of esters and alcohols, respectively. Similarly to what was observed in malt wort, *Pichia kudriavzevii* L1 stood out for the higher alcohol and aldehyde contents. The addition of puree resulted in the production of lower levels of esters with an increase in alcohols by *H. pseudoguilliermondii*. Of note, the addition of apricot puree resulted in significant changes in the aromatic profile of the unfermented

substrates, evidenced by the relative reduction of alcohol and esters and an increase in ketones and terpenes (Table 3).

Table 3. Relative abundance (%) of organic compounds in unfermented malt wort (W) and malt wort added with 5% apricot puree (WPP).

	W	WPP	*p*-Value
Carboxylics acids	1.0420 ± 0.287	1.082 ± 0.469	0.869
Alcohols *	12.161 ± 0.246	8.344 ± 0.375	0.001
Esters *	16.198 ± 0.394	8.221 ± 0.089	0.002
Aldehydes	19.345 ± 0.538	16.990 ± 0.965	0.086
Ketones *	11.587 ± 0.117	14.326 ± 0.112	0.000
Terpenes *	25.127 ± 0.302	43.867 ± 0.869	0.001
Others *	14.192 ± 0.246	7.008 ± 0.662	0.003

* Denotes significant differences as determined by *t*-test (Bonferroni adjusted *p*-value = 0.05). Data are means ± SD of the percentage of total sum area of the integrated peak area (As) compared to the area of the internal standard (AIS) of three independent replicates.

Finally, as a proof of concept, the fresh apricot puree (FP) was fermented by LAB strains with GRAS status, isolated from dairy products, to evaluate the feasibility of puree fermentation by LAB to enhance its nutritional and sensorial quality, while at the same time reducing the risk of microbiological spoilage before its addition to fruit beers. Notwithstanding the initial low pH of apricot puree (3.74), all the tested LAB strains were able to grow in PP (data not shown). Particularly, samples fermented by *Lacticaseibacillus paracasei* S13 and *L. ramnosus* R7 showed pH values of 3.37± 0.07 and 3.49± 0.07, respectively. In addition, strain-dependent aromatic profiles were highlighted at the end of puree fermentation (Figure S1).

4. Discussion

4.1. Bacterial and Fungal Communities Isolated from Bisucciu Apricot Fruit

The epiphytic microbial communities of *Bisucciu* fruit were characterized by high variability in total mesophilic bacteria, LAB, and fungi viable counts. This result is in accordance with that reported by Badosa et al. [28], who found that the aerobic counts and the fungal counts in the same fruit species ranged from <1 to 9 $\log_{10}$ CFU/g, based on the specimen analyzed. The high variability in the extent of microbial colonization of fruit surface is dependent on many factors, thus it is conceivable that different fruits harbor significant diverse microbial loads depending on their degree of maturity, exposure to UV rays, and specific growing conditions [29]. Similarly, the observed low numbers of epiphytic bacteria in most of the *Bisucciu* fruit samples are in accordance with previous studies. For instance, Rahman et al. [30], in a comprehensive assessment of the microbial quality of apple, lemon, grape, and pineapple fruits, found that the amount of total viable bacteria was on the order of 4 $\log_{10}$ CFU/g. Regarding the community structure of the cultivable microbiota, the main bacterial and fungal species isolated from the *Bisucciu* cultivar were already identified among the dominant epiphytes of other fruit species [31,32]. These similarities are in accordance with the observation that the surface of fruits, providing shelter and nutrients to specific fungal and bacterial taxa, represents a selective environment that shapes the endophytic microbiota in a very peculiar way [33,34].

Particularly, the microbiota of *Bisucciu* fruit was constituted of bacterial species already identified in the carposphere. For instance, the surface of ripe grapevine berries was characterized by the presence of *Frigoribacterium* and *Bacillus* genera, among others [35]. Isolates belonging to the *Staphylococcus* genus were isolated from apple fruit skins [32]. *Enterococcus* is among the lactic acid bacteria most frequently isolated as epiphytes within fruit microbiota, depending on the plant and fruit origin [36].

Interestingly, the bacterial species identified on *Bisucciu* fruit could be regarded as non-pathogenic. Indeed, *Staphylococcus equorum* belongs to coagulase-negative staphylococci that are generally considered safe organisms, in contrast to the coagulase-producing

Staphylococcus aureus. No evidence of *S. equorum* pathogenicity or food poisoning has been reported [37]. Enterococci are involved in the fermentation of traditionally manufactured cheese and sausages, and some species are used as probiotics and as feed additives [38].

Regarding the fungal communities, the largest proportion of isolates found on *Bisucciu* apricot fruit was unicellular yeasts of the genera *Aureobasidium*, *Hanseniaspora, Metschnikowia*, *Pichia*, *Starmerella,* and *Zygosaccharomyces* (Ascomycota), as well as *Rhodotorula, Sporobolomyces,* and *Vishniacozyma* (Basidiomycota). Similar fungal communities were found associated with apple, grape, and nectarine fruits [39,40]. Vadkertiová et al. [31] identified *A. pullulans, H. guilliermondii, H. uvarum, M. pulcherrima, P. kudriavzevii, Z. bailii,* and the basidiomycetous genera *Rhodotorula*, in ripe fruits of plum. Similarly, Bösch et al. [32] found that apple fruit was colonized by yeasts of the genera *Aureobasidium*, *Metschnikowia*, and *Rhodotorula*.

It has been observed that fermentative yeast populations (e.g., *Hanseniaspora, Metschnikowia,* and *Pichia*) are rarely detected in the early stage of fruit development but predominate during the ripening [40]. Indeed, *H. uvarum* and *H. guilliermondii* dominated the yeast microbiota of ripe plum fruits [41]. The occurrence of *P. kudriavzevii* has been associated with ripped fruits of apple, pear, and plum [31]. Similarly, *M. pulcherrima* dominates the very first stages of grape must fermentation [39]. Of relevance is that plant pathogenic fungi (e.g., *Penicillium*, *Aspergillus*, and *Alternaria*) were not identified among the main fungal genera of *Bisucciu* fruit. This result could be related to the co-occurrence on the fruits analyzed of fungi previously identified as biocontrol organisms, like *Aureobasidium*, *Sporobolomyces, Filobasidium*, and *Vishniacozyma* [42,43]. Indeed, *A. pullulans* isolates showed antagonist activity against *Monilinia*, *Botrytis*, *Penicillium,* and *Colletotrichum* [44,45]. *Sporobolomyces* has been reported to exhibit antifungal and growth-promoting activity of plants [46,47]. Similarly, *Filobasidium* and *Vishniacozyma* have been shown to induce plant resistance [34]. *M. pulcherrima* is another relevant antagonist detected in the *Bisucciu* mycobiota. This yeast species has been shown effective against fruit spoilage fungi and postharvest rots [48]. In our study, we did not observe any isolate belonging to the *Saccharomyces* genus. This is in accordance with the observation that *S. cerevisiae* occurs rarely on fruit surfaces, approximately one out of every 1000 fruit surfaces analyzed [49].

4.2. Yeast Isolated from Apricot Fruits as Potential Starters for Beer Production

The identification of non-*Saccharomyces* yeasts within the epiphytes of apricot fruits suggests their possible application as starters to produce fruit beers. Indeed, it is now widely accepted that non-*Saccharomyces* yeasts, when used in mixed fermentation with *S. cerevisiae*, positively contribute to the sensorial quality of the fermented beverage [13,50]. In this respect, enrichment cultures of *Bisucciu* apricot puree allowed for identifying isolates of *P. kudriavzevii, H. uvarum, H. guilliermondii,* and *H. clermontiae* as capable of dominating the indigenous microbiota. *P. kudriavzevii* was already associated with natural fermentation of ripped and rotten fruits, such as Indian plum (*Ziziphus mauritiana*), pineapples, and Camu-Camu (*Myrciaria dubia*) [51–53]. Previously, it has been shown that strains of *P. kudriavzevii* isolated from fermented sugar cane led to a high degree of attenuation (up to 85%) when used as starters for beer fermentation [54]. In comparison, the isolate *P. kudriavzevii* L1, here characterized, performed poorly (7.9% attenuation). This can be attributed to a lower capacity to assimilate the main sugars in beer wort (maltose, maltotriose, and dextrins), as already described for this species [55].

Hanseniaspora spp. are mainly associated with grape surfaces. Particularly, *Hanseniaspora uvarum* is the dominant yeast in the first stages of wine fermentation [39]. Grape isolates of *H. clermontiae* contributed to the polyphenolic composition of wines subjected to spontaneous fermentation [54]. In brewing, the role of *Hanseniaspora* spp. is less investigated. *Hanseniaspora valbyensis* and *Hanseniaspora vineae* were evaluated for the production of alcohol-free beer [56]. A fermentative behavior similar to that here displayed by *Hanseniaspora uvarum* L2 and *H. pseudoguilliermondii* L3, which produced 0.69 and 0.77 g of CO_2/100 mL of wort, respectively, was described by Matraxia et al. [57]. Particularly, the authors showed that two *Hanseniaspora uvarum* strains, isolated from honey by-products,

produced from 0.35 to 0.37 g of CO_2/100 mL of wort, when used as a starter for craft beer production [57]. As a comparison, *S. cerevisiae* US-05 released 4.077 g of CO_2/100 mL of wort. The authors suggested that the low fermentation rate of *H. uvarum* strains does not represent a limitation for beer production as these yeasts can be used in mixed fermentation with *S. cerevisiae*

The use of non-*Saccharomyces* yeast to produce fermented beverages has been associated with an increase in the levels of aroma-active compounds, such as higher alcohols, esters, carboxylic acids, sulfur compounds, and volatile phenols [12]. Particularly, the use of *H. guilliermondii* in mixed fermentation increased the levels of phenylethyl acetate ('rose' aroma) in beer. dos Santos et al. [54] found that *P. kudriavzevii* used as a starter for beer production led to levels of 1-octanol and 1-pentanol higher than those obtained by using *S. cerevisiae* as a single starter. The yeast *H. uvarum* used in co-inoculum with S. *cerevisiae* resulted in the production of considerable amounts of isoamyl acetate, ethyl acetate, ethyl octanoate, and ethyl exanoate in the final beer [57]. Terpenes are one of the main chemical families in the hop oil and are responsible for the fruity and floral aromas of beers ('citrus', 'floral', 'aniseed', 'lime', 'hyacinth', 'rose', etc.). The levels of terpenes in beer are dependent on different factors, the yeast being used during fermentation one of the most important [58]. Particularly, geraniol and linalool could be synthesized de novo by *Hanseniaspora uvarum* [59]. In addition, yeasts can convert hop-derived monoterpene alcohols through esterification or hydrolysis [60]. Following these observations, the beer wort fermented by non-*Saccharomyces* yeasts isolated from *Bisucciu* fruit was characterized by a lower content of terpenes and increased levels of esters and alcohols, suggesting that the tested yeasts may contribute significantly to the definition of the taste and flavor of beers when used in mixed fermentations with *Saccharomyces*.

5. Conclusions

In the present study, we uncovered for the first time the structure of the cultivable bacterial and fungal communities colonizing the surface of apricot fruits at pre-harvest. However, with significant differences in the extent of microbial colonization, the studied apricot fruits were homogeneous enough to identify a specific epiphytic microbiome assembly, characterized by the absence of pathogens and the presence of isolates with interesting biotechnological potential. Particularly, we identified four yeast isolates belonging to *P. kudriavzevii*, *H. uvarum*, *H. guilliermondii* and *H. clermontiae* that could be used as brewing starters in mixed fermentations to specifically characterize the aroma profile of craft beers. In addition, we evaluated the suitability of apricot puree fermentation by GRAS LAB strains to increase the shelf-life and add more aromatic complexity to fruit beers. Results obtained are relevant for decision-making in fruit beer brewing, particularly the choice of the fermentation starters and fruit addition (pasteurized puree or fermented puree).

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fermentation8080364/s1, Figure S1: Heatmaps of the relative abundance (%) of volatiles organic compounds in apricot puree fermented by four LAB strains.

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