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Novel starters for old processes: use of *Saccharomyces cerevisiae* strains isolated from artisanal sourdough for craft beer production at a brewery scale

Antonella Marongiu · Giacomo Zara · Jean-Luc Legras · Alessandra Del Caro · Ilaria Mascia · Costantino Fadda · Marilena Budroni

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Abstract The deliberate inoculation of yeast strains isolated from food matrices such as wine or bread, could allow the transfer of novel properties to beer. In this work, the feasibility of the use of baker's yeast strains as starters for craft beer production has been evaluated at laboratory and brewery scale. Nine out of 12 *Saccharomyces cerevisiae* strains isolated from artisanal sourdoughs metabolized 2 % maltose, glucose and trehalose and showed growth rates and cell populations higher than those of the brewer's strain Safbrew-S33. Analysis of allelic variation at 12 microsatellite loci clustered seven baker's strains and Safbrew-S33 in the main group of bread isolates. Chemical analyses of beers produced at a brewery scale showed significant differences among the beers produced with the baker's strain S38 or Safbrew-S33, while no significant differences were observed when S38 or the brewer's strain Safbrew-F2 was used for re-fermentation. The sensory profile of beers obtained with S38 or the brewer's yeasts did not show significant differences, thus suggesting that baker's strains of *S. cerevisiae* could represent a reservoir of biodiversity for the selection of starter strains for craft beer production.

A. Marongiu and G. Zara contributed equally to this work.

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A. Marongiu · G. Zara · A. Del Caro · I. Mascia · C. Fadda · M. Budroni (✉)
Dipartimento di Agraria, University of Sassari, Viale Italia 39, 07100 Sassari, Italy
e-mail: mbudroni@uniss.it

J.-L. Legras
INRA, UMR1083 Science Pour L'Œnologie, 2 Place Viala, 34060 Montpellier, France

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Introduction

Craft brewing is a term that applies to relatively small breweries, usually focused on the production of traditional ale beers, which compete with the mass-market breweries on the basis of quality and diversity, instead of low prices. Most craft brewers are continuing to see strong growth in production, sales, brewing capacity and employment [9].

Considering that craft beers are generally unfiltered, unpasteurized and re-fermented in bottle, the choice of yeast strains for wort fermentation and beer re-fermentation is crucial [7, 11]. *Saccharomyces cerevisiae* and *Saccharomyces pastorianus* are the two species that are most often used as starter cultures for 'ale' and 'lager' beers, respectively [8]. While several yeast strains are commercially accessible, the availability of new starter strains could be an important differentiating factor among craft beers produced in different microbreweries. Indeed, while larger brewing companies tend to manage their own in-house strains, microbrewers usually rely on commercial strains [29].

The development of new starters requires the improvement of already available yeast strains or the selection of new strains. Genetic improvements of brewing yeasts have been already carried out; however, practical application of these techniques is limited by the concerns of consumers for such modified yeasts [5, 31]. At the same time, the selection of new strains from wort/beer is problematic due to the boiling of wort, which drastically reduces the viability of wild yeasts, and to the widespread use of commercial starter strains in breweries [7].

In this context, the characterization of *S. cerevisiae* strains isolated from food matrices other than beer could represent a valid approach for the selection of starters for brewing. One criterion considered in selecting industrial strains for brewing is their ability to rapidly and completely utilize the fermentable carbohydrates available [6]. Canonico and coworkers tested 33 *S. cerevisiae* strains isolated from a winery environment for their ability to ferment wort and beer [11]. While interesting results were obtained regarding the re-fermentation process, wine isolates performed poorly in wort fermentation compared to the brewer's strain used as control.

Sourdoughs, intermediate fermented products used for the production of bread and sweet, leavened, baked goods [16], may represent another source for the isolation of starter yeasts for brewing. The environment of sourdoughs is dominated by different yeast species, among which is *S. Cerevisiae* [19]. Strains of this species showed an efficient uptake and utilization of trehalose that shares common transporters with maltose [33]. Thus, those strains may be able to ferment maltose, the main carbohydrate of beer [18].

In this context, the aim of the present study was to evaluate the feasibility of the use of *S. cerevisiae* yeast strains isolated from artisanal sourdoughs as starters for craft beer production at a brewery scale during both wort fermentation and beer re-fermentation.

Materials and methods

Yeast strains

Twelve yeast strains isolated from homemade sourdoughs and belonging to the culture collection of Dipartimento di Agraria, University of Sassari, were used. As representative commercial beer strains, Safbrew-S33 and Safbrew-F2 (Fermentis, Lesaffre Italia S.p.A., Italy) were used (Table 1). Cultures were maintained on YPD plates (yeast extract 2 %, peptone 2 %, glucose 2 %, bacto-agar 2 %) and WL plates (Wallerstein Laboratory Nutrient Agar, OXOID). Yeast strains were identified as belonging to the *S. cerevisiae* species by PCR–RFLP analysis of the rDNA region [15].

Growth on different carbon sources

Yeast strains were inoculated at a starting concentration of 10^5 cell/ml on YNB (0.67 % Yeast Nitrogen Base, DIFCO) added with 2 % (w/v) glucose or 2 % (w/v) maltose as carbon sources [24]. Assimilation of 2 % (w/v) trehalose was evaluated in YNB buffered with sodium succinate at pH 4 according to Jules [21]. Yeast growth was determined by measuring optical absorbance at 600 nm after 24 h of growth with shaking (300 rpm) at 25 °C. Yeast growth kinetics were assessed in 96-well microplates during 48 h of growth on YNB + 2 % (w/v) maltose (YNBM) by measuring variations in the optical absorbance (600 nm) every 10 min with a SpectroStar Nano (BMG Labtech, Germany). Each yeast strain was inoculated in eight wells at a starting concentration of 10^5 cell/ml. To determine the length of the lag phase (λ), the maximum specific growth rate (μ) and the maximum population (A) of each of the strains tested, the package grofit v1.1.1 of the R v3.0.2 statistical environment was used [22, 30].

Molecular typing

Saccharomyces cerevisiae strains isolated from homemade sourdoughs were characterized for their allelic variation at four minisatellite *loci* [26] and at 12 microsatellite *loci* [25]. The Bruvo distance among strains was calculated by analyzing the microsatellite data with the package polysat v1.3-2 of the R v3.0.2 statistical environment [12, 30]. A Jackknife procedure was used to evaluate the reliability of the nodes. The neighbor-joining tree was obtained from the distance matrices with the R package ape v3.0.2 [27], and drawn using MEGA v5.05 [35].

Beer wort fermentation

Fermentations were carried out in a hopped wort mashed by a local microbrewery (95 % barley malt, 5 % raw wheat, 2 % hop). Lab-scale fermentations were carried out in 500-ml Erlenmeyer flasks filled with 300-ml wort and stopped with Müller valves. Yeast strains were inoculated at a starting concentration of 1×10^6 cells/ml and kept in static at 20 °C for 2 days, 18 °C from second to fifth day and 20 °C from sixth to

Table 1 Yeast strains used in this study

S. cerevisiae strains	Source of isolation
S1, S3	Strains isolated from sourdoughs collected in Marmilla (Sardinia, Italy) from producer A
S10, S15, S16	Strains isolated from sourdoughs collected in Nurra (Sardinia, Italy) from producers B and C
S20, S25, S34, S38, S42	Strains isolated from sourdoughs collected in Mejlogu from the producers D and E
S44	Strains isolated from sourdoughs collected in Lombardy (Italy)
S49	Strains isolated from sourdoughs collected in Sicily (Italy)
Safbrew S-33, Safbrew F2	Commercial strains used for wort and beer fermentation (Fermentis, Lesaffre Italia S.p.A., Parma, Italy)

eighth day of fermentation according to the brewery protocol. Fermentation progression was monitored daily by weight loss.

Pilot-scale fermentations were carried out at a local microbrewery in 30-L stainless steel fermenters. Strain S38 was inoculated at a starting concentration of 5×10^5 cells/ml and strain Safbrew-S33 was used following manufacturer's instructions (3.5×10^6 cells/ml). Fermentations were carried out in triplicate at 18 °C. At the end of fermentation, beers obtained were added with 50 g/l glucose, transferred in 500 ml bottles and inoculated with strain S38 (1×10^6 cell/ml) and Safbrew-F2 (following manufacturer's instructions). Re-fermentations were carried out at 20 °C for 7 days, 10 °C for the next 7 days and at 4 °C for one month according to the brewery's protocol. Four different protocols were tested in triplicate: (1) strain Safbrew-S33 for primary fermentation followed by strain S38 for the re-fermentation (S33-S38); (2) strain Safbrew-S33 for primary fermentation followed by strain F2 for the re-fermentation (S33-F2); (3) strain S38 for primary fermentation followed by strain S38 for the re-fermentation (S38-S38); (4) strain S38 for primary fermentation followed by strain F2 for the re-fermentation (S38-F2).

Analytical determinations

Beers obtained were analyzed by the Italian Brewing Research Center (University of Perugia, Italy) to evaluate: apparent extract (°P), real extract (g/100 g), alcohol (% v/v), apparent degree of attenuation (%), real degree of attenuation (%), and bitterness (BU). Two-way ANOVA was carried out to evaluate the influence of the fermenting yeast strain (first independent variable with two levels: S33 and S38) and the refermenting yeast strain (second independent variable with three levels: no inoculation, S38, F2) on each of the analytical parameters (dependent variable). All calculations were performed using the R software v 3.0.2 [30].

Sensory evaluation

Sensory evaluation was performed on the beers obtained after the re-fermentation in the microbrewery using a descriptive test with a trained panel and a preference-ranking test on consumers. Descriptive sensory analysis was performed using a panel of 12 assessors, selected and trained following the ASBC methodology [2]. The chosen attributes to describe the products were the following: presence, color and persistency of the foam, fruity, malt and yeast character for the odor, bitter and acid for the taste and at last, freshness and fullness for the mouth-feel sensations. Assessors evaluated these attributes on a 10-cm unstructured scale (0 = absence or low intensity and 10 = maximum intensity). Assessors were calibrated using reference standards for the chosen attributes, for at least two points of the scale. A one-way ANOVA was used to point out significant differences among the samples in the sensory profile. Significant means were separated using Tukey's test ($p < 0.05$).

The preference ranking test was carried out by asking 100 consumers to put in order of preference the four beer analyzed. Preference test was elaborated using the Friedman statistic (T) test followed by LSD test at a significant level of 5 % [23].

Results and discussion

Most of the baker's strains of *S. cerevisiae* are able to assimilate maltose

Maltose is one of the main fermentable sugars in sourdough due to the activity of amylase, which degrades starch, constantly generating glucose and maltose [32]. Therefore, maltose utilization should be a common feature of baker's strains of *S. cerevisiae*. Considering that maltose is also the main carbohydrate of wort, followed by maltotriose and glucose [18], baker's strains able to ferment maltose should represent good starters for beer production. In this work, most of the *S. cerevisiae* strains isolated from homemade sourdoughs were able to ferment glucose, maltose and trehalose (Table 2). Only strains S10, S15 and S16 were not able to use and ferment maltose and were therefore unsuitable for brewing. Assimilation of maltose by *Saccharomyces* requires a least one of five MAL permeases (MAL1–MAL4 and MAL6) [13]. Also, the uptake of trehalose is mediated by a carrier involved in the transport of maltose and maltotriose. In particular, the high-affinity symporter AGT1/MAL11, controlled by the MAL system, is able to

Table 2 Optical density at 600 nm (OD600) in media containing glucose, maltose and trehalose after 48 h of fermentation by 12 *Saccharomyces cerevisiae* strains isolated form homemade sourdoughs

Strains	Growth on 2 % glucose (OD600)	Growth on 2 % maltose (OD600)	Growth on 2 % trehalose (OD600)
S1	$1.704^{c} \pm 0.102$	$1.404^{e} \pm 0.098$	$2.000^{f} \pm 0.140$
S3	$1.568^{b} \pm 0.109$	$1.242^{d} \pm 0.111$	$1.721^{e} \pm 0.154$
S20	$1.567^{b} \pm 0.188$	$1.452^{e} \pm 0.0731$	$1.997^{f} \pm 0.139$
S25	$1.558^{b} \pm 0.078$	$1.604^{f} \pm 0.176$	$1.678^{e} \pm 0.151$
S34	$1.195^{a} \pm 0.119$	$1.700^{f} \pm 0.102$	$1.503^{d} \pm 0.150$
S38	$1.508^{b} \pm 0.150$	$1.642^{f} \pm 0.180$	$1.815^{e} \pm 0.163$
S42	$1.621^{bc} \pm 0.129$	$1.217^{d} \pm 0.061$	$2.003^{f} \pm 0.200$
S44	$1.257^{a} \pm 0.113$	$1.073^{c} \pm 0.086$	$2.012^{f} \pm 0.120$
S49	$1.223^{a} \pm 0.122$	$0.719^{b} \pm 0.065$	$1.734^{e} \pm 0.191$
S10	$1.986^{d} \pm 0.119$	$0.048^{a} \pm 0.004$	$0.035^{a} \pm 0.002$
S15	$1.728^{c} \pm 0.155$	$0.022^{a} \pm 0.002$	$0.984^{b} \pm 0.088$
S16	$1.899^{d} \pm 0.094$	$0.058^{a} \pm 0.006$	$1.235^{c} \pm 0.111$
Safbrew S-33	$2.250^{e} \pm 0.071$	$2.108^{g} \pm 0.120$	$0.584^{b} \pm 0.272$

Same superscript letters in the same column indicate not significant differences ($p > 0.05$) as determined by ANOVA followed by Tukey HSD test

transport trehalose [33]. Consequently, the majority of the strains able to ferment maltose were also able to grow on trehalose (Table 2). While the brewer's strain Safbrew-S33 was also able to ferment the three carbohydrates tested, it showed an assimilation pattern different from that of the baker's strains. In particular, strain Safbrew-S33 showed the highest growth on maltose and glucose and a low assimilation of trehalose. In strains S15 and S16, which were able to grow on trehalose but not on maltose, trehalose uptake could be due to a different system, probably the already identified low-affinity transporter system whose corresponding gene remains to be characterized [21].

To better evaluate the rate of maltose utilization, growth curves of the nine strains of *S. cerevisiae* able to ferment maltose were carried out and the main kinetic parameters determined by applying the Gompertz equation [20]. With the exception of S1 and S3, all the baker strains were able to quickly start cellular multiplication in presence of maltose as unique carbon source, as demonstrated by the relative low lag phase (λ) (Table 3). Except for S20, all the strains showed specific growth rates (μ_{max}) higher than the commercial strain Safbrew S33. Finally, strain S38 attained a cellular population (*A*) significantly higher ($p < 0.05$) than the representative beer strain.

Table 3 Main growth parameters of strains S1, S20, S25, S3, S34, S38, S42, S44, S49 and Safbrew S-33 grown on YNBM

Strain	μ_{max} (OD600 min^{-1})	λ (min)	*A* (OD600)
S1	$0.43^{b} \pm 0.2$	$154.88^{a} \pm 5.98$	$1.42^{c} \pm 0.37$
S20	$0.26^{d} \pm 0.01$	$76.70^{c} \pm 4.30$	$1.46^{c} \pm 0.12$
S25	$0.48^{a} \pm 0.1$	$77.49^{cd} \pm 23.20$	$1.89^{b} \pm 0.24$
S3	$0.33^{c} \pm 0.01$	$102.89^{b} \pm 8.46$	$1.95^{b} \pm 0.20$
S34	$0.38^{b} \pm 0.2$	$67.13^{cd} \pm 12.22$	$1.87^{b} \pm 0.16$
S38	$0.41^{b} \pm 0.1$	$70.58^{cd} \pm 11.80$	$2.24^{a} \pm 0.04$
S42	$0.32^{c} \pm 0.01$	$53.30^{d} \pm 5.59$	$2.05^{a} \pm 0.17$
S44	$0.41^{b} \pm 0.1$	$82.26^{c} \pm 6.24$	$1.32^{d} \pm 0.06$
S49	$0.38^{b} \pm 0.2$	$70.35^{cd} \pm 9.32$	$1.27^{d} \pm 0.06$
Safbrew S-33	$0.28^{d} \pm 0.01$	$59.82^{d} \pm 7.16$	$1.88^{b} \pm 0.17$

Same superscript letters in the same column indicate not significant differences ($p > 0.05$) as determined by ANOVA followed by Tukey HSD test

Data are mean ± standard deviations of five independent measurements

μ_{max} maximum specific growth rate, λ length of lag phase (min), and *A* maximum cell growth (OD600)

Baker's strains are able to ferment hopped wort

To verify the feasibility of the use of baker's strains as starters for beer production, fermentation trials were carried out on a commercial wort at a lab scale using the nine strains able to ferment maltose. Fermentation progression was followed by measuring the fermenter weight loss as this parameter allows the calculation of some physico-chemical parameters of beer [28]. All the baker's strains were able to ferment the hopped wort, showing in some cases fermentation performances higher than those of the commercial strain utilized as control (Table 4). At the third day of fermentation, strains S20 and S44 produced more alcohol than Safbrew-S33, suggesting their ability to quickly dominate the fermentation. At the end of fermentation, lower extract values were measured in wort fermented by strains S20, S25, S34 and S49, while higher values were measured

Table 4 Basic physiochemical parameters of beers fermented by baker's strains S1, S3, S20, S25, S34, S38, S42, S44, S49 and the representative brewer strain Safbrew S-33 after 3, 5 and 9 days of fermentation at laboratory scale

Strain	Day 3		Day 5		Day 9	
	Real extract (g/100 g)	**Alcohol (vol/vol)**	**Real extract (g/100 g)**	**Alcohol (vol/vol)**	**Real extract (g/100 g)**	**Alcohol (vol/vol)**
S1	$8.32^{a} \pm 0.30$	$2.40^{a} \pm 0.15$	$5.18^{a} \pm 0.21$	$4.14^{a} \pm 0.12$	$3.56^{c} \pm 0.07$	$4.89^{c} \pm 0.09$
S3	$8.87^{a} \pm 0.53$	$2.09^{a} \pm 0.12$	$4.97^{ab} \pm 0.25$	$4.17^{a} \pm 0.11$	$3.58^{c} \pm 0.15$	$4.80^{c} \pm 0.13$
S20	$6.81^{b} \pm 0.37$	$3.18^{c} \pm 0.14$	$4.45^{c} \pm 0.08$	$4.44^{b} \pm 0.14$	$1.81^{a} \pm 0.08$	$5.84^{a} \pm 0.25$
S25	$7.94^{a} \pm 0.34$	$2.56^{a} \pm 0.18$	$5.20^{a} \pm 0.23$	$4.04^{a} \pm 0.12$	$2.03^{a} \pm 0.09$	$5.73^{a} \pm 0.27$
S34	$7.44^{b} \pm 0.37$	$2.89^{b} \pm 0.10$	$3.48^{d} \pm 0.17$	$4.96^{c} \pm 0.26$	$1.41^{a} \pm 0.07$	$6.05^{a} \pm 0.31$
S38	$8.30^{a} \pm 0.40$	$2.44^{a} \pm 0.12$	$5.12^{a} \pm 0.28$	$4.08^{a} \pm 0.20$	$2.72^{ab} \pm 0.19$	$5.41^{ab} \pm 0.30$
S42	$8.34^{a} \pm 0.41$	$2.37^{a} \pm 0.14$	$4.99^{ab} \pm 0.30$	$4.15^{a} \pm 0.08$	$3.22^{b} \pm 0.19$	$5.09^{bc} \pm 0.29$
S44	$6.45^{b} \pm 0.45$	$3.37^{c} \pm 0.21$	$3.72^{d} \pm 0.20$	$4.83^{c} \pm 0.34$	$3.10^{b} \pm 0.17$	$5.16^{bc} \pm 0.26$
S49	$8.34^{a} \pm 0.45$	$2.37^{a} \pm 0.11$	$4.75^{b} \pm 0.21$	$4.28^{ab} \pm 0.23$	$1.99^{a} \pm 0.09$	$5.75^{a} \pm 0.24$
Safbrew S-33	$7.23^{b} \pm 0.29$	$2.96^{b} \pm 0.09$	$4.27^{c} \pm 0.17$	$4.53^{bc} \pm 0.18$	$3.02^{b} \pm 0.15$	$5.20^{b} \pm 0.21$

Data are mean ± standard deviations of three independent measurements

Same superscript letters in the same column indicate not significant differences ($p > 0.05$) as determined by ANOVA followed by Tukey HSD test

in wort fermented by S3 and S1. Based on these results, baker's strains could be classified as strong (S20, S25, S34 and S49), mild (S38, S42, S44) and weak (S1 and S3) fermenters. Also Safbrew-S33, routinely used as starter strain for craft beer production, could be included in the group of the mild fermenters as its use resulted in beers with a real extract not significantly different ($p < 0.05$) from that of the beers fermented by S38, S42 and S44. Considering that extract measures sugar content, beers with high extract are sweet and subjected to microbial contamination. On the other hand, if the residual sugars are too low, the beer will remain flat [3]. Thus, the baker's strains with fermentation performance similar to the Safbrew-S33 were considered the more interesting as beer starter strains.

Molecular typing reveals the genetic architecture of *S. cerevisiae* baker strains

Molecular characterization of the nine baker's strains at AGA1, SED1, HSP150 and DAN4 *loci* showed that all

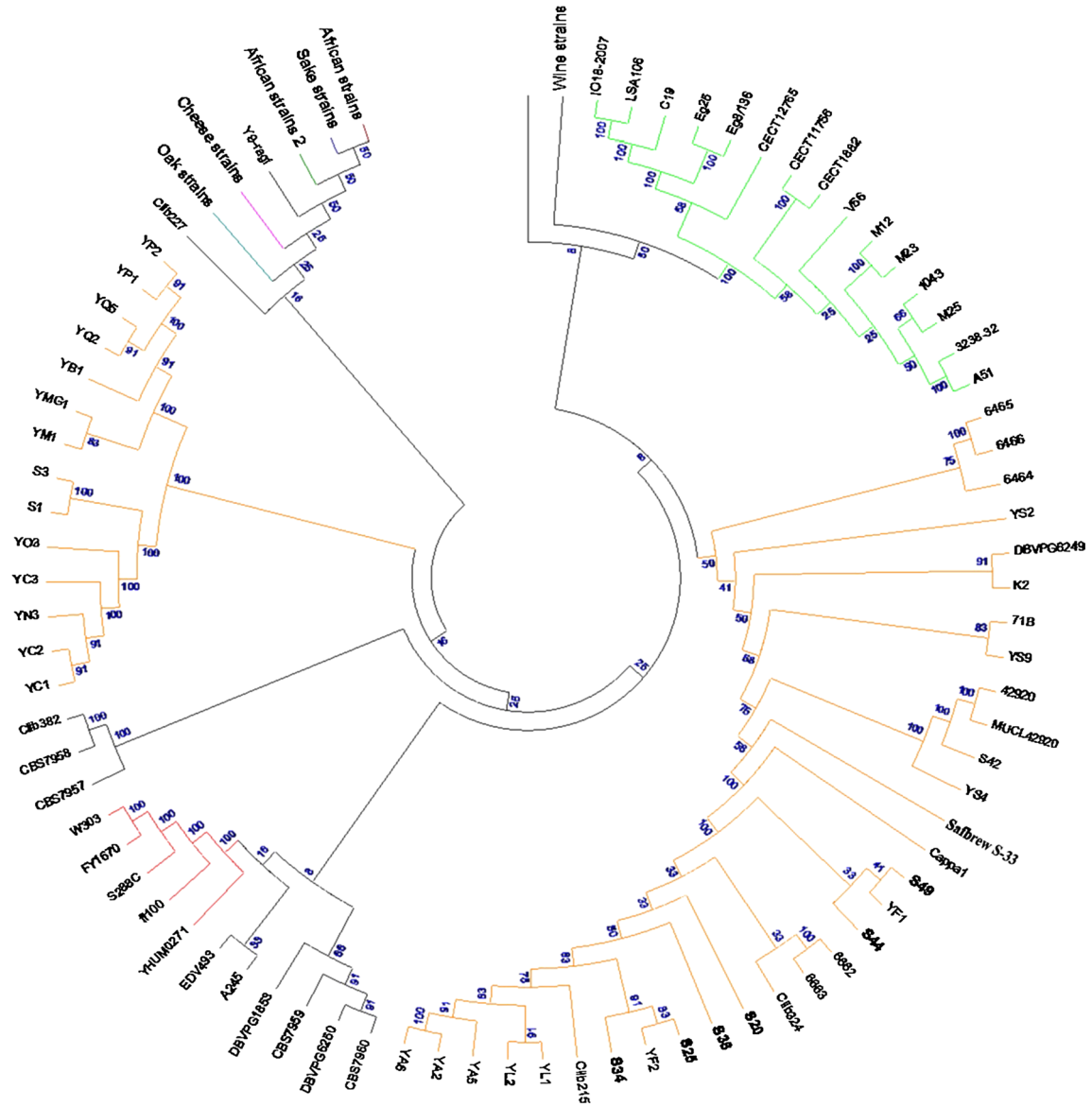

Fig. 1 Consensus neighbor-joining tree showing the clustering of 10 sardinian bread strains in comparison to 190 other yeast strains isolated from different sources (Bread strains are in *orange*, wine strains in *green*, flor strains in *light green*, sake strains in *dark blue*, rum strains in *purple*, cheese strains in *pink*, and oak strains in *blue–green*). The tree was constructed from the Bruvo distance between strains based on the polymorphism at 12 loci and is rooted on the group of Asian and African strain. Sardinian isolates are in *bold letters*. The reliability of the nodes were obtained with the Jackknife method. In particular the estimated parameter was calculated by systematically removing each of the 12 loci from the dataset. (color figure online)

the isolates are different strains (Supp. Table S1). In the sequence of the cell wall-related genes analyzed, minisatellite regions act as sites for genetic recombination and generate alleles with high-length polymorphism. Thus, analysis of these alleles allows the discrimination of *S. cerevisiae* isolates at the strain level [26].

The neighbor-joining tree obtained after genotyping the nine baker's strains at 12 microsatellite loci, allowed the comparison of these strains with Safbrew S33 and a database of 200 *S. cerevisiae* strains of different origin, both geographical and technological, including sourdoughs isolates (Fig. 1 and Supp. Table S2). The great intraspecific variability that characterizes *S. cerevisiae* has allowed the selection of strains with genetic characteristics adapted to different substrates and production technologies [4]. Furthermore, some authors have shown that *S. cerevisiae* has genetic, genomic and metabolic peculiarities that differ depending on their technological use [10, 14, 25]. Seven baker's strains were clustered with the main group (30 strains) of tetraploid bread strains isolated from France, Japan, Sicily and Spain [1, 25]. Strains S1 and S3, isolated in Sardinia, share genetic similarities with strains isolated from Sicilian sourdoughs and belong to the second minor cluster of bread isolates (14 strains). The hypothesis that baker's strains may be used as starters for beer production is supported by the observation that all the strains were clustered on the basis of their technological use (wine, bread, sake fermentation, etc.) and that the representative beer strain Safbrew-S33 belongs to the main cluster of bread strains.

Novel strain to ferment wort

The baker's strain S38 was used for wort fermentation in brewery as it showed good fermentation performances at the laboratory scale. Moreover, beers obtained with this strain were characterized by interesting aromatic properties (data not shown). Fermentation of wort lasted 6 days for the control strain Safbrew S33 and 11 days for the baker's strain S38. In this last case, while at day 3 and 6 the extract values were higher than the control, at the end of fermentation lower extract values were observed in the beer fermented by S38 (Table 5). Significant differences were also observed for alcohol production and attenuation, which were higher in beer fermented by S38. Interestingly, the beer produced with S38 resulted significantly less bitter than that produced with Safbrew S33. After the re-fermentation in bottle, the use of the representative strain Safbrew-F2 or S38 did not significantly affect any of the parameters measured, including bitterness. Thus, S38 can be used for wort fermentation and/or beer re-fermentation without negatively affecting the physico-chemical composition of beer. As a confirmation of this conclusion, one-way ANOVA on the sensory profile of beers did not show significant differences among the four beers obtained (Fig. 2). With regard to the odor it can be observed that the S33-S38 beer is the less fruity and the S38-F2 beer presents a less intense yeast character but not in a significant manner. Regarding taste sensations, the S33-F2 beer is the least acid and the S33-S38 beer is the least bitter, even if the differences are not significant. Finally, no differences were found in the mouth-feel sensations.

Table 5 Two-way ANOVA table of the main physiochemical parameters measured in beers fermented by the baker strain S38 and the representative beer strain Safbrew-S33 at the end of fermentation (A) and re-fermentation (B) at a brewery scale

	Principal effects							Interaction AB *p* value
	A: fermentation strain			B: re-fermentation strain				
	p value	Mean		*p* value	Mean			
		S33	S38		No	F2	S38	
Extract								
Apparent	>0.0000	3.54[a]	2.06[b]	0.0003	2.91[a]	2.72[b]	2.77[b]	0.0529
Real	>0.0000	5.20[a]	4.17[b]	0.0008	4.82[b]	4.64[a]	4.73[a]	0.0282
Alcohol								
Volume	>0.0000	5.27[a]	6.12[b]	0.0003	5.49[a]	5.79[b]	5.80[b]	0.0779
Attenuation								
Apparent	>0.0000	73.23[a]	84.61[b]	0.5712	77.80[a]	79.3[a]	79.65[a]	0.9185
Real	>0.0000	61.56[a]	70.41[b]	0.0001	64.75[a]	66.4[b]	66.80[b]	0.0422
Bitterness	0.0185	29.33[a]	25.66[b]	0.3119	29.00[a]	27.00[a]	26.50[a]	0.4736

Data are means of three to six independent fermentations

For each condition (fermentation and re-fermentation), same superscript letters in the same row indicate not significant differences ($p < 0.05$) as determined by Tukey HSD test

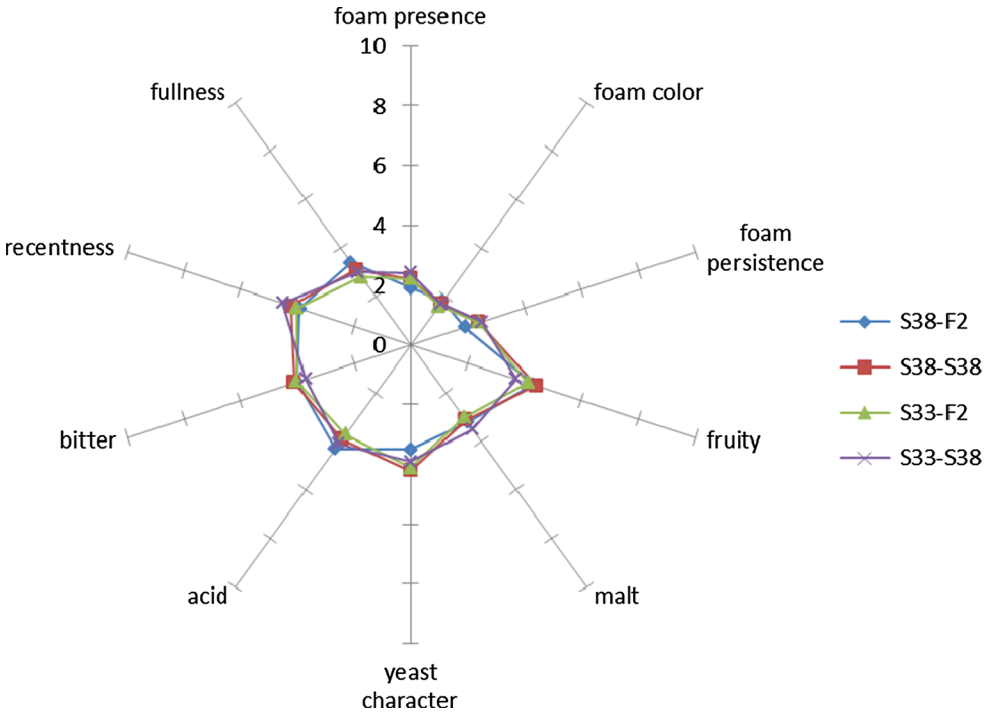

Fig. 2 Sensory profile of beers obtained at brewery scale using S38 as fermentative and F2 as refermentative strain (S38-F2), S38 as fermentative and S38 as refermentative strain (S38-S38), Safbrew S33 as fermentative and F2 as refermentative strain (S33-F2) and Safbrew S33 as fermentative and F2 as refermentative strain (S33-S38)

These data were also confirmed by the preference ranking test whereas the beers did not show any significant difference for preference. In this case, it can be evidenced that the two beers, which were subjected to the secondary fermentation in bottle with the S38 strain, were more appreciated than the others even if the score obtained is not statistically significant.

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