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Research Article

Viability and Acidification by Promising Yeasts Intended as Potential Starter Cultures for Rice-based Beverages

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Abstract: Over the last years, some innovative cereal-based beverages were designed using beneficial lactic acid bacteria; however, few data are available on the potential role of yeasts. The main topic of this research was to investigate the suitability of four promising yeast strains (*Saccharomyces cerevisiae* var. *boulardii*, *Kluyveromyces lactis*, *Saccharomyces pastorianus* and *Kazachstania exigua*) as potential starter cultures for rice-based beverages. This aim was achieved through some intermediate scientific aims, i.e., by assessing cell viability and acidification in different cereal substrates (malt extract, soft wheat, rice and kamut flours); thereafter by studying acidification and persistence in an organic rice drink during a prolonged storage at 25 and 4°C. Rice flour provided appropriate growth for all the strains. *K. exigua* and *S. pastorianus* experienced a relatively fast acidification within 24 h. After 40 d the yeasts showed similar cell counts (*ca.* 7 log cfu/mL) and acidification (ΔpH of *ca.* 2.7 at 25°C and *ca.* 1.2-1.4 at 4°C) in the organic rice drink. The evaluation of viability and acidification by promising candidates should be a simple procedure to screen yeast strains for potential use as starter cultures to design new rice-fermented functional beverages.

Keywords: Cereals, functional beverages, health, rice, starter, yeasts

INTRODUCTION

In recent years, the development of new food products with improved quality and health benefits has been gaining a particular focus (Dallagnol *et al*., 2013). An increasing trend is the use of rice flour as a good substrate to prepare gluten-free products due to some interesting properties such as natural, hypoallergenic, colourless and bland taste (Ronda *et al*., 2014). Moreover, rice is considered as a suitable carrier in new probiotic formulations due to its ability to well support the growth of probiotic bacteria and its protective bile resistance effect (Subhasree *et al*., 2013). Similarly, interest in rice fermented product is growing globally among general population (Ghosh *et al*., 2015). Alcoholic beverages such as *sake* in Japan, *jiu* in China, *yakju* in Korea, *tapuy* in Philippines, *ruou nep than* in Vietnam, *tapai* in Malaysia and Indonesia, *tapae* in Cambodia and *sato* in Thailand are produced with rice as the main ingredient, often as the only cereal source (Luangkhlaypho *et al*., 2014).

In addition to traditional beverages, there have recently been efforts to design new functional beverages with health-promoting potential (Marsh *et al*., 2014). This kind of products are by far the most active functional food category because of convenience and possibility to meet consumer demands for container contents, size, shape and appearance, as well as ease of distribution and storage for refrigerated and shelf-stable products. Moreover, they are excellent carriers for nutrients and bioactive compounds including vitamins, minerals, antioxidants, ω-3 fatty acids, plant extracts and fiber, prebiotics and probiotics (Corbo *et al*., 2014). Thus some innovative cereal-based beverages were designed using beneficial Lactic Acid Bacteria (LAB) (Coda *et al*., 2012); however, a drawback in the literature is that few data are available on the potential role of yeasts (Corbo *et al*., 2014). Although they were not usually considered for processing to avoid the presence of ethanol in the beverages (Kreisz *et al*., 2008), recent reports evaluated the possibility of using yeasts as they can generate desirable aromatic compounds and can aid bacterial growth by producing amino acids, vitamins and other metabolites, as well as for the health-promoting potential of some species (Marsh *et al*., 2014).

Therefore, the main aim of this study was to assess the suitability of four yeasts as starter strains for rice-based beverages; the yeasts were selected on the basis of some beneficial effects on human health reported in the literature such as probiotic activity (*Saccharomyces cerevisiae* var. *boulardii*), improvement of bioavailability of minerals (*Saccharomyces pastorianus*

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and *Kazachstania exigua*) and folate biofortification (*Kluyveromyces lactis*) (Moslehi-Jenabian *et al*., 2010). This goal was achieved through some intermediate scientific aims, i.e., by assessing cell viability and acidification in different cereal substrates (malt extract, soft wheat, rice and kamut flours); thereafter by studying acidification and persistence of yeasts in an organic rice drink during a prolonged storage at 25 and 4°C.

MATERIALS AND METHODS

Yeast strains and growth conditions: The following microorganisms were used throughout this study: *Saccharomyces cerevisiae* var. *boulardii* ATCC MYA-796 from American Type Culture Collection (Manassas, USA) and *Kluyveromyces lactis* DBVPG 6530, *Saccharomyces pastorianus* DBVPG 6033, *Kazachstania exigua* DBVPG 4384 from the Industrial Yeast Collection, University of Perugia (Perugia, Italy). The strains, stored at 4°C on YPG slant (bacteriological peptone, 20 g/L; yeast extract, 10 g/L; glucose, 20 g/L; all the ingredients were from Oxoid, Milan, Italy), were grown in YPG broth, incubated at 25°C for 24 h before each assay; then, the cultures were centrifuged at 1000 g for15 min and washed twice with sterile distilled water.

Screening of cereal-based media: Malt extract from Oxoid and soft wheat, rice and Khorasan wheat (kamut) flours purchased from a local supplier were singly used for the preparation of cereal extracts. Briefly, 2 g of each substrate was mixed with 98 mL of distilled water and filtered under vacuum (pore size 0.45-µm; Sigma-Aldrich, Milan, Italy). After that, appropriate amounts of filtered extracts were added to 20 mL of distilled water in order to obtain culture media with 15% (w/w) of cereal concentration. Immediately after the inoculation (*ca.* 6.4-7.7 log cfu/mL), the samples were incubated at 4°C for 24 h to assess periodically pH values and viable cells. All the analyses were performed in duplicate over two different batches. Aliquots of cereal media not inoculated with the microbial targets were used as negative controls.

Cell viability and pH change in an organic rice drink: A commercial organic rice-drink gluten and dairy free and with no added sugar was purchased from a local supplier. Immediately after the inoculation (*ca.* 7.4-8.3 log cfu/mL), the samples (30 mL) were stored at 4°C or 25 for 40 d to assess periodically pH and viable cell counts. The analyses were done in duplicate over two different batches. Aliquots of rice drink, not inoculated with the microbial targets, were used as negative controls.

Determination of pH and kinetics of acidification: pH was determined through a pH-meter Crison 2001 (Crison Instruments, Barcelona, Spain); kinetics of acidification were reported as pH decrease referred to the initial value (ΔpH) and modeled through a modified Gompertz equation as follows:

$$y = \Delta pH_{\max} \exp\left\{-\exp\left[\left(\frac{d_{\max} e}{\Delta pH_{\max}}\right)(\alpha - time) + 1\right]\right\}$$

where, ΔpH_{max} is the maximal extent of acidification (maximal decrease of pH within the running time), d_{max} the acidificatione rate (ΔpH/h) and α (h) the time before the beginning of acidification.

Viable count: Yeast cell concentrations (log cfu/mL) were determined by using the standard plate count (YPG agar incubated at 25°C for 48-72 h).

Statistical analysis: A two-way Analysis of Variance (ANOVA) and Tukey's test as a *post-hoc* comparison test ($p<0.05$) were used to determine statistically significant differences. Statistical analysis and data fitting were performed through the software STATISTICA for Windows (StatSoft, Inc., Tulsa, OK, USA; software version 10.0.1011.0).

RESULTS AND DISCUSSION

Cereal fermentation processes depend on specific determinants, which have to be strictly controlled to get standardized and acceptable products; the type of flour is one of the most important determinant. It affects fermentation through the level and type of fermentable carbohydrates, nitrogen sources and growth factors (Pontonio *et al*., 2014). Therefore, yeasts were preliminarily studied for their ability to grow infourcereal-based substrates (malt extract, soft wheat, rice and kamut flours); a fast acidification was considered as a discriminant technological property. Table 1 shows the statistical effects of two-way ANOVA on pH decrease after 24 h; yeast and kind of substrate were used as input factors. Fig. 1a to d shows the acidification profile.

Both the targets and the substrate were significant, as well their interaction; namely, *K. exigua* and *S. pastorianus* experienced the highest acidification after

Table 1: Two way ANOVA for the effects of yeasts and kind of medium on the decrease of pH after 24 h. SS, sum of squares; MS, mean sum of squares (SS/degree of freedom)

Effect	SS	Degree of freedom	MS	F	P
Intercept	5.51	1	5.51	2215.56	<0.01
Yeast	0.37	3	0.12	49.86	<0.01
Medium	1.83	3	0.61	245.56	<0.01
Yeast * medium	0.63	9	0.07	28.27	<0.01
Error	0.04	16	0.002		

24 h (1.22 and 0.75, respectively), since they are respectively a typical sourdough-specific yeast species (De Vuyst *et al*., 2014) and a lager beer strain (Nuobariene *et al*., 2011), indeed high adapted to cereal-based environments. Moreover, yeasts attained after 24 h a cell count of 7.0-7.8 log cfu/mL (data not shown).

An important trait for the selection of suitable strains is their persistence and viability (Tripathi and Giri, 2014), particularly for cereal-type beverages that would traditionally be stored at room temperature (Marsh *et al*., 2014). Thus the viability of target yeasts in a commercial rice drink was assessed for 40 d both at 25 (room temperature) and 4°C (refrigeration) (Fig. 2); yeast count was *ca.* 7 log cfu/mL, thus the targets fulfilled the basic requirement of a minimal level of useful microorganisms in food (Corbo *et al*., 2014).

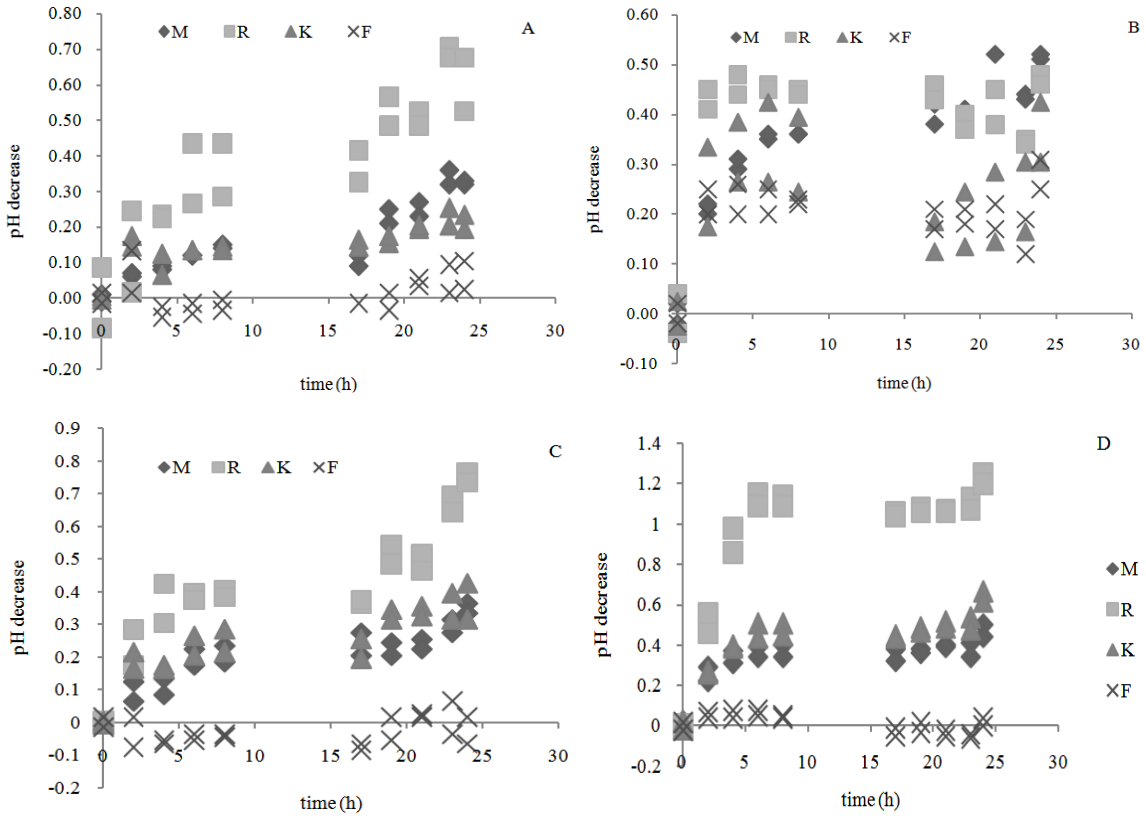
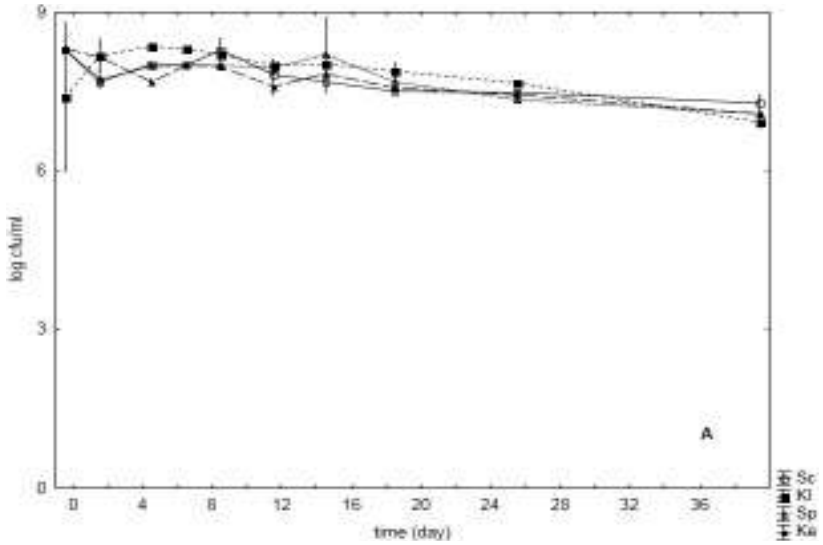


Fig. 1: pH decrease in the lab media containing the different extracts. M, Malt extract; R, rice; K, khorasan; F, wheat flour. For each sampling points, both the replicates were reported A, *S. cerevisiae* var. *boulardii*; B, *Kl. lactis*; C, *S. pastorianus*; D, *K. exigua*



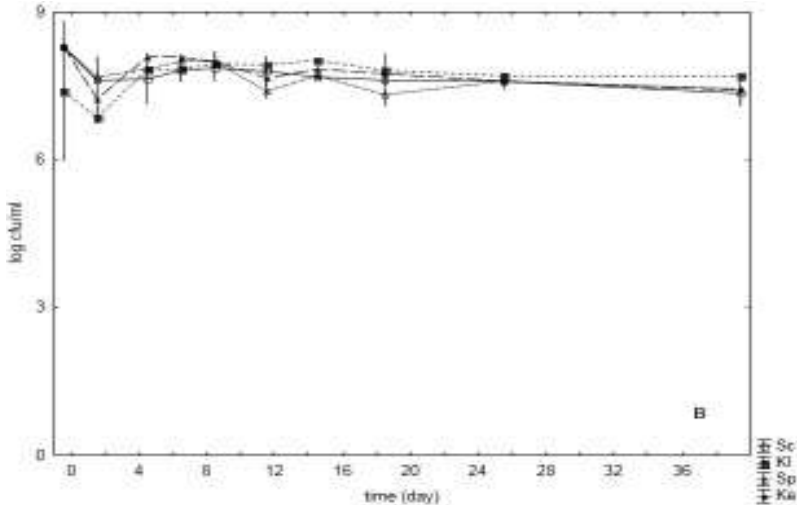


Fig. 2: Cell count of target yeasts in the commercial rice drink stored at 25°C (A) and 4°C (B) for 40 d. Mean values±standard deviation. Sc, *S. cerevisiae* var. *boulardii*; Kl, *Kl. lactis*; Sp, *S. pastorianus*; Ke, *K. Exigua*

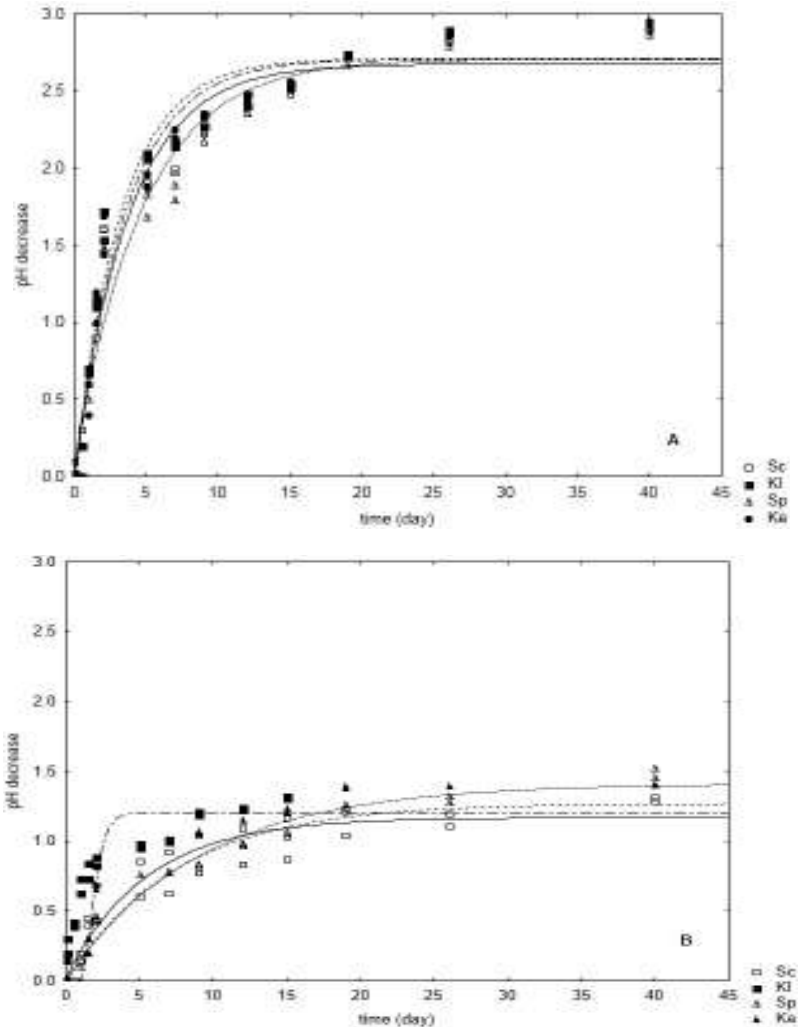


Fig. 3: Acidification profiles of target yeasts in the rice beverage stored at 25°C (A) or 4°C (B) for 40 d. For each sampling points, both the replicates were reported; lines represent the best fit through the Gompertz equation. Sc, *S. cerevisiae* var. *boulardii*; Kl, *Kl. lactis*; Sp, *S. pastorianus*; Ke, *K. exigua*

A topic of great concern in food applications relies upon cell viability of many probiotics during refrigerated storage (Zhang *et al*., 2014). The results from this study showed that rice constituents had a positive effect on the survival of target yeasts throughout the cold storage; moreover, storage temperature was not significant.

This effect was also found on lactic acid bacteria (*Lactobacillus plantarum* and *Lactobacillus reuteri*), inoculated in the same rice beverage, thus suggesting that some undefined compounds could exerted a protective effect against cell aging (A. Bevilacqua, unpublished data). Nevertheless, further studies are required to confirm this hypothesis.

Finally, we focused on the acidification, modeled through a modified Gompertz equation as it could be described by a sigmoidal trend, with three different steps: an initial phase when acidification did not occur (α), a tumultuous metabolic step and a final steady state (ΔpH_{max}). At 25°C yeasts reduced pH by 2.5-2.7 over 15 d; acidification occurred mainly within 5 d with a reduction of pH by 2-2.1. A further reduction was found for 5-10 d and then pH attained a steady state (Fig. 3A). Under refrigeration, *Saccharomyces* spp. and *Kl. lactis* experienced a similar trend with a ΔpH_{max} of 1.2-1.5 and acidification occurred within 15-20 days. The trend by *K. exigua* was quite different, with a significantly higher acidification rate within 2-3 days (0.87 ΔpH per day *vs* 0.12-0.20 ΔpH per day for the other strains), followed by a steady state after 5 days.

The design of new foods with enhanced healthyproperties is a challenge for food industry; some beverages with functional lactic acid bacteria are commercially available, but to date there is a lacking of details on yeast suitability, mainly for cereal-based beverages. The results of this research suggest that yeasts (*S. cerevisiae* var. *boulardii*, *Kl. lactis*, *S. pastorianus* and *K. exigua*) fulfilled both the requirements for a robust starter: acidification and prolonged cell viability; in addition, rice seems to provide a protective effect against cell aging during refrigerated storage. Further studies on yeast metabolism are required to understand their role during fermentation and storage conditions, as well as to design a controlled process using a selected culture (Fig. 3B).

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