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FUNCTIONAL CHARACTERISTICS OF *SACCHAROMYCES BOULARDII* STRAINS TO PRODUCE SOY BASED FERMENTED FOOD PRODUCTS

Beyza SAYMAN, Remziye YILMAZ*

FoodOmics Research Laboratory, Department of Food Engineering, Hacettepe University,
Beytepe Campus, 06800 Ankara, TURKEY

*remziye@hacettepe.edu.tr

INTRODUCTION

With the development of healthy nutrition awareness among consumers worldwide, foods are not merely a means of nutrition but have also started to be noticed as products with benefits. In addition, the trend of plant-based nutrition is growing as they are a much more sustainable and environmentally friendly alternative, especially compared with food of animal origin. To meet these demands simultaneously, this study aims to focus on the identification of *Saccharomyces cerevisiae var. boulardii* and determine its characteristics in order to be used in soy-based products that were developed with a probiotic yeast and can be used as the most used strain worldwide, *Saccharomyces cerevisiae*, but may also provide functionality.

METHODOLOGY

- For biochemical tests, optimum growth temperatures of all 51 strains of *S.cerevisiae* isolated from certain regions of Anatolia provided by FoodOmics Research Laboratory Culture Collection, were investigated.
- To understand heat stability, strains were incubated for 48 h in YPD (Yeast Peptone Dextrose) Broth at 30, 37, and 45°C in 48-well plates [1].
- For the investigation of glucose and galactose fermentation at the optimum growth temperature of *S.boulardii*, all strains were inoculated in media which contained 20g/L glucose or galactose, 20 mL bromothymol blue (50mg/75mL), 2.25g powdered yeast extract and 3.75g peptone in 1 liter of demineralized water and containing Durham tubes. Incubated at 37°C for 20 days [2].
- Lastly, for the low pH viability test, all experiments were carried out at certain pH values of 1.5, 2.0, 2.5, and 3.0, respectively. All strains were inoculated in YPD Broth in corresponded pH, adjusted with 1M HCl. All samples were collected at 0, 4, 8, 12, 16 h, and to evaluate the yeast viability, spectrophotometric readings at 600 nm were taken [3].
- For the High Resolution Melting (HRM) analysis, genomic DNAs from broth cultures of all samples were isolated using GeneMATRIX Bacterial & Yeast Genomic DNA Purification Kit with minor modifications. Selected genes for differentiation between *S. cerevisiae* and *S. boulardii*, *AAD15* and *MAL11* gene sequences [4] were downloaded from NCBI. Two new primers were designed specifically for this study using the Primer 3 program (Table 1).

Table 1. Designed Primers and Selected Genes for HRM Analysis.

Systematic Name	Gene	Function	Primers (5'-3')
YOL165C	***AAD15***	Aryl-Alcohol Dehydrogenase	ggatgtcatgggaggtggaa actcagtgccatgttcctca
YGR289C	***MAL11***	Alpha-glucoside transporter	tgggttagcgggtacacttt aaccaccggcaccattacta

- As control, *S. cerevisiae var. boulardii* CNCM I-745® and *S. cerevisiae* ATCC®9763 strains were used. The Solis Biodyne Hot FIREPol® EvaGreen® HRM Mix (ROX) was used for qPCR (Roche Light Cycler® 96), followed by the instruction manual.

REFERENCES

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RESULTS

- **Biochemical Tests**

Specific biochemical tests such as low pH viability, optimum growth temperature, and glucose-galactose fermentation ability were applied to differentiate *S. boulardii* strains from *S. cerevisiae* [2]. Table 2 presents *S.boulardii* and *S.cerevisiae* controls with four of the potential *S.boulardii* strains showing viability at 37°C. The selected strains also have the ability to utilize glucose while they cannot ferment galactose.

Table 2. Biochemical Test Results For Potential *S.boulardii* Strains and Reference Strains.

		Viability	*Low pH Viability*				*Optimum Growth Temperature*			*Fermentation Ability*	
Strains	Source	**YPD Broth 28˚C 48 h**	**pH 1.5**	**pH 2.0**	**pH 2.5**	**pH 3.0**	**28 ˚C**	**37 ˚C**	**45 ˚C**	**Glucose**	**Glactose**
***S. cerevisiae var. boulardii* CNCM I-745**	Commercial (Control)	+	-	-	+	+	+	+	+	+	-
***S. cerevisiae* ATCC®9763**	Commercial (Control)	+	-	-	-	+	+	+	-	+	+
***S. Cerevisiae* HUF16M3B11021**	Kırıkkale Grape	+	-	-	-	-	+	+	+	+	-
***S. cerevisiae* HUF16M3D11047**	Nevşehir Grape	+	-	-	-	-	+	+	-	+	-
***S. cerevisiae* HUF16M3H11104**	Çankırı Grape	+	-	-	+	+	+	+	+	+	-
***S. cerevisiae* HUF17M3D31089**	Nevşehir Grape	+	-	-	-	-	+	+	+	+	-

One of the potential *S.boulardii* strains (HUF16M3H11104) showed the desired characteristics as 2.5 pH viability, the growth temperature of 37˚C, and not utilizing galactose. Showing similar results as the control *S.boulardii*; therefore, it was selected for HRM analysis.

- **HRM Analysis**

HRM was performed to identify selected strains as *S. boulardii* by using qPCR to be used in future studies for fermentation of soy-based products.

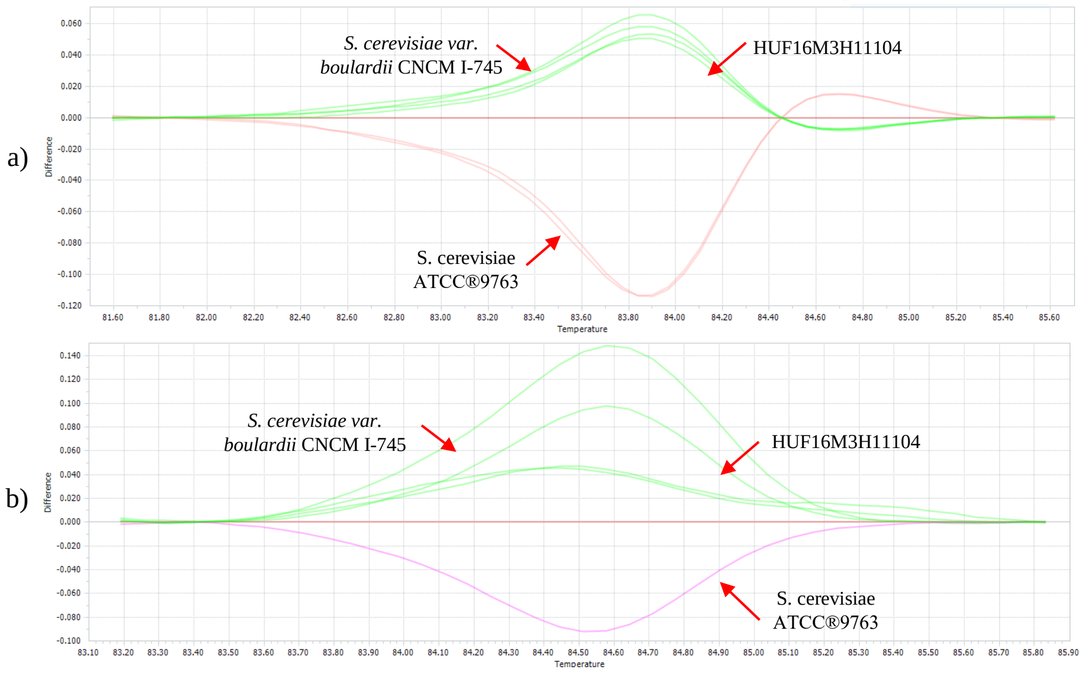

Figure 1. HRM Difference Plots for *AAD15* Gene (a) and *MAL11* Gene (b)

As seen in Figure 1, difference plots for *AAD15* and *MAL11* genes were obtained. The *AAD15* plot represents more reliable results when compared to *MAL11*. Nevertheless, both results satisfy the identification of *S.boulardii* successfully. As a result HUF16M3H11104 was identified as *S.boulardii.*

CONCLUSION

- The HUF16M3H11104 *S.boulardii* strain selected due to biochemical test results and identified by using HRM Analysis can be suitable for fermentation in replacement of *S. cerevisiae* to improve plant-based product quality.
- Considering the results, it has been observed that HRM can be successfully performed using primers designed for *AAD15* and *MAL11* genes.
- Future steps include an investigation of the products obtained from fermentation with identified *S. boulardii* strains. For further investigation, sensory and chemical differences should be considered while comparing traditional fermented products with *S. boulardii.*

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