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Review

Adaptive response and tolerance to sugar and salt stress in the food yeast *Zygosaccharomyces rouxii*

Tikam Chand Dakal, Lisa Solieri, Paolo Giudici *

Department of Life Sciences, University of Modena and Reggio Emilia, Via Amendola 2, 42122, Reggio Emilia, Italy

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ABSTRACT

The osmotolerant and halotolerant food yeast *Zygosaccharomyces rouxii* is known for its ability to grow and survive in the face of stress caused by high concentrations of non-ionic (sugars and polyols) and ionic (mainly Na^+ cations) solutes. This ability determines the success of fermentation on high osmolarity food matrices and leads to spoilage of high sugar and high salt foods. The knowledge about the genes, the metabolic pathways, and the regulatory circuits shaping the *Z. rouxii* sugar and salt-tolerance, is a prerequisite to develop effective strategies for fermentation control, optimization of food starter culture, and prevention of food spoilage. This review summarizes recent insights on the mechanisms used by *Z. rouxii* and other osmo and halotolerant food yeasts to endure salts and sugars stresses. Using the information gathered from *S. cerevisiae* as guide, we highlight how these non-conventional yeasts integrate general and osmoticum-specific adaptive responses under sugar and salts stresses, including regulation of Na^+ and K^+-fluxes across the plasma membrane, modulation of cell wall properties, compatible osmolyte production and accumulation, and stress signalling pathways. We suggest how an integrated and system-based knowledge on these mechanisms may impact food and biotechnological industries, by improving the yeast spoilage control in food, enhancing the yeast-based bioprocess yields, and engineering the osmotolerance in other organisms.

Contents

Abbreviations: CDRE, calcineurin dependent response element; CNV, copy number variation; CWI, cell wall integrity; DHA, dihydroxyacetone; DHAP, dihydroxyacetone phosphate; HOG, high-osmolarity glycerol; MAPK, mitogen-activated protein kinase; MAPKK, mitogen-activated protein kinase kinase; MAPKKK, mitogen-activated protein kinase kinase kinase; P-Hog1, phosphorylated Hog 1; STRE, stress responsive element; SWI/SNF complex, switch/sucrose non-fermenting complex.

* Corresponding author. Tel.: +39 0522522057; fax +39 0522522027.
E-mail address: paolo.giudici@unimore.it (P. Giudici).

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1. Introduction

The high concentrations of ionic (mainly Na^+) and non-ionic (mainly sugars and polyols) solutes reduce water activity (a_w) in food and are two of the major abiotic stressors, both limiting the yeast growth. High external osmolarity has been used for centuries for food preservation, because it causes water outflow from the cell and results in a higher intracellular concentration of ions and metabolites and in an eventual arrest of cellular activity. The yeast ability to cope with these environmental insults determines both the success of certain food and beverage fermentation and the thriving of food spoilage.

Since the sequencing of strain S288c (Goffeau et al., 1996), impressive advances in genomics, proteomics, and systems biology have made *S. cerevisiae* the paradigm for understanding these osmo-adaptive mechanisms, which have been exhaustively summarized by several reviews (Nevoigt and Stahl, 1997; Hohmann, 2002; Ariño et al., 2010; Kühn and Klipp, 2012). As a result, the *S. cerevisiae* response to high external solute concentrations has been described as a system-level coordination between the extracellular environment and the genetic make-up inside the cell. The following interconnected modules are involved: (i) receiving information from external environment (sensing); (ii) conducting it to the inside (signal transduction); (iii) integrating it with internal genetic information in order to mount an appropriate response (effector processes) (de Nadal et al., 2011). This system-level knowledge has been exploited in food industry to improve yeast fermentations on highly salty and sugary matrices or to decrease the food spoilage by sugar and salt resistant-yeast species. However, as being moderately halotolerant and osmotolerant, *S. cerevisiae* could be inappropriate to describe the yeast response to hypersaline and hyperosmotic food.

Zygosaccharomyces rouxii is the osmotolerant and halotolerant yeast most phylogenetically related to *S. cerevisiae* and inhabits a variety of highly sugary and salty food, where it carries out fermentation or determines food spoilage. It belongs to the genus *Zygosaccharomyces*, which includes the highest number of salt and sugar-tolerant yeasts. The majority of these species are osmotolerant (positive growth at high sugar concentration up to 60–70% glucose), whereas only a few are both highly osmo and halotolerant (Table 1). Recently, the complete genome sequences of *Z. rouxii* (Souciet et al., 2009) and other highly osmo and halotolerant yeasts, such as *Millerozyma farinosa* (formerly *Pichia sorbitophila*) (Louis et al., 2012), *Debaryomyces hansenii* (Kumar et al., 2012), and *Zygosaccharomyces bailii* (Galeote et al., 2013), have become available. Furthermore, 'omics' tools and genetic manipulation protocols have been recently employed to analyze the relationships of osmostress phenotype to genetic and molecular determinants (Prybilova et al., 2007a,b; Watanabe et al., 2010). From these intense efforts, the yeast osmostress adaptation emerges as a complex mechanism that integrates genes, regulatory networks, and signalling pathways, and that differs depending upon the species and the osmoticum in the surrounding medium. Comparison of species with different sugar and salt tolerance highlighted how yeasts exploit different strategies to survive under osmotic and salt stress (Ramos et al., 2011). For example, *Z. rouxii* resembles *S. cerevisiae* in extruding Na^+ cations out of the cell or driving them into the vacuole (Ramos, 1999), while the halotolerant yeast *Debaryomyces hansenii* is a sodium includer, which accumulates intracellularly Na^+ without getting intoxicated (Ramos, 1999). Beyond the species-specific strategies, other osmostress responses, such as the osmolytes accumulation, are ubiquitous among yeasts to avoid outflow of cellular water in low a_w environments (Nevoigt and Sthal, 1997; Lages et al., 1999; Silva-Graça and Lucas, 2003). Another emerging issue concerns how salt and sugars elicit distinct or partial overlapping responses in yeasts. Whereas sugars and polyols modify osmotic pressure, salts induce alterations both in osmotic pressure and ion homeostasis. The result is that partially different mechanisms become operational in response to sugar and salts. Since halo and osmotolerance could be paired and unpaired phenotypes in *Z. rouxii* and relatives, these yeasts are very attractive models for deciphering genetic circuits and functional pathways underlying halotolerance and osmotolerance.

Here, we review recent insights on the mechanisms that govern halotolerance and osmotolerance in *Z. rouxii* and compare them to those active in *S. cerevisiae* and in other osmo and halotolerant food yeasts at genetic, metabolic, signalling, and epigenetic level. Furthermore, we highlight how these yeasts can achieve generic and osmoticum-specific responses to sugar and salt stresses. Finally, we point out how the understanding of osmostress responsive mechanisms can advantage microbial fermentation and food quality.

2. A matter of nomenclature

Tolerance to high ionic and non-ionic solute concentrations is a specific cellular adaptability to sudden and severe fluctuations in water availability and a tendency of cells to restore or maintain normal physiology, morphology and biological functions (Yancey, 2005; Klipp et al., 2005). Microbial growth under high external osmolarity is frequently described in terms of a_w that is the chemical potential of free water in solution. Microorganisms able to colonize food with high osmolarity and, consequently, low a_w, were collectively indicated as xerotolerant (no absolute requirement of low a_w), and xerophilic ("lovers of low a_w") (Pitt and Hocking, 2009) (Table 1). A more appropriate microbial classification would consider the kind of osmoticum and include the following categories: osmophilic, absolute requirement for non-ionic solutes and ability to grow up to solute concentrations approaching saturation; osmotolerant, no absolute requirement of non-ionic solutes for viability and ability to tolerate a wide range of osmolarity, from hypo-osmotic to hyper-osmotic solutions; osmosensitive, sensitive to excess concentration of non-ionic solutes; halophilic, absolute requirement for high salt and ability to grow up to salt concentrations approaching saturation; halotolerant, no absolute requirement of salt for viability and ability to tolerate a wide range of salinity, from hypo-saline to hyper-saline solution; and halosensitive, sensitive to excess concentration of salt.

Most food yeasts can develop well at a_w values around 0.95–0.90. A cut-off of $a_w < 0.70$ has been frequently used to delineate osmotolerant and halotolerant yeasts. In the past, yeasts isolated from sugary and salty food with a_w lower than 0.70 were referred to as "osmophilic" and "halophilic" (Tokuoka, 1993). For instance, *Debaryomyces hansenii* has been described as halophilic yeast based on the ability to grow at 1.0 M of salt with growth rate and final biomass close to the values obtained without salt (Almagro et al., 2000; González-Hernández et al., 2004; Aggarwal and Mondal, 2009). Other yeasts were classified as halophilic or osmophilic, such as *M. farinosa* (formerly *P. sorbitophila*) (Rodrigues de Miranda et al., 1980), *Candida etchellsii* (formerly *Candida halonitratophila*), *Candida versatilis* (Barnett et al., 2000), and the black yeast *Hortea werneckii* (Gunde-Cimerman et al., 2000). However, differently from halophilic and osmophilic bacteria, none of these yeasts satisfies the true definition of osmophily or halophily, because they