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Comparative Regulation of Invertase Activity during Short- and Long-Term Glucose Derepression across Yeast Species

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Abstract

Glucose repression and derepression play a central role in regulating carbon source utilization in yeasts. However, the strength and dynamics of this regulation differ markedly among yeast species. In this study, invertase regulation was examined under short- and long-term derepression in several non-conventional yeasts (*Pichia farinosa*, *Pichia anomala*, *Pichia jadinii* and *Candida milleri*). *Saccharomyces cerevisiae* was included as a reference yeast species. Yeast cells were first grown under fermentable and nonfermentable conditions to establish short-term and long-term derepressed metabolic states, and subsequently transferred to repressed (2% glucose) or derepressed (0.05% glucose) media prior to invertase activity measurements. Growth analysis revealed clear interspecies differences in metabolic performance. Non-conventional yeasts, particularly *P. jadinii*, maintained relatively high growth rates under both rich and minimal conditions. Invertase activity showed distinct regulatory patterns among species. *S. cerevisiae* displayed strong glucose-dependent repression and robust induction under derepressed conditions, as expected. In contrast, *P. farinosa* exhibited no detectable invertase activity. *P. anomala* showed a transient increase in activity during short-term derepression, but this response was not sustained after long-term derepression. *P. jadinii* exhibited high and persistent invertase activity, especially following long-term derepression. *C. milleri* maintained elevated invertase activity under both repressed and derepressed conditions, indicating reduced sensitivity to glucose availability. Time-course experiments demonstrated that invertase regulation is highly dynamic and strongly influenced by prior growth conditions. Overall, these findings highlight the diversity of carbon regulation strategies in yeasts and emphasize the metabolic flexibility of non-conventional yeast species.

Keywords: Carbon-catabolite regulation; Glucose repression; Invertase; Metabolic adaptation; Non-*Saccharomyces* yeasts

1. Introduction

Carbon source availability is a central determinant of growth, metabolic organization, and gene expression in yeast. Glucose is preferentially utilized and supports rapid fermentative growth. When glucose is abundant, genes required for the utilization of alternative carbon sources are repressed, a regulatory phenomenon known as glucose repression or carbon-catabolite repression [1-3].

In *Saccharomyces cerevisiae*, glucose repression is controlled by a well-characterized regulatory network that links carbon availability to transcriptional regulation. The Snf1 protein kinase, the yeast homolog of AMP-activated protein kinase (AMPK), plays a central role in this regulation process. Under glucose-rich conditions, Snf1 remains inactive, allowing the transcriptional repressor Mig1 to accumulate in the nucleus and repress target genes involved in alternative carbon metabolism, including *SUC2*, which encodes extracellular invertase [4,5]. Upon glucose depletion, Snf1 becomes activated, leading to phosphorylation-dependent nuclear export of Mig1 and relief of transcriptional repression [6,7].

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Glucose-responsive transcription is further shaped by additional nutrient-sensing pathways. The cAMP-protein kinase A (PKA) pathway responds to extracellular glucose signals and promotes fermentative growth while repressing stress-responsive gene expression [2,8,9]. In parallel, the target of rapamycin (TOR) pathway integrates carbon and nitrogen availability to regulate anabolic growth. Under nutrient-rich conditions, TOR signalling supports biosynthesis, whereas nutrient limitation results in TOR inhibition and activation of catabolic and stress-associated programs [10,11,12]. Together, these pathways coordinate growth and metabolism in response to nutrient status.

Within this regulatory framework, invertase occupies a central position as a classical readout of glucose repression and derepression. Invertase hydrolyzes sucrose into glucose and fructose outside the cell, enabling subsequent uptake and metabolism. In *S. cerevisiae*, invertase synthesis is tightly regulated at the transcriptional level and responds sensitively to glucose availability. Low basal activity under glucose-replete conditions and strong induction upon glucose limitation are characteristic features of this system [13,14,15]. Because invertase is secreted and its activity can be readily quantified, *SUC2* expression and invertase activity have been widely used as experimental markers for monitoring glucose repression dynamics.

Glucose derepression can occur under different physiological contexts. Short-term derepression refers to the acute response following glucose depletion and involves rapid signalling and transcriptional changes mediated primarily by Snf1 activation and Mig1 relocalization [7]. Long-term derepression develops after prolonged growth on nonfermentable carbon sources and is associated with broader metabolic adjustments, including increased respiratory activity and changes in enzyme expression [4,16,17,18].

Non-conventional yeast exhibit substantial diversity in carbon metabolism, regulatory sensitivity, and ecological adaptation. Comparative physiological and genomic studies indicate that while core components of glucose signalling pathways may be conserved, regulatory architectures and metabolic outputs differ among yeast species [19,20,21]. *Pichia farinosa* has primarily been investigated in relation to stress tolerance and alternative metabolic traits, whereas information on its glucose-responsive regulation of extracellular carbohydrases remains limited. *Pichia anomala* is known for its metabolic versatility and ability to utilize a broad range of carbon sources. *Pichia jadinii* (syn. *Candida utilis*) has been widely used as a model for respiratory metabolism and biomass production on nonfermentable substrates. *Candida milleri* (syn. *Candida humilis*) is frequently associated with sourdough and other mixed microbial ecosystems characterized by fluctuating sugar availability, where carbon utilization strategies differ from those observed under stable, glucose-rich laboratory conditions [19,22,23].

In this study, invertase regulation under short-term and long-term derepression was examined in non-conventional yeast species and compared with the well-characterized *S. cerevisiae* model. By combining growth kinetics, invertase activity measurements, and time-course analyses, this approach allows direct comparison of glucose-responsive regulatory behavior across species and highlights differences in carbon-catabolite regulation beyond the *S. cerevisiae* framework.

2. Materials and Methods

2.1. Yeast strains

The yeast strains used in this study included non-conventional yeasts *Pichia farinosa* (DGVPG-3626/CBS185), *Pichia anomala* (DBVPG-3511), *Pichia jadinii* (DBVPG-6160/CBS621), and *Candida milleri* (DBVPG-6754/CBS8195) which were obtained from the DBVPG yeast collection (University of Perugia, Italy). *Saccharomyces cerevisiae* (BY4741) strain used as the reference strain, was obtained from the EUROSCARF (European *Saccharomyces cerevisiae* Archive for Functional Analysis). All non-conventional yeast strains were wild type isolates. The genotype of *S. cerevisiae* strain used in this study is MATa, *his3Δ1; leu2Δ0; met15Δ0; ura3Δ0*.

2.2. Growth conditions

Yeast strains were grown in different media according to their nutritional requirements. Growth in rich YP medium (10 g/L Yeast Extract and 20 g/L Bacto-Peptone) supplemented with either glucose (YPD) or 2% glycerol and 2% lactate (YPGL) was used for *P. farinosa P. anomala,* and *S. cerevisiae*. In contrast, *P. jadinii* and *C. milleri* were grown in MP medium (5 g/L Malt Extract and 20 g/L Bacto-Peptone) supplemented with the same carbon sources as described previously [24]. Cultures were incubated with constant shaking (250 rpm) at 30°C.

For short-term derepression (STD), yeast species were grown in rich medium (YP or MP) containing 2% glucose, whereas for long-term derepression cells were grown in rich medium supplemented with 2% glycerol and 2% lactate,

both until mid-logarithmic phase (OD_{600}~0.5-0.6). Cultures were then divided into two portions, harvested, washed and resuspended in fresh medium containing either 2% glucose (repressed) or 0.05% glucose (derepressed). Cells were subsequently incubated for an additional 3 h under the same growth conditions prior to enzyme activity determination.

For minimal growth conditions, non-conventional yeast species were grown in nitrogen base medium (YNB) (1.6 g/L Yeast Nitrogen Base and 5.0 g/L $NH_4)_2SO_4$) containing either glucose (YNBD) and glycerol-lactate (YNBGL). *S. cerevisiae* yeast cells were grown in YNB medium supplemented with appropriate amino acids (0.02 g/L histidine, 0.06 g/L leucine, 0.02 g/L methionine, and 0.02 g/L uracil), and with appropriate fermentable (glucose) and nonfermentable (glycerol-lactate) carbon sources.

2.3. Growth characteristics: Doubling time and specific growth rates

Growth characteristics were determined by growing yeast strains in YP, MP, or YNB media supplemented with either fermentable or nonfermentable carbon sources at the same conditions up to stationary phase. During the growth period, OD_{600} was measured at 30 min time intervals, growth curves were generated for calculating doubling times (T_d) and specific growth rates (μ). Mean doubling times were calculated from three independent experiments for each strain and condition.

2.4. Invertase enzyme assay and time-course analysis

Secreted invertase activity was determined using whole yeast cells with a previously described protocol [25]. Yeast cells were grown to logarithmic phase (OD_{600}~0.5-0.6) in rich media supplemented with fermentable (2% glucose) and nonfermentable (2% glycerol and 2% lactate) carbon sources. Yeast cells were harvested, washed and transferred to repressed and derepressed conditions and incubated for 3 h. Then, they were harvested, washed, and resuspended in 200 μL sodium acetate buffer containing 2 mM PMSF (50 mM, pH 5.0). Aliquot of cell suspension was incubated with sucrose (200 mM) for 15 min at 37°C. The reaction was terminated by adding 50 μL of 1M Tris-HCl (pH 8.8), and released glucose was quantified enzymatically using the glucose oxidase-peroxidase system using a commercial kit (Fluitest®- GLU, Biocon, Germany). Invertase activity was expressed as units, defined as μmol of glucose released per minute per 100 mg dry weight of yeast cells.

For time-course experiments, logarithmically growing cells were transferred to fresh repressed and derepressed media containing either fermentable or nonfermentable carbon sources. Invertase activity was then measured at 1 h time intervals from logarithmic growth to stationary phase.

2.5. Effects of temperature and pH on invertase activity

To evaluate the effect of temperature, logarithmically growing yeast cells were harvested, washed, and resuspended in repressed and derepressed media. Cultures were incubated for 3 hours at 25°C, 30°C, 37°C, and 42°C with constant shaking, and invertase activity was subsequently determined as described above. To assess the effect of pH, repressed and derepressed cultures were prepared in the same manner and divided into aliquots. The pH of each aliquot was adjusted to 4.0, 5.0, 6.0, 7.0, or 8.0 using 1 M HCl or 1 M NaOH. Cultures were incubated for 3 hours at 30°C and invertase activity was determined.

2.6. Statistical analysis

All experiments were performed at least three independent biological replicates. Data are presented as mean ± standard deviation (SD).

3. Results

3.1. Growth kinetics of yeast species

To enable a quantitative comparison of growth performance among yeast species, growth kinetics evaluated under defined nutritional conditions using fermentable (glucose) and nonfermentable (glycerol-lactate) carbon sources. Doubling times (Td) were determined during logarithmic growth and specific growth rates (μ) were calculated to allow direct comparison of growth efficiency across species and media (Table 1). *P. farinosa* displayed relatively uniform growth across all tested conditions, with doubling time increasing from rich medium (176 min) to nonfermentable and minimal condition (up to 291 min). Correspondingly, specific growth rates gradually declined from 3.95×10^{-3} to 2.38×10^{-3} min^{-1} with increasing nutritional restriction. *P. anomala* exhibited consistently rapid growth, with short doubling times in rich medium (up to 113 min) and moderate increases under minimal conditions (132 min in glucose and 161 min in glycerol-lactate). Specific growth rates remained relatively high (>4.0×10^{-3} min^{-1}) across all conditions.

Among the tested species, *P. jadinii* showed the highest growth efficiency. Doubling times were low in rich medium (102 min and 129 min), and growth in minimal medium was even faster (80 min), corresponding to the highest specific growth rate value observed (>4.0×10^{-3} min^{-1}). The reference strain *S. cerevisiae* grew rapidly in rich medium (113 min) as expected but pronounced growth impairment on nonfermentable carbon sources and under minimal condition, with doubling times exceeding 460 min and specific growth rate value below 2.0×10^{-3} min^{-1}.

Overall, comparison of doubling times and specific growth rates revealed substantial interspecies variation in growth efficiency. Non-conventional yeast, particularly *P. jadinii* and *P. anomala*, maintained higher growth rates across diverse nutritional conditions, whereas *C. milleri* showed pronounced reduction in growth efficiency under minimal and nonfermentable conditions like *S. cerevisiae*.

Table 1 Specific growth rate (μ) of yeast species under different media and carbon sources

Yeast species	Rich medium		Minimal Medium	
	Fermentable	**Nonfermentable**	**Fermentable**	**Nonfermentable**
P. farinosa	3.95×10^{-3}	3.14×10^{-3}	2.71×10^{-3}	2.38×10^{-3}
P. anomala	6.66×10^{-3}	6.16×10^{-3}	5.23×10^{-3}	4.31×10^{-3}
P. jadinii	6.78×10^{-3}	5.39×10^{-3}	8.62×10^{-3}	4.62×10^{-3}
C. milleri	4.29×10^{-3}	3.43×10^{-3}	2.65×10^{-3}	1.03×10^{-3}
S. cerevisiae	6.15×10^{-3}	2.57×10^{-3}	1.51×10^{-3}	1.27×10^{-3}

3.2. Carbon source-dependent invertase activity in yeast species

To distinguish immediate glucose-dependent regulation of invertase activity from longer-term metabolic adaptation and evaluate species-specific differences in carbon catabolite regulation, invertase activities were analysed under both short-term and long-term derepression conditions. Short-term derepression (STD) was defined as the acute response following transfer from glucose-rich to glucose-limited conditions, whereas long-term derepression (LTD) reflected metabolic adaptation after growth on nonfermentable carbon sources. For STD, cells were initially grown under repressed conditions (2% glucose) and then transferred to derepressed conditions (0.05% glucose). In contrast, for LTD, they were grown on nonfermentable carbon sources (2% glycerol and 2% lactate), representing a transcriptionally and metabolically derepressed state, and subsequently transferred to repressed or derepressed conditions.

As a well-characterized yeast species in which glucose repression and derepression are extensively studied, *S. cerevisiae* was included as a reference strain to validate the experimental conditions and to provide a benchmark for comparison with non-conventional yeast species. Invertase activity in *S. cerevisiae* showed a clear and pronounced response to glucose derepression under both STD and LTD conditions (Figure 1). Under STD, repressed cells exhibited only very low basal invertase activity, whereas derepressed cells displayed a strong induction, reaching 1041 units. This difference was further amplified under LTD, where invertase activity increased around 2356 units in derepressed cultures, while repressed cells remained at comparatively low activity levels. The distinct separation between repressed and derepressed conditions demonstrates robust glucose-dependent regulation of invertase in *S. cerevisiae* and supports its use as a reference strain for comparison with non-conventional yeast species.

In *P. farinosa*, invertase activity remained extremely low under both repressed and derepressed conditions, and no consistent induction was detected under either STD or LTD (Figure 2). In clear contrast to the strong derepression-dependent induction observed in *S. cerevisiae*, invertase does not appear to contribute to carbon source utilization in this yeast species.

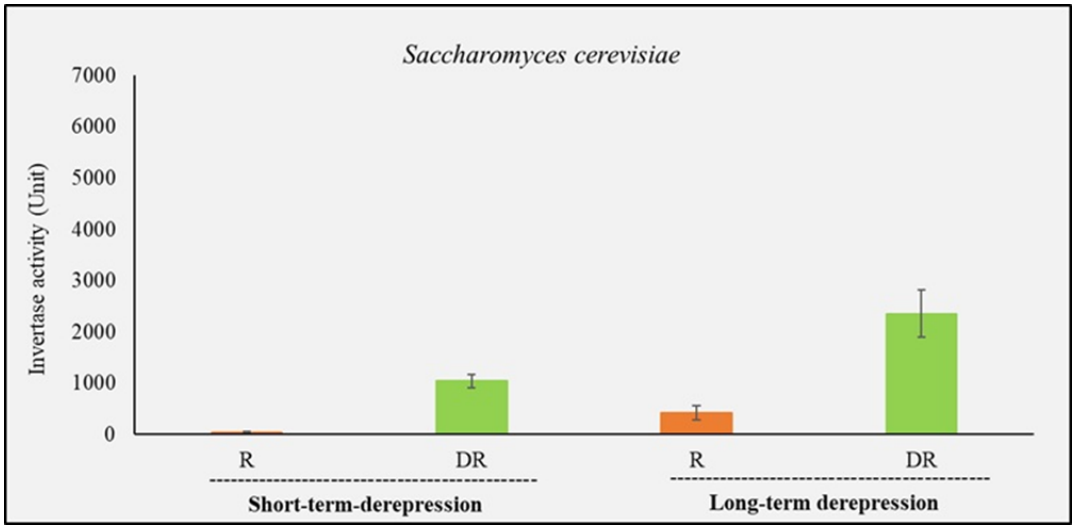

Figure 1 Invertase activity of *S. cerevisiae* under short-term and long-term derepression conditions. Invertase activity was expressed as units (µmol glucose/minute/100 mg dry weight of yeast cells)

Figure 2 Invertase activity of *P. farinosa* under short-term and long-term derepression conditions. Invertase activity was expressed as units (µmol glucose/minute/100 mg dry weight of yeast cells)

Invertase activity in *P. anomala* exhibited a clear but condition-dependent response to glucose derepression (Figure 3). Under STD, derepressed cells showed a pronounced increase in invertase activity, reaching 404 units, whereas repressed cells displayed only low basal activity. In contrast, this induction was not maintained under LTD, where invertase activity remained low following growth on nonfermentable carbon sources, even after transfer to derepressed conditions. These results indicate that *P. anomala* is capable of a rapid and transient invertase response upon acute glucose depletion but displays a delayed or limited reactivation of invertase synthesis following prolonged growth on nonfermentable substrates. Compared to *S. cerevisiae*, the invertase response in *P. anomala* appears weaker and more dependent on the metabolic history of the cells.

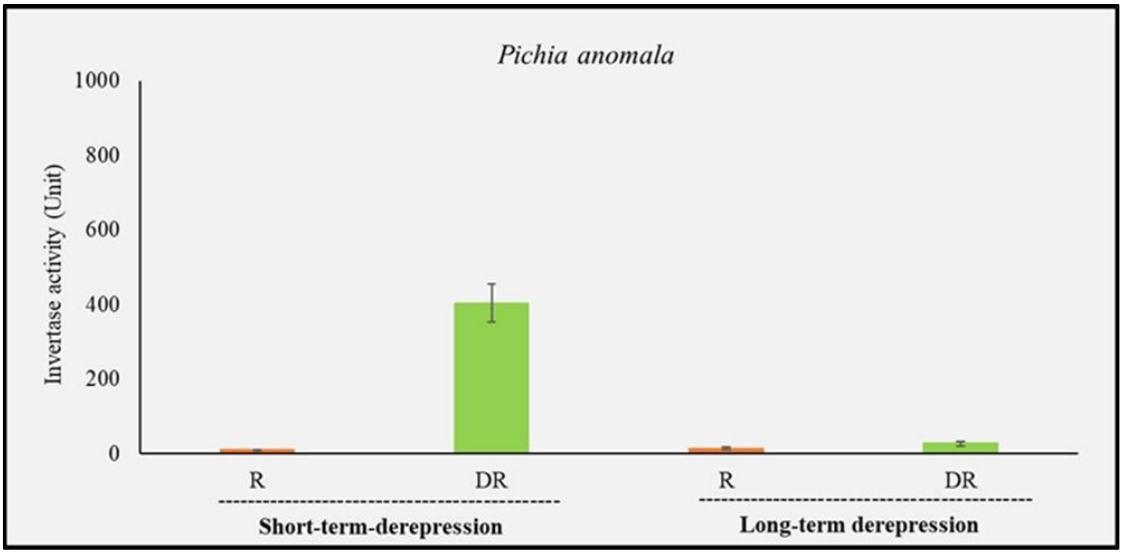

Figure 3 Invertase activity of *P. anomala* under short-term and long-term derepression conditions. Invertase activity was expressed as units (µmol glucose/minute/100 mg dry weight of yeast cells)

Invertase activity in *P. jadinii* showed a strong and sustained response to glucose derepression under both STD and LTD (Figure 4). Under STD, derepressed cells displayed a clear increase in activity compared to repressed cells, with activity levels rising from 486 to over 1100 units. This response became markedly more pronounced under LTD, where invertase activity reached 6010 units in derepressed cultures, while repressed cells also exhibited substantial but lower activity levels (3585 units). The magnitude of induction under LTD indicates that *P. jadinii* not only responds rapidly to glucose depletion but is also capable of maintaining high invertase activity during prolonged growth under derepressed conditions. In comparison to *S. cerevisiae*, *P. jadinii* exhibited similarly strong derepression-dependent regulation, with even higher absolute activity levels under LTD.

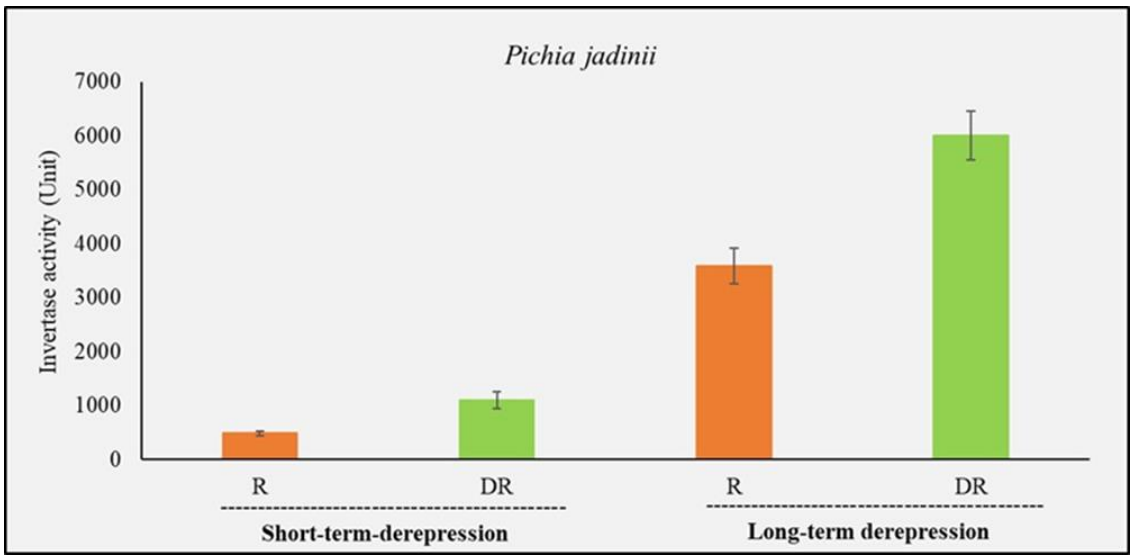

Figure 4 Invertase activity of *P. jadinii* under short-term and long-term derepression glucose repression and derepression conditions. Invertase activity was expressed as units (µmol glucose/minute/100 mg dry weight of yeast cells)

Invertase activity in *C. milleri* was detected under both repressed and derepressed conditions, but the overall response to glucose derepression was less pronounced than that observed in some other yeast species (Figure 5). Under STD, derepressed cells exhibited higher invertase activity compared to repressed cells, increasing from 1472 to 3066 units. However, this difference was not maintained under LTD, where invertase activities in repressed and derepressed cultures were comparable, remaining close to 3000 units. In contrast to the strong and sustained derepression-

dependent induction observed in *S. cerevisiae* and *P. jadinii*, *C. milleri* displays a more limited and condition-dependent regulation of invertase activity.

Taken together, these results demonstrate marked interspecies differences in invertase regulation. While *P. anomala* exhibits a clear but transient invertase induction under short-term derepression, resembling an acute glucose-responsive behaviour, it fails to sustain this response after long-term growth on nonfermentable carbon sources, in contrast to the tightly regulated and persistent derepression observed in *S. cerevisiae*. In comparison, *P. jadinii* and *C. milleri* maintain high invertase activity across both repressed and derepressed conditions, indicating reduced sensitivity to glucose availability.

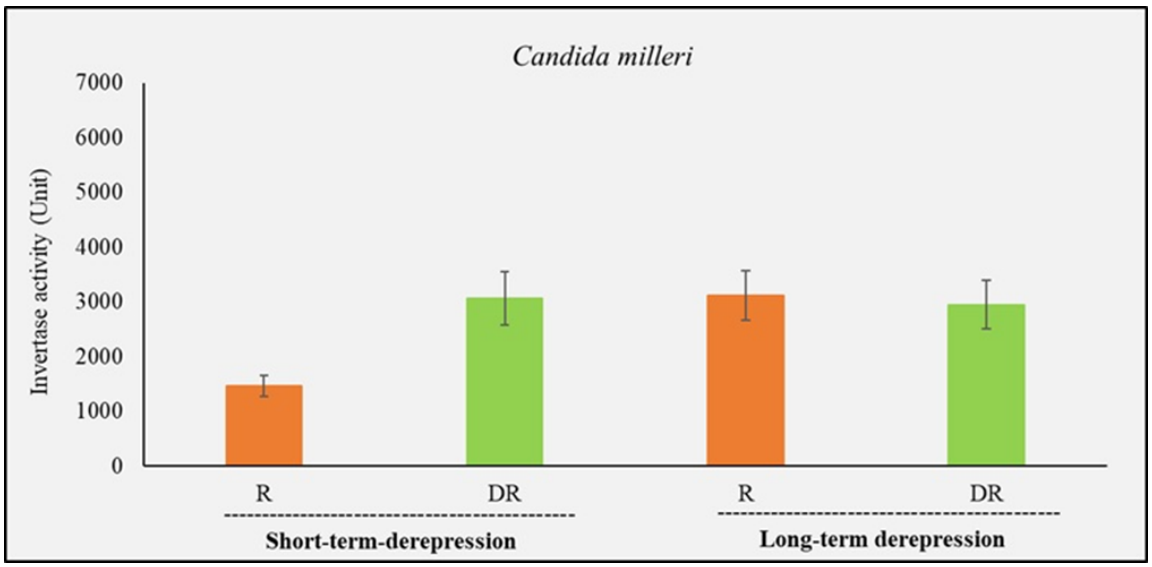

Figure 5 Invertase activity of *C. milleri* under short-term and long-term derepression glucose repression and derepression conditions. Invertase activity was expressed as units (µmol glucose/minute/100 mg dry weight of yeast cells)

3.3. Time-course analysis of invertase activity under STD and LTD

To examine the temporal dynamics of invertase activity and to determine whether short-term or long-term derepression triggers rapid or sustained changes in enzyme levels, invertase activities were monitored over time under repressed and derepressed conditions in non-conventional yeast species. Although invertase activity was not detectable in *P. farinosa* during the initial 3 h incubation period, this species was included in the time-course analyses to assess the possibility of a delayed or low-level invertase activity at later time points.

Under short-term derepression while cells were maintained under repressed conditions, invertase activity exhibited distinct species-specific patterns over the 10 h time-course (Figure 6). *S. cerevisiae* maintained very low invertase activity throughout the experiment, consistent with strong glucose repression. Similarly, *P. farinosa* and *P. anomala* showed only minimal activity with no pronounced temporal changes. In contrast, *P. jadinii* displayed high invertase activity (3105 units) at the onset of the experiment, which declined rapidly within the first 2-3 h and subsequently stabilized at low levels. *C. milleri* exhibited moderate invertase activity under repressed conditions, with gradual increase during the first half of the time-course followed by a slight decline at later time intervals.

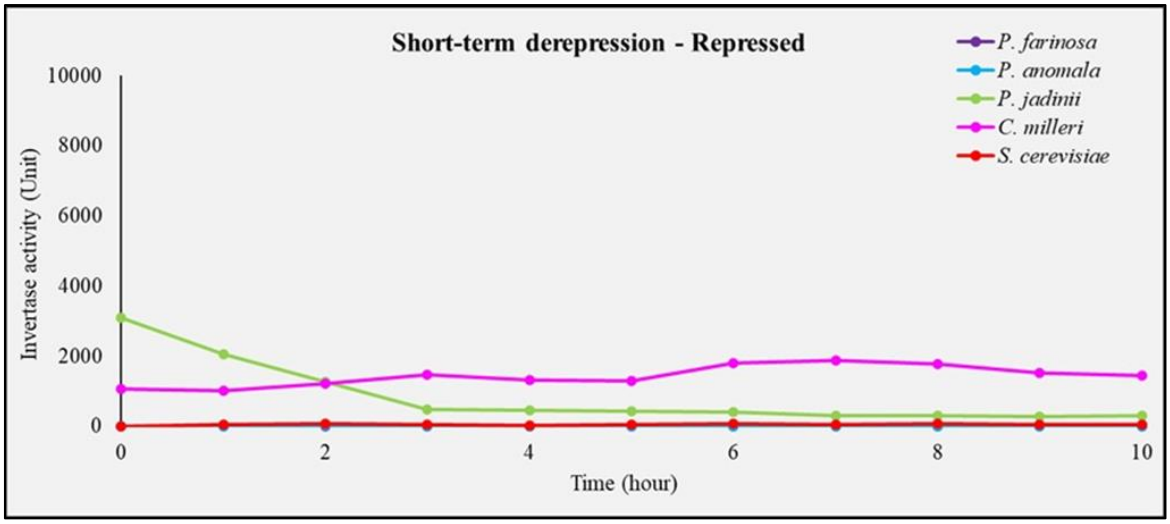

Figure 6 Time-course invertase activity under short-term derepression while cells were maintained under repressed conditions. Invertase activity was expressed as units (µmol glucose/minute/100 mg dry weight of yeast cells)

Under STD in glucose-limited conditions (0.05% glucose), invertase activity exhibited pronounced and species-specific temporal patterns (Figure 7). As expected, *S. cerevisiae* showed a rapid induction of invertase activity, reaching maximal levels (1395 units) within the first 4-5 h, followed by a gradual stabilization at later time points. *P. farinosa* displayed no invertase activity throughout the time-course. *P. anomala* showed a modest and delayed increase in invertase activity, peaking at intermediate time points before declining slightly toward the end of the experiment. Notably, *P. jadinii* exhibited high initial invertase activity (4129 units) that progressively decreased over time, suggesting rapid activation followed by downregulation under prolonged derepressed conditions. In comparison, *C. milleri* displayed a strong and sustained induction of invertase activity, with activity levels steadily increasing throughout the time-course and reaching the highest value (5246 units) among the non-conventional yeast species.

Under long-term derepression followed by exposure to repressed condition, invertase activity profiles differed markedly among the yeast species over time-course (Figure 8). *S. cerevisiae* did not immediately adapt to repress conditions following long-term derepression, as reflected by sustained invertase activity during early hours, followed by a marked decrease after 6 h. Similarly, *P. anomala* exhibited negligible enzyme activity at all time points, indicating strong repression and limited residual enzyme expression. In contrast, *P. jadinii* displayed very high invertase activity (7010 units) at the onset of the experiment, which declined sharply within the first 3-4 h (2090 units at 4 h) and subsequently stabilized at lower levels, suggesting incomplete or delayed repression following long-term growth on nonfermentable carbon sources. *C. milleri* showed sustained and relatively high invertase activity under repressed conditions, suggesting reduced sensitivity to glucose-mediated repression rather than delayed repression alone. *P. farinosa* again exhibited no invertase activity across the entire time-course.

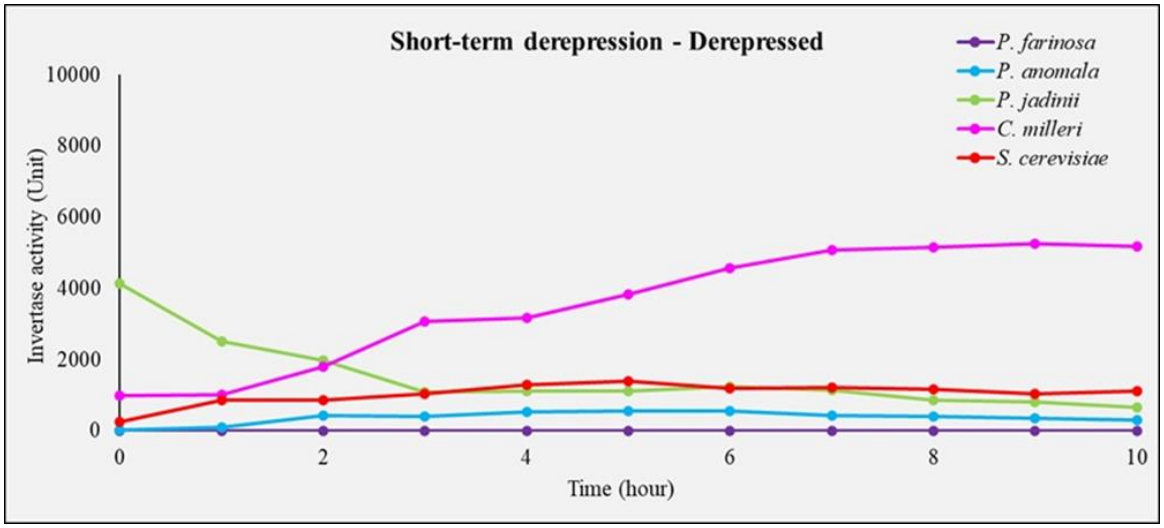

Figure 7 Time-course invertase activity under short-term derepression while cells were maintained under derepressed conditions. Invertase activity was expressed as units (µmol glucose/minute/100 mg dry weight of yeast cells)

Under long-term derepression followed by incubation under derepressed conditions, invertase activity displayed pronounced and sustained species-specific patterns over time (Figure 9). *S. cerevisiae* maintained moderately high invertase activity throughout the experiment, with only minor fluctuations, indicating effective re-induction of invertase synthesis after prolonged growth on nonfermentable carbon sources. In contrast, *P. anomala* and *P. farinosa* showed no invertase activity at all time points. *P. jadinii* exhibited very high enzyme activity at early time points (around 8000 units) which gradually declined over the course of the experiment but remained substantially elevated compared to most other species. Notably, *C. milleri* showed a strong and dynamic response, with invertase activity increasing sharply to a pronounced peak at intermediate time points (7869 units at 6 h) before gradually decreasing at later stages.

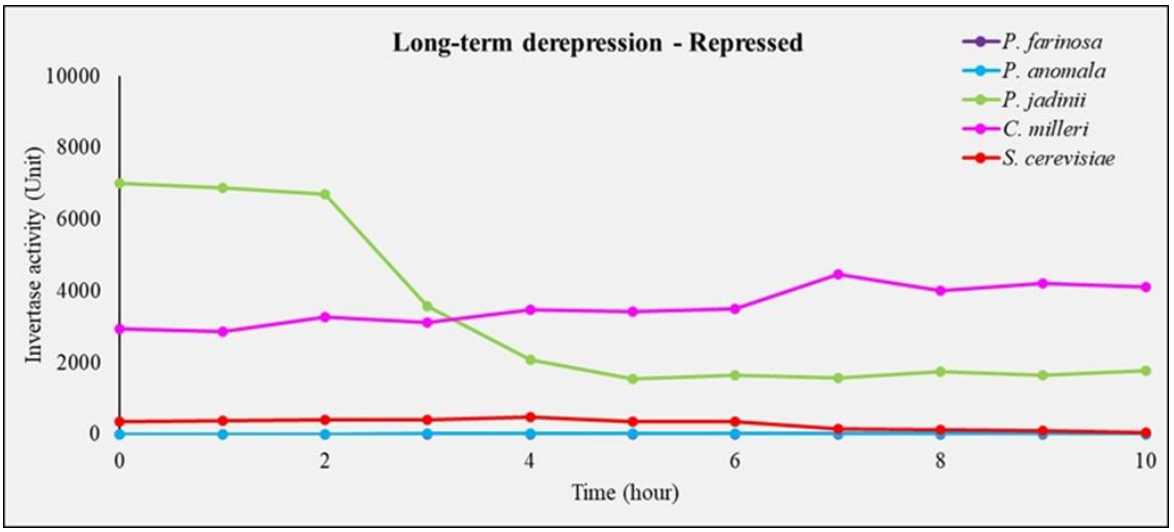

Figure 8 Time-course invertase activity under long-term derepression while cells were maintained under repressed conditions. Invertase activity was expressed as units (µmol glucose/minute/100 mg dry weight of yeast cells)

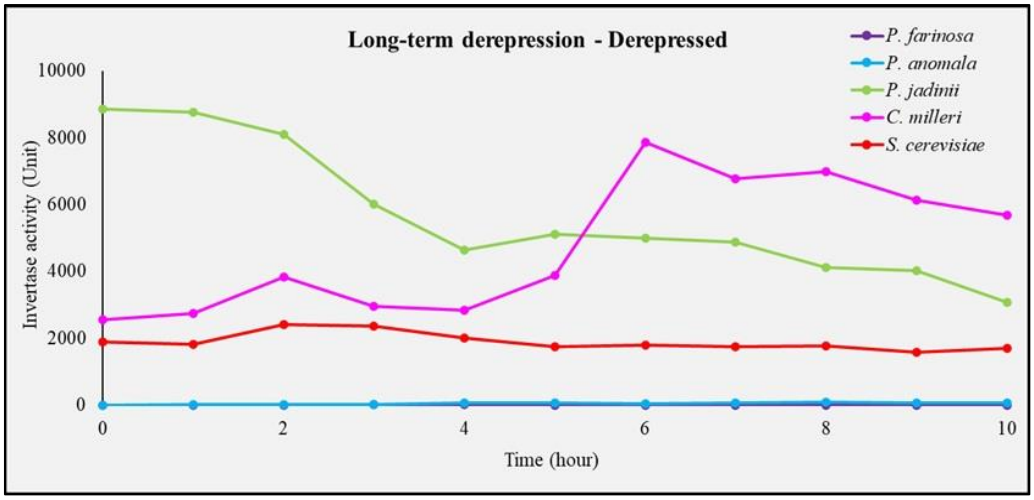

Figure 9 Time-course invertase activity under long-term derepression while cells were maintained under derepressed conditions. Invertase activity was expressed as units (µmol glucose/minute/100 mg dry weight of yeast cells)

Overall, the time-course analyses reveal marked species-specific differences in both the kinetics and magnitude of invertase regulation in response to glucose availability. Whereas *S. cerevisiae* exhibits rapid and tightly controlled repression-derepression dynamics, non-conventional yeast species display more diverse and often delayed responses, highlighting distinct regulatory strategies underlying carbon source adaptation.

4. Discussion

Glucose repression and derepression provide a well-established framework for assessing how yeast cells regulate the utilization of alternative carbon sources. In this study repression-derepression analyses were applied to distinguish between rapid regulatory responses to glucose limitation (short-term derepression) and longer-term metabolic adaptation following growth on nonfermentable carbon sources (long-term derepression). Using the canonical *S. cerevisiae* model as a reference, this approach enabled direct comparison of the regulatory capacity, kinetics, and sensitivity of invertase regulation among non-conventional yeast species.

Differences in specific growth rates among the yeast species examined in this study provide an essential basis for understanding their carbon utilization strategies. As an integrative physiological parameter, specific growth rate reflects the efficiency of carbon uptake, respiratory capacity, and metabolic regulation. S. cerevisiae showed high growth rates on fermentable carbon sources but a marked decline under nonfermentable and minimal conditions. This pattern is consistent with its strong dependence on fermentative metabolism and tight glucose repression, which limits respiratory growth when glucose availability is reduced [16,26,27].

In contrast, non-conventional yeast such as *P. anomala* and *P. jadinii* maintained relatively high growth rate across both fermentable and nonfermentable conditions, including minimal media. This indicates a greater metabolic flexibility and an enhanced ability to support respiratory growth. Recent studies have shown that many non-*Saccharomyces* yeasts possess increased mitochondrial activity and reduced sensitivity to glucose triggered repression, allowing sustained growth under carbon-limited or fluctuating environments [19,28,29]. The high growth efficiency of *P. jadinii* observed here is in agreement with these findings and suggests that its growth is less constrained by carbon source identity. Notably, *C. milleri* exhibited a pronounced reduction in growth rate under nonfermentable and minimal conditions despite maintaining high invertase activity. This indicates that elevated extracellular enzyme activity alone does not guarantee efficient growth and highlights the importance of downstream metabolic integration and energy generation. Similar uncoupling between enzyme activity and growth efficiency has been reported in other yeast species, where carbon catabolite regulation is often linked to stress adaptation rather than optimization of growth rate [30,31]. These observations suggest that growth rate and enzyme regulation represent partially independent traits shaped by species-specific metabolic priorities.

Invertase regulation in S. cerevisiae followed the well-established model of strict glucose repression and derepression. Low basal activity under repressed conditions and strong induction upon glucose limitation, particularly after long-term derepression, reflect tight transcriptional control of *SUC2* via Snf1-Mig1 signalling pathway [5,14,15]. The

enhanced activity observed following prolonged growth on nonfermentable carbon sources is consistent with previous reports linking derepressed metabolic states to sustained transcriptional activation [4,8].

In non-conventional yeasts, invertase regulation was more variable and species-specific. *P. farinosa* showed no detectable invertase activity under any condition, suggesting that sucrose utilization is either absent or mediated through alternative pathways. The ability to utilize sucrose and the level of extracellular invertase activity vary widely among yeast species, indicating that sucrose metabolism is not universally conserved across yeasts. *P. anomala* displayed a transient invertase induction under short-term derepression but failed to sustain activity after long-term growth on nonfermentable carbon sources, indicating limited long-term maintenance of derepressed states. The variable derepression patterns observed in Pichia species suggest the glucose-dependent regulation differs in magnitude and timing across species, without implying a conserved underlying signalling pathway [25].

Among the species examined, *P. jadinii* exhibited the strongest and most persistent invertase activity, particularly under long-term derepression, exceeding that observed in S. cerevisiae. High basal activity following growth on nonfermentable carbon sources and delayed repression upon glucose re-addition suggest reduced sensitivity to glucose-mediated repression. Such regulatory behaviour is characteristic of yeasts adapted to nutrient-poor or variable environments, where sustained expression of carbohydrate-degrading enzymes provides a selective advantage [26,32]. The parallel observation of high invertase activity and high growth rates further supports an integrated strategy linking carbon acquisition to growth efficiency in this species.

The other non-conventional yeast, *C. milleri*, maintained relatively high invertase activity under both repressed and derepressed conditions, with limited discrimination between glucose availability states. This attenuated repression response contrasts with the strict control observed in *S. cerevisiae* and suggest the glucose repression in *C. milleri* operates through relaxed or alternative regulatory mechanisms. Glucose-responsive regulation in *Candida* (including glucose repression mediated by Mig proteins) shows distinctive features and is tuned to host-relevant sugar levels, supporting metabolic flexibility and niche adaptation rather than simply maximizing growth on glucose. In contrast, glucose repression is not uniformly attenuated across the genus *Candida*. In *Candida albicans*, glucose repression is mediated by Mig-family repressors, and genetic evidence supports a functional Snf1-Mig circuit in controlling glucose-responsive transcription [33]. Moreover, classical reviews of yeast carbon catabolite repression describe glucose-dependent repression of sugar utilization systems in *C. albicans*, indicating that strict repression can occur in at least some *Candida* lineages [1,34,35]. Therefore, the sustained invertase activity observed in *C. milleri* under both repressed and derepressed conditions in our experiments likely reflects species-specific regulatory wiring rather than a genus-wide trait. Considering that *C. milleri* (syn. *Candida humilis*) is frequently associated with sourdough ecosystems, where sugar availability fluctuates and co-metabolism with lactic acid bacteria is common, relaxed repression or incomplete downregulation of extracellular carbohydrase activity could be advantageous in this niche [22].

Time-course analyses further demonstrate that invertase regulation is highly dynamic and dependent on metabolic history. While *S. cerevisiae* showed rapid induction and repression consistent with tight regulatory control, non-conventional yeasts frequently exhibited delayed or sustained activity profiles. These differences likely reflect variation in transcriptional control, enzyme stability, and post-translational regulation. Overall, the combined analyses of growth kinetics and invertase activity indicates that carbon catabolite regulation in yeasts spans a continuum of strategies rather than single conserved.

5. Conclusion

This study demonstrates that yeast species differ markedly in their growth behavior and invertase regulation in response to carbon source availability and environmental conditions. Non-conventional yeast, particularly *P. jadinii* and *C. milleri*, exhibited greater metabolic flexibility, maintaining robust growth and sustained invertase activity under minimal, nonfermentable, and environmentally variable conditions. In contrast, *P. anomala*, like *S. cerevisiae*, displayed tighter glucose-dependent regulation, characterized by strong repression of invertase activity under high-glucose conditions and pronounced sensitivity to nutrient limitation. Time-course analysis further revealed that the dynamics of derepression and persistence of enzyme activity are species-specific and influenced by prior carbon source history. Together, these findings highlight the diversity of regulatory strategies governing carbon utilization in yeasts.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] Gancedo JM. Yeast carbon catabolite repression. Microbiol Mol Biol Rev. 1998;62(2):334-361. doi:10.1128/MMBR.62.2.334-361.1998

[2] Conrad M, Schothorst J, Kankipati HN, Van Zeebroeck G, Rubio-Texeira M, Thevelein JM. Nutrient sensing and signaling in the yeast *Saccharomyces cerevisiae*. FEMS Microbiol Rev. 2014;38(2):254-299. doi:10.1111/1574-6976.12065

[3] Wu D, Cheng Y, Jiang J, Liu Y, Song Y. Mechanism of glucose repression in yeast. Acta Microbiologica Sinica, 2023;63(9): 3409-3427. doi: 10.13343/j.cnki.wsxb.20230042

[4] De Vit MJ, Waddle JA, Johnston M. Regulated nuclear translocation of the Mig1 glucose repressor. Mol Biol Cell. 1997;8(8):1603-1618. doi:10.1091/mbc.8.8.1603

[5] Carlson M. Glucose repression in yeast. Curr Opin Microbiol. 1999;2(2):202-207. doi:10.1016/S1369-5274(99)80035-6

[6] Hedbacker K, Carlson M. SNF1/AMPK pathways in yeast. Front Biosci. 2008;13:2408-2420. doi:10.2741/2854

[7] Braam S, Tripodi F, Österberg L, Persson S, Welkenhuysen N, Coccetti P, Cvijovic M. Exploring carbon source related localization and phosphorylation in the Snf1/Mig1 network using population and single cell-based approaches. Microb Cell. 2024;11:143-154. Published 2024 May 16. doi:10.15698/mic2024.05.822

[8] Santangelo GM. Glucose signaling in *Saccharomyces cerevisiae*. Microbiol Mol Biol Rev. 2006;70(1):253-282. doi:10.1128/MMBR.70.1.253-282.2006

[9] Rødkaer SV, Faergeman NJ. Glucose- and nitrogen sensing and regulatory mechanisms in *Saccharomyces cerevisiae*. FEMS Yeast Res. 2014;14(5):683-696. doi:10.1111/1567-1364.12157

[10] De Virgilio C, Loewith R. The TOR signalling network from yeast to man. Int J Biochem Cell Biol. 2006;38(9):1476-1481. doi:10.1016/j.biocel.2006.02.013

[11] Loewith R, Hall MN. Target of rapamycin (TOR) in nutrient signaling and growth control. Genetics. 2011;189(4):1177-1201. doi:10.1534/genetics.111.133363

[12] Kessi-Pérez EI, Salinas F, Molinet J, González A, Muñiz S, Guillamón JM, Hall MN, Larrondo LF, Martínez C. Indirect monitoring of TORC1 signalling pathway reveals molecular diversity among different yeast strains. Yeast. 2019;36(1):65-74. doi:10.1002/yea.3351

[13] Trumbly RJ. Glucose repression in the yeast Saccharomyces cerevisiae. Mol Microbiol. 1992;6(1):15-21. doi:10.1111/j.1365-2958.1992.tb00832.x

[14] Elbing K, Ståhlberg A, Hohmann S, Gustafsson L. Transcriptional responses to glucose at different glycolytic rates in *Saccharomyces cerevisiae*. Eur J Biochem. 2004;271(23-24):4855-4864. doi:10.1111/j.1432-1033.2004.04451.x

[15] Persson S, Welkenhuysen N, Shashkova S, Cvijovic M. Fine-Tuning of Energy Levels Regulates SUC2 via a SNF1-Dependent Feedback Loop. Front Physiol. 2020;11:954. doi:10.3389/fphys.2020.00954

[16] Otterstedt K, Larsson C, Bill RM, Ståhlberg A, Boles E, Hohmann S, Gustafsson L. Switching the mode of metabolism in the yeast *Saccharomyces cerevisiae*. EMBO Rep. 2004;5(5):532-537. doi:10.1038/sj.embor.7400132

[17] Huberts DH, González J, Lee SS, Litsios A, Hubmann G, Wit EC, Heinemann M. Calorie restriction does not elicit a robust extension of replicative lifespan in *Saccharomyces cerevisiae*. Proc Natl Acad Sci U S A. 2014;111(32):11727-11731. doi:10.1073/pnas.1410024111

[18] García-Salcedo R, Lubitz T, Beltran G, Elbing K, Tian Y, Frey S, Wolkenhauer O, Krantz M, Klipp E, Hohmann S. Glucose de-repression by yeast AMP-activated protein kinase *SNF1* is controlled via at least two independent steps. FEBS J. 2014;281(7):1901-1917. doi:10.1111/febs.12753

[19] Flores CL, Rodríguez C, Petit T, Gancedo C. Carbohydrate and energy-yielding metabolism in non-conventional yeasts. FEMS Microbiol Rev. 2000;24(4):507-529. doi:10.1111/j.1574-6976.2000.tb00553.x

[20] Hittinger CT, Rokas A, Bai FY, Boekhout, T., Gonçalves P, Jeffries TW, Kominek J, Lachance MA, Libkind D, Rosa CA, Sampaio JP, Kurtzman CP. Genomics and the making of yeast biodiversity. Curr Opin Genet Dev. 2015;35:100-109. doi:10.1016/j.gde.2015.10.008

[21] Riley R, Haridas S, Wolfe KH, Lopes MR, Hittinger CT, Göker M, Salamov AA, Wisecaver JH, Long TM, Calvey CH, Aerts AL, Barry KW, Choi C, Clum A, Coughlan AY, Deshpande S, Douglass AP, Hanson SJ, Klenk HP, LaButti KM, et al. Comparative genomics of biotechnologically important yeasts. Proc Natl Acad Sci U S A. 2016;113(35):9882-9887. doi:10.1073/pnas.1603941113

[22] Meroth CB, Hammes WP, Hertel C. Identification and population dynamics of yeasts in sourdough fermentation processes by PCR-denaturing gradient gel electrophoresis. Appl Environ Microbiol. 2003;69(12):7453-7461. doi:10.1128/AEM.69.12.7453-7461.2003.

[23] Kutzman CP, Fell J. W, Boekhout,T. (Eds). The Yeasts: A taxonomic study (5th ed.). 2011; Elsevier.

[24] Granström TB, Aristidou AA, Jokela J, Leisola M. Growth characteristics and metabolic flux analysis of Candida milleri. Biotechnol Bioeng. 2000;70(2):197-207. doi:10.1002/1097-0290(20001020)70:2<197::aid-bit9>3.0.co;2-d

[25] Türkel S, Turgut T, Kayakent N. Effect of osmotic stress on the derepression of invertase synthesis in nonconventional yeasts. Lett Appl Microbiol. 2006;42(1):78-82. doi:10.1111/j.1472-765X.2005.01806.x

[26] Pfeiffer T, Morley A. An evolutionary perspective on the Crabtree effect. Front Mol Biosci. 2014;1:17. doi:10.3389/fmolb.2014.00017

[27] Kayikci Ö, Nielsen J. Glucose repression in *Saccharomyces cerevisiae*. FEMS Yeast Res. 2015;15(6):fov068. doi:10.1093/femsyr/fov068

[28] Nair A, Sarma SJ. The impact of carbon and nitrogen catabolite repression in microorganisms. Microbiol Res. 2021;251:126831. doi:10.1016/j.micres.2021.126831

[29] Xiao Z, Zhao Y, Wang Y, Tan X, Wang L, Mao J, Zhang S, Lu Q, Hu F, Zuo S, Liu J, Shan Y. Sucrose-driven carbon redox rebalancing eliminates the Crabtree effect and boosts energy metabolism in yeast. Nat Commun. 2025;16(1):5211. doi:10.1038/s41467-025-60578-8.

[30] García M, Crespo J, Cabellos JM, Arroyo T. Growth of Non-*Saccharomyces* Native Strains under Different Fermentative Stress Conditions. Fermentation, 2021; 7:124. doi:10.3390/fermentation7030124.

[31] Sevillano ME, Morata VI, Martín MC. Growth Kinetics and Extracellular Enzyme Secretion of *Aureobasidium pullulans* m11-2 as an Alternative Source of Polysaccharidases for Winemaking. Fermentation, 2025; 11:520. doi:10.3390/fermentation11090520.

[32] Van Dijken JP, Scheffers WA. Redox balances in the metabolism of sugars by yeasts. FEMS Microbiology Reviews, 1986; 1(3-4):199–224. doi:10.1111/j.1574-6968.1986.tb01194.x

[33] Lagree K, Woolford CA, Huang MY, et al. Roles of *Candida albicans* Mig1 and Mig2 in glucose repression, pathogenicity traits, and *SNF1* essentiality. PLoS Genet. 2020;16(1):e1008582. doi:10.1371/journal.pgen.1008582.

[34] Lorenz MC. Carbon catabolite control in *Candida albicans*: new wrinkles in metabolism. mBio. 2013;4(1):e00034-e13. doi:10.1128/mBio.00034-13

[35] Van Ende M, Wijnants S, Van Dijck P. Sugar Sensing and Signaling in *Candida albicans* and *Candida glabrata*. Front Microbiol. 2019;10:99. doi:10.3389/fmicb.2019.00099