

Ecological, biological and biotechnological aspects of *Saccharomyces cerevisiae* biomass production

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ABSTRACT

Baker's yeast *Saccharomyces cerevisiae*, belonging to the Ascomycota yeast type and being facultatively anaerobic, plays a key role in ecology, fundamental and evolutionary biology, biotechnology and industrial fermentations, in particular, in the production of fermented food and beverages. *S. cerevisiae* grows on substrates with a high sugar content and is an important ingredient in flour bakery and confectionery products. This study reveals the fundamental and applied biology of baker's yeast *S. cerevisiae* and reveals the technological methods of enriching beet molasses with nutrients in order to increase the yield of biomass. Nowadays, as shown in the study, technological methods for enriching beet molasses with nutrients have different solutions. In the technology of *S. cerevisiae* biomass production, a population of diploid cells is used, since compared with haploid cells they are genetically more stable, characterized by faster and more active metabolism, and larger sizes.

Keywords: *Saccharomyces cerevisiae*, Biochemistry of baker's yeast, Carbon metabolism, Cultivation, Molasses, Biomass, Ecology.

Article type: Review Article.

INTRODUCTION

Baker's yeast, *Saccharomyces cerevisiae*, belonging to the group of yeasts Ascomycota (subclass Saccharomycotina: class Saccharomycetes: order Saccharomycetales; Peris *et al.* 2023) and with a domestication history of 9000 years (McGovern *et al.* 2004), plays a crucial role in ecology, fundamental and evolutionary biology, biotechnology, and industrial fermentations, particularly in the production of fermented food and beverages (Parapouli *et al.* 2020; Bai *et al.* 2022). Baker's yeast, *S. cerevisiae*, as the most famous type of yeast due to its use as a baking powder, is an important ingredient in flour bakery and confectionery products (André *et al.* 2010; Wunsch *et al.* 2022;). The combination of high growth rates and biomass yield with a high ability to loosen the dough are important characteristics for the efficient commercial production of baking yeast (Shevchenko *et al.* 2014). In the world, the industrial production of baking yeast exceeds the production of any other microorganism by more than 100 times (Johnson *et al.* 2011) and reaches 2.0 (Shima *et al.* 2009) and 2.3 million ton/year in dry weight (Azmuda *et al.* 2006). The economic value of yeast is projected to reach about 7.5-9 billion US dollars in the next five years (Azmuda *et al.* 2006). Bread baking, according to archaeological data, is a historically developed industrial and biochemical process, since its application geographically was scientifically recorded (González Carretero *et al.* 2017; Arranz-Otaegui *et al.* 2018):

— On the Giza plateau in 2575 BC;

— In ancient Egypt in the 2nd and in the 1st millennium BC and in the Ancient Kingdom of Egypt, in the tombs of Niankhhotep and Khnumhotep (approximately 2450 and 2400 BC);

— In Northwestern China (1st millennium BC) and at the site of Subei (500-300 BC), located in the Turfan Basin (Xinjiang Uygur Autonomous Region of China), a sample was found that included baker's yeast;

— In Europe, the Neolithic culture of Cortaillod and Horgen (from 4500 to 2800 BC), and in the Neolithic parking lot of the Zurich opera "Parkhouse" (Switzerland; 3234 and 3226 remains of a tortilla were found;

— In the settlement of Çatalhöyük (Chatalheyuk), in south-central Turkey (7100-5950 BC);

— In Shubayki 1, a Natufian hunter-gatherer camp located in the northeast of Jordan (14.6–11.6 thousand years BC).

Population growth in the XI-XII centuries contributed to the industrial production of baker's yeast, and since the XIX century, when *S. cerevisiae* was obtained from the remnants of beer production, they began to produce starter cultures based on baker's yeast. While oxygen is available for aerobic respiration, *S. cerevisiae* on the basis of anaerobic fermentation converts sugar into ethanol and CO₂ (Mamun-Or-Rashid *et al.* 2013). In the food industry, this ability of *S. cerevisiae* has been selected for centuries due to the selection of stress-resistant strains during fermentation. To date, yeast strains with a higher maltose utilization capacity have been obtained by selection methods and they are used for the fermentation of dough without sugar. Two classes are used in yeast production, one of which (LS class) is capable of switching to the use of maltose (instead of sucrose) when the sugar content in the medium is low, and the second (HS class) is able to survive in conditions with high sugar content in the medium (Tilak *et al.* 2020). Bread production technology is based on mixing flour, water, and sourdough with fermenting yeast. The latter, from the total number of ingredients, are inoculated into bread dough at a concentration of 2%. When kneading, oxygen is quickly consumed for the respiration of yeast cells, then, due to the transition to anaerobic mode, the reproduction of yeast cells slows down and a fermentation reaction occurs. Favourable conditions for the fermentation of fresh yeast in the dough are 34-38 °C and pH 4.0–5.2; negative: the addition of fat, salt, or spices to the batch (Stewart 2014). The breeding characteristics of baker's yeast (Stewart 2014) are: the ability to adapt to changing cultivation conditions, because the composition of molasses has high variability; the efficiency of respiratory metabolism during yeast production, which determines the yield of biomass; biomass production; cell growth rate; the ability to ferment; the ability to quickly convert carbohydrates into CO₂; ethanol production; dehydration; the volume of the final product, structure, colour (carbohydrates, amino acids); stress resistance to environmental conditions, in particular, to cold stress; shelf life (acids, glycerine). The purpose of the analytical review is to establish the key aspects of modern knowledge on yeast cultivation on improved nutrient media based on beet molasses in the context of biotechnological production of baker's yeast, *S. cerevisiae*.

Fundamental biology of Baker's yeast, *S. cerevisiae*

The study of baker's yeast has a centuries-old scientific history, because, after its isolation by E. Hansen in 1888 and designation in the XIX century of its niche in nature as an agent of alcoholic fermentation, *S. cerevisiae* became a popular object, both in fundamental biology and in scientific and industrial technologies (Chambers *et al.* 2010). The development of the fundamental biology of *S. cerevisiae* is based on the following key scientific events (Gryganskyi *et al.* 2023): Pure cultures were isolated (by Hansen 1888); active dry yeast was developed (in 1920s); evidence of the existence of a sexual cycle was obtained and the possibility of hybridization of commercial yeast was determined (1935-1940); the foundations of yeast genetics were established (1935-1955); the existence of a system of mating types was proved (1943); the genome was sequenced (1996, strain S288c); three independent gene ontologies were created (2000) and continue to be developed including biological process, molecular functions and cellular components; key regulators of the cell cycle were discovered (2001); a collection (library) of gene deletions (2003), a collection (library) of strains where the reported genes are labelled with a green fluorescent protein for visualization of localization and interactions of proteins (2003) and a collection (library) of mutants with overexpression (2006) were created. *S. cerevisiae* is the most studied and best-characterized unicellular eukaryotes.

Ecology of baker's yeast *S. cerevisiae*

The genus *Saccharomyces* includes eight species (*S. arboricola*, *S. cerevisiae*, *S. eubayanus*, *S. jurei*, *S. kudriavzevii*, *S. mikatae*, *S. paradoxus*, and *S. uvarum*; Naseeb *et al.* 2017). It grows on substrates with high sugar

content, but it is rarely found on fruits (*S. cerevisiae* is found in ripe damaged grapes (25%), whereas before ripening in berries *S. cerevisiae* is almost absent (~0.05%; Naseeb *et al.* 2017). More often it is isolated from the bark of hardwoods, leaf surfaces, rotten wood and soil (Wang *et al.* 2023). It was found that *S. cerevisiae* and *S. paradoxus* often live together in nature (Bing *et al.* 2014). The best source of yeast is citrus juice and sugar cane juice. The dispersal of *Saccharomyces* in nature is carried out by insects (Stefanini *et al.* 2012).

Key ecological features of *S. cerevisiae*. (Naseeb *et al.* 2017)

—Natural strains live in harsher conditions than laboratory ones, because favourable cultural conditions are created for the latter;

— By aerobic cultivation on glucose or fructose, they break down glucose through respiration, and on mannose or galactose degradation proceeds simultaneously through respiration and fermentation. This regulation system, called the "Crabtree effect", is the result of the suppression of the synthesis of respiratory enzymes by high fermentation rates. Thus, glucose and sucrose are the preferred carbon source for *S. cerevisiae*, and fermentation is the main way of energy production, even in aerobic conditions;

— To capture a certain niche and ensure their own growth, they produce and accumulate ethanol, which is toxic to most other types of microorganisms competing for sugar compounds.

According to environmental properties, there are (Feng *et al.* 2023):

1) Three classes of yeast according to their respiratory ability: respiration prevails (feed yeast); respiration and fermentation processes are equal (pathogenic, baking and brewing yeast); fermentation prevails (alcoholic, wine and beer yeast of low fermentation). In the first group, respiration provides 100% catabolism, in the second, respiration corresponds to 40-50% catabolism and in the third – respiration accounts for 10-15% catabolism.

2) Five classes of yeast according to their sugar catabolism: the short-term Crabtree effect is characterized by a weakening of respiration due to the presence of glucose; the long-term Crabtree effect is characterized by inhibition and inactivation of respiration due to the presence of glucose; the Pasteur effect is explained by a decrease in glycolysis under aerobic conditions; the Kluver effect is determined by the mandatory aerobic use of disaccharides; the Custers effect is characterized by aerobic stimulation of fermentation glucose.

3) There are two types according to the method of sugar metabolism: respiratory, when more than 70% of sugar is metabolized during respiration; enzymatic, at less than 10%.

Morphology of baker's yeast *S. cerevisiae*

S. cerevisiae are unicellular eukaryotes surrounded by a 100-120 nm thick cell wall (Dupres *et al.* 2010) and contain membrane-bound organelles such as the nucleus, endomembrane system, and mitochondria (Duina *et al.* 2014). *S. cerevisiae* cells transport mitochondria via actin filaments. Yeast cells have a diameter of 5-10 microns (Overbeck *et al.* 2015). Non-budded cells have a diameter of about 5 microns (Duina *et al.* 2014). The content of intracellular organelles is influenced by environmental factors. Thus, at a low growth rate, a high content of ribosomes is noted (Zakhartsev *et al.* 2018). At higher growth rates, yeast cells have lower mechanical strength (Duina *et al.* 2014). Carbohydrates in yeast are stored in the form of glycogen granules, so the cytosol is optically heterogeneous (Zakhartsev *et al.* 2018). *S. cerevisiae* cells are surrounded by a cytoplasmic membrane, a periplasmic space, and a cell wall. *S. cerevisiae* has a strong cell wall, cellular and intracellular membranes (Zakhartsev *et al.* 2018). The cell wall of *S. cerevisiae* supports the structure, lysis and structure of cells, absorbs nutrients (Esquivel *et al.* 2023) and, depending on the conditions of cultivation and the process, is 10-25% (Yousefi 2023); 10-30 % (Kim *et al.* 2006) of the dry mass of the cell. The cell wall consists of three types of polymers that combine to form a modular complex structure. The structure of the cell wall of *S. cerevisiae* consists mainly of polysaccharides, the content of which can be 20-90% dry matter. Of the total amount of polysaccharides, glucose residues consist of 80-90%, mannose 10-20% and N-acetylglucosamine (chitin) 1-2%. Proteins comprise about 15% of the cell wall, while glycoproteins about 66% in terms of the total amount of sugars (Bzducha-Wróbel *et al.* 2012). The polysaccharides found in the cell wall of the genus *Saccharomyces* are mainly β -glucans, which make up 30-60% of the dry matter of the cell wall (Bzducha-Wróbel *et al.* 2012). The dry weight of the yeast cell wall consists of 30-60 % (Martiniano *et al.* 2020), 50-60% β -glucans (β -1,3- and β -1,6-glucans), 1-2 – 4% chitin and various proteins associated with glycans (Martiniano *et al.* 2020). The two classes of β -glucan differ from each other by the site of the glycoside bond between β -D-glucopyranose molecules: β -1,3-glucan consists of

approximately 1,500 β -D-glucopyranose subunits and β -1,6-glucan 130-150 glucose subunits (Bzducha-Wróbel *et al.* 2012). The inner layer of the cell wall responsible for mechanical strength consists of a network of β -1,3-glucans (80-90% of the total number of β -glucans) branched by chitin (Dague *et al.* 2010), β -1,6-linked glucans (8-18% of the total number of β -glucans) branched into β -1,3-glucan networks and are covered with mannoproteins that make up the outer layer (Pillet *et al.* 2014). The mechanical strength of the cell wall is based on the inner layer of moderately branched β -1,3-glucan molecules, which form a three-dimensional network and have several side chains. β -1,6-glucan is a highly branched molecule and water-soluble. Chitin is deposited in the side walls after cytokinesis and is less than 10% of the total chitin content in the cell walls and can be associated with β -glucan (both β -1,3- and β -1,6-glucan; Horváthová *et al.* 2022). The second important group of structural compounds of the yeast cell wall is a heterogeneous group of glycoproteins – mannoproteins, which can make up 20-50% of the dry matter of the cell wall. About 40 of them have been identified. The peptide fragment in mannoproteins is 3-50% of the molecule, whereas phosphorus is about 2% (Bzducha-Wróbel *et al.* 2012). When glycoprotein molecules pass through the Golgi apparatus, the side chains of oligosaccharides are modified by transferring sugar residues from sugar nucleotide donors. In *S. cerevisiae*, only mannose residues are involved in these modifications. The binding of metal ions by yeast *S. cerevisiae* involves mannoproteins present in the outer layer of the cell wall, as well as β -glucans and chitin. The components of the cell wall play an essential role in chemisorption, which is carried out due to the interaction of metal ions with functional groups of compounds forming cells (carboxyl, phosphate, amine, hydroxyl, sulfhydryl and carbonyl groups). Thus, the cell wall consists of mannoproteins and β -glucans (85-90% of the dry mass of the cell wall). β -glucans form the inner layer of the cell wall, and mannoproteins are embedded in this glucan layer and cover it (Bzducha-Wróbel *et al.* 2012). The yeast cell wall is elastic and is constantly remodelled during morphogenetic processes and growth, as well as in response to environmental stresses such as ethanol and oxidative stress, thermal and osmotic stress, as well as antifungal drugs (allicin, caspofungin; Horváthová *et al.* 2022). During stress, such biochemical modifications occur in the cell wall as the deposition of chitin, the formation of cross-links between chitin and β -1,3-glucan, as well as the appearance of new bonds between proteins and chitin through β -1,6-glucan (Horváthová *et al.* 2022). Dead cells of microorganisms usually have a greater ability to bind metal ions from the environment (Kordialik-Bogacka 2011). The chemical composition of *S. cerevisiae* includes the following elements: 47% C, 32% O₂, 6% H₂, 7.7% N₂, 2% K, 1.2% P, 1% S, 0.2% Mg, 0.1% Na and other trace elements. In addition, yeast cells contain a small amount of a complex of B vitamins, of which D-pantothenic acid, D-biotin and M-inositol are necessary because yeast cells cannot synthesize them (Al-Jasass *et al.* 2010).

Genetics and life cycle of baker's yeast *S. cerevisiae*

The production strains of *S. cerevisiae* yeast differ genetically and phenotypically from natural isolates. The nuclear genome of *S. cerevisiae*, 12068 kb in size, has 16 chromosomes and contains approximately 6000 genes, of which 5570 are protein coding. Such genetic transformations as single nucleotide polymorphism (SNP), gene duplication, horizontal gene transfer, and genomic hybridization take place in their genome (Legras *et al.* 2018). A number of genes in the genome of *S. cerevisiae* are included horizontally and have prokaryotic (about 10 genes) and eukaryotic (for instance, the FSY1 gene highly utilizes fructose in conditions of low hexose concentrations (glucose, galactose and fructose) origin (Legras *et al.* 2018). Extra-chromosomal elements of the *S. cerevisiae* genome include variable mitochondrial DNA molecules (about 85780 bp), copy (up to about 60 copies per cell), double-stranded DNA (about 6318 bp; reduce the growth rate of the host) and other extra-chromosomal single- and double-stranded RNA molecules and retroviruses. The genome of *S. cerevisiae* has a high level of genetic diversity and, therefore, a complex population structure (Zhu *et al.* 2016). Its genome database is publicly available. Approximately 68% of production of bakery strains are characterized by higher ploidy (above 2n) and 17% have a higher level of aneuploidy (Bigey *et al.* 2021). The high ploidy of *S. cerevisiae* is useful for the adaptation of the strain to the baking medium, since it leads to a much faster start of fermentation compared to diploid strains, however, it hinders breeding work, leading to low spore formation efficiency, low spore viability and unstable mating types (Bigey *et al.* 2021). The life cycle of *S. cerevisiae*, which consists of homothallic and heterothallic periods, is described in Table 1 (Fischer *et al.* 2021), and the processes included in the life cycle in Table 2.

Table 1. Life cycle of *S. cerevisiae*.

| Life cycle period | Homothallic | | Heterothallic* |
|---------------------------------------|---|---|---|
| Creation during mating | diploids "a/alpha" | | "a/a" or "alpha/alpha" |
| Stages included in the life cycle | The cell cycle | Nuclear cycle | The cell cycle |
| The type of cells entering this cycle | Haploid "a" and "alpha", diploid "a/alpha" | Diploid "a/alpha" | Haploid "a" and "alpha", diploid "a/a" and "α/α" (alpha/alpha) |
| Cycle phases | G ₁ , S, G ₂ , mitosis | G ₁ , S, G ₂ , meiosis, morphogenesis of spores | G ₁ , S, G ₂ , mitosis |
| Entering the cycle | Between phases G ₁ and S | Between phases G ₁ and S | Between phases G ₁ and S |
| Exiting the cycle | Between G ₁ and mitosis (transition to the resting phase of G ₀) | At the end of the sporulation | Between G ₁ and mitosis (transition to the resting phase of G ₀) |

*There is no nuclear cycle in the heterothallic period, because diploids "a/a" and "alpha/alpha" do not sporulate (meiosis does not occur).

Table 2. Processes included in the life cycle of *S. cerevisiae* (Katz Ezov *et al.* 2010; Börner *et al.* 2023).

| Process | Pairing (creating a diploid) | Mitosis (proliferation and budding) | Sporulation (gametogenesis; meiosis with the formation of spores) |
|-------------------------|--|--|--|
| Process triggers | The presence of a mating partner in the vicinity and favourable conditions | Favourable conditions | Lack of nutrition (in nitrogen; in carbon) |
| Mechanism | <p>Formation of receptors and signalling molecules - alpha factors (a peptide of 13 amino acids).</p> <p>The production of a-factor (a peptide of 12 amino acids) leads to the stopping of the cell cycle stage at the G₁ phase, immediately before the start of DNA replication and the synthesis of proteins necessary for the nuclear and cell cycles.</p> <p>The production of these factors (a- or alpha-) is associated with the absence or presence of activator proteins (expressed by the alpha1 and alpha2 genes), respectively</p> | <p>Mitosis occurs in two directions:</p> <p>1) budding (the original "mother" cell gives rise to an ellipsoid daughter cell made of a completely new cell surface material),</p> <p>2) proliferation (the original cell increases and then splits into two daughter cells, which are slightly smaller than the mother cell and which must increase in size before initiating chromosome duplication)</p> | <p>With nutritional starvation, in the presence of the regulatory element a 1-alpha 2, gene expression for mating and activation of the sporulation process occurs due to suppression of the haploid-specific gene of the meiosis inhibitor RME1</p> |
| Results | Diploids "a/a", diploids "alpha/alpha" and diploids "a/alpha" | Ellipsoid or identical two daughter cells | Four haploid meiotic cells (each enclosed in a spore sheath) packed together in a pouch |

As shown in Table 1, the life cycle of *S. cerevisiae*, due to the presence of germ cell types "a" and "alpha", has two types (heterothallic and homothallic; Fischer *et al.* 2021). With a homothallic cycle, cells (haploid "a", "alpha"; diploid "a/alpha") are capable of mitosis, while two haploid cells can form a diploid cell that can enter the nuclear cycle stage and sporulate. The initiation of gametogenesis is controlled by internal and external signals of the cell, which together regulate the main transcription factor – inducer of meiosis I, IME1 (Fischer *et al.* 2021). In the heterothallic cycle, cells can mate with unrelated haploids (amphimixis), between spores from the same tetrad (intra-tetrad crossing or automixis), but they cannot mate according to the "mother-daughter" type, as in the homothallic life cycle (Katz Ezov *et al.* 2010). As shown in Table 2, during the life cycle (heterothallic, homometallic) reproduction occurs by mating, mitosis and sporulation. In the first case, from two cells "a" and "alpha", due to their joint location in a favourable nutrient medium, a third type of specialized cell is formed, i.e., diploid (mating efficiency can reach 100%); in the second, mitosis, proliferation and budding are possible (during

budding, ellipsoid cells are formed); in the third, in the process of sporulation, a complex of processes of meiosis and the formation of spores is carried out, which exists in the form of a nuclear cycle. With a lack of nutrition, this process gives rise to four haploid meiotic cells, each of which is enclosed in a spore shell. All four cells of the same meiosis are packed together in a pouch – ask (Katz Ezov *et al.* 2010). In addition to Tables 1 and 2, notably, the type of mating is determined by the presence of the MAT locus encoding the genes "a1" (MATa), "alpha1" and "alpha2" (MATalfa; Katz Ezov *et al.* 2010). Therefore, *S. cerevisiae* is characterized by the presence of three types of specialized cells important for the life cycle (Katz Ezov *et al.* 2010): mating type "a" (sexual); mating type "alpha" (sexual); diploid cell "a"/"alpha". Each cell type is capable of simple mitosis, which follows the cycle of phases G₁, S, and G₂, accompanied by noticeable changes in shape and lifestyle. The transition from the cell cycle to the nuclear one occurs between the G₁ and S phases. In this nuclear cycle, meiosis and sporulation take place.

Thus, the life cycle of yeast (homothallic, heterothallic; Börner *et al.* 2023):

— is characterized as haplo-diplontic, because during the life cycle there is an alternation of haploid and diploid stages;

— has intertwining between such phrases as "asexual reproduction", "sexual reproduction" and "rest";

— consists of sexual (includes inbreeding and outbreeding) and asexual reproduction;

— diploid cells multiply mitotically in an environment rich in nutrients, and during starvation they undergo meiosis and sporulation with the formation of haploid spores (ascospores) enclosed in a pouch that are resistant to environmental stresses.

Under favourable nutritional conditions, ascospores can germinate into haploid cells, which, soon after germination, can multiply mitotically or merge with another haploid cell of the opposite type of mating, forming a diploid vegetative cell. Mating can occur both between haploids obtained as a result of the same meiosis (intra-tetrad mating, or automixis), and between haploids from different notebooks, which may be more or less related (inter-tetrad mating). Haploids that have already undergone mitosis may, at the next mitotic division, change the type of mating to between clones, forming completely homozygous diploids (auto-diploidization; Börner *et al.* 2023). Signs of the actual onset of meiosis are: an increase in the nucleus, and an elevation in the protein content in the cell by about 10% (Börner *et al.* 2023). In the technology of *S. cerevisiae* biomass production, a population of diploid cells is used, since compared with haploid cells they are genetically more stable, characterized by faster and more active metabolism, and larger sizes.

Physiology of baker's yeast *S. cerevisiae*

S. cerevisiae are facultatively anaerobic yeasts and are one of the few yeasts capable of anaerobic growth in the presence of added sterols and unsaturated fatty acids, since they are not synthesized by them in the absence of oxygen. Baker's yeast has an osmotrophic type of nutrition and this feature is based on the Crabtree effect: in aerobic conditions, it does not use a breathing apparatus to metabolize saccharides and stimulate biomass growth, but produces ethanol and other two-carbon compounds through pyruvate (Börner *et al.* 2023). The Crabtree effect observed in yeast is quantitative (anaerobic metabolic activity is proportional to the initial concentration of soluble solids in the wort) and low-quality (lack of aerobic metabolism in the wort with a high concentration of sugars) behaviour. *S. cerevisiae* strains are thermotolerant and mesophilic to microorganisms (Martiniano *et al.* 2020).

Baker's yeast cells can change their level of metabolic activity (carbon and nitrogen metabolisms) and stress response to environmental conditions. *S. cerevisiae* isolated from beetroot may have amino acid auxotrophy and have a weak ability to synthesize some amino acids (Tanaka *et al.* 2006). The metabolic response to oxygen availability requires changes in intracellular flows, which are mediated by transcription, increased glucose levels as well as metabolic regulation and reproduce the integrated response of regulated interactions between enzymes and metabolites. Detection of mitochondrial production in comparison with phosphorylation at the substrate level serves as a more effective means of ATP respiration. Even with an oxygen content of only 0.5%, ATP is produced by respiration (Jouhten *et al.* 2008).

The key features of the physiological and biochemical activity of *S. cerevisiae* are as follows (Gómez-Pastor *et al.* 2011):

— alcohol dehydrogenase activity, induction of the catalase enzyme and the rate of alcohol formation increases by an elevation in temperature to 40 °C;

— in the presence of glucose and ethanol, glucose is first consumed until it is completely consumed;

- an upraise in the mixing rate leads to an increase in the mass of cells;
- the effectiveness of strains of various origins (industrial, laboratory and natural) under fermentation conditions of wort originating from a sugar-rich medium differs significantly, since industrial strains, compared with laboratory and natural ones, can complete the fermentation process, however, laboratory strains are more resistant to ethanol and pressure than industrial ones;
- utilising sugars as a substrate for their metabolism;
- they can catabolize sugars for the production of cellular chemical energy (ATP) by both aerobic and anaerobic ways: during aerobic (respiration), the sucrose molecule is oxidized in the presence of oxygen, resulting in the formation of carbon dioxide and water. During anaerobic (alcoholic fermentation), the sucrose molecule is oxidized in the absence of oxygen, resulting in the formation of ethanol and carbon dioxide;
- in the presence of excess sugar in the medium, aerobic fermentation of glucose to ethanol provides a significant part of the energy needed for the formation of biomass;
- maltose, a disaccharide consisting of two glucose molecules bound by a 1,4-bond, is the main source of carbon during the loosening of bread dough;
- maltose metabolism differs from glucose metabolism only in the first two stages, namely, its transport and subsequent hydrolysis into glucose;
- the net yield of ATP in alcohol fermentation of maltose is lower than in glucose fermentation (1,5 instead of 2 ATP molecules per glucose unit);
- unlimited absorption of maltose can lead to intracellular acidification, contributing to cell lysis, which is observed in cultures exposed to maltose stress;
- industrial strains do not exhibit their inherent osmotolerance, and with a higher sugar content, osmotic pressure suppresses their activity;
- stressful conditions (oxidative and fasting cycles) during the reproduction process can increase the activity of yeast in the baking powder of the dough;
- the periodic phase provides accumulation of important reserve metabolites in cells;
- the presence of O₂ allows yeast to oxidize the resulting ethanol during sucrose depletion, which triggers the transition from fermentation to respiration;
- the content of minerals in molasses, although it varies greatly, generally favour calcium in the ratio of c (Mg) / c (Ca) 0.1:1 (calcium ions are involved in stimulating agglomeration in resuspended pressed yeast, with an increase in the concentration of magnesium in the nutrient medium from molasses, cellular calcium decreases, excessively high levels of magnesium contribute to the formation of granularity due to the nonspecific action of a large number of cations that reduce the total negative charge on the surface of yeast cells and ensure cell adhesion).

Technology of industrial cultivation of baker's yeast *S. cerevisiae*

Fundamental knowledge in the field of biology of *S. cerevisiae* allowed this microbial species to realize its genetic resources in a wide range of applications of applied biology, from molecular scientific research to commercial industrial technologies. By fermentation, these yeasts convert starch into complex sugars based on the enzyme alpha-amylase, then complex sugars are broken down by glucoamylase into simple sugars. From one mole of glucose (180 g), thanks to *S. cerevisiae*, two moles of ethanol (92 g) + two moles of carbon dioxide (88 g) + energy (26.4 kcal) are formed (Sharma *et al.* 2022). For thousands of years, the products of this reaction have been used in the food industry, in particular, for baking and beverage production. Thanks to metabolic engineering, *S. cerevisiae* can be used in harsh industrial environments. These yeasts produce approximately 20% of biopharmaceuticals (Nielsen *et al.* 2013), ferment xylose contained in cellulosic biomass, such as agricultural, wood and paper waste (Brat *et al.* 2009), collect aqueous palladium Pd (II) ions by bio-reductive deposition and biosorption at room temperature, are used as a pesticide and biostimulants (Dima *et al.* 2020), etc. The development of the program for utilizing *S. cerevisiae* has been going on since ancient times from the preparation of bread, beverages and other products to the usefulness of baker's yeast as a systemic biological model in biotechnological processes.

Behaviour of baker's yeast *S. cerevisiae* in culture medium.

In industrial production, *S. cerevisiae* baker's yeast is obtained either from collection centres at the initial stages, or its own strains are isolated and cultivated, and subsequently, cultures are maintained, ensuring consistency of quality and productivity (Dima *et al.* 2020). The successful commercial production of *S. cerevisiae* is determined by biological (*S. cerevisiae* strain with good breeding indicators; crop size, etc.) and technological (molasses quality: nature, composition and concentration of substrate; cheap, suitable for cultivation carbon source; easily controlled cultivation process to obtain the most viable biomass, physico-chemical conditions of cultivation, first of all, temperature, pH; cultivation period; development of the canning process, etc. by conditions (Nakata *et al.* 2014). During cultivation, baker's yeast catabolizes sugar from molasses by both aerobic and anaerobic metabolism. At the same time, the higher the concentration of sugars in molasses, the higher the activity of anaerobic metabolism and lower the aerobic one (Dima *et al.* 2020). Molasses, a by-product of the sugar industry, is one of the cheapest sources of carbohydrates. Monosaccharides (glucose, galactose and fructose) and disaccharides (maltose, sucrose, trehalose and melibiose) are of particular importance for *S. cerevisiae* in the culture medium. Compared with other carbohydrates, glucose is the preferred substrate for *S. cerevisiae*. Two glucose uptake systems have been described in *S. cerevisiae* including those with low (constitutive system; high Michaelis constant, from 15 to 20 mM) and high (low Michaelis constant, from 1 to 2 mM) affinity. Glucose, even at low concentrations, in the presence of acetate, strongly suppresses sporulation. Studies on the consumption of hexose (glucose, galactose and fructose) in *S. cerevisiae* have shown that glucose is consumed faster than fructose when both of them (9:1 fructose to glucose) are present in the medium (Díaz-Campillo *et al.* 2012). Increased glucose uptake is necessary for *S. cerevisiae* to synthesize glycerol, the main osmo-protector in yeast. Baker's yeast grows well in a wide range of glucose concentrations (from a few mM to 2 mM). Glucose transport in cells grown on galactose is strongly inhibited by galactose, which is a monosaccharide widely distributed in nature (a component of lactose disaccharide in dairy products, as well as raffinose trisaccharide and melibiose disaccharide in cereals and other plants. When 1% glucose is added to a medium with galactose, *S. cerevisiae* first consumes glucose, followed by beginning to metabolize galactose (Harrison *et al.* 2022). Aerobic galactose cultures produce more biomass and fewer fermentation products than glucose cultures. Fructose is a simple monosaccharide sugar (Gaily *et al.* 2010), which, according to the sweetness index, is 1.3-2.0 times sweeter than glucose. Fructose, compared with glucose, reduces the reproductive capacity of *S. cerevisiae* more and increases their mortality. However, *S. cerevisiae* grown on fructose showed a higher survival rate under stress caused by H₂O₂ compared to yeast grown in a glucose medium (Semchyshyn *et al.* 2011). Maltose is a competitive inhibitor of glucose transport, an important source of carbon for *S. cerevisiae* in bread baking. Maltose metabolism is initiated by the transportation of carbohydrate through the plasma membrane, followed by its hydrolysis by glucosidase. The resulting glucose is catabolized by glycolytic pathway. Industrial *S. cerevisiae* has a better ability to utilize maltose compared to natural and laboratory yeast. Maltose transport uses the electrochemical gradient of the plasma membrane. The Michaelis constant for maltose is 1-5 mM (Semchyshyn *et al.* 2011). Sucrose is the most common, cheap and important disaccharide in the industrial use of *S. cerevisiae* (Berg *et al.* 2022). Sucrose is hydrolysed by extracellular invertase (β -D-fructosidase) of yeast into glucose and fructose, which are transported into the cell by hexose carriers and metabolized by glycolysis (Korshunova *et al.* 2005). Yeast expresses two forms of invertase (Sing *et al.* 2022); the first is localized in the cytoplasm and requires sucrose absorption for functioning, while the second form is localized in the periplasmic space between the plasma membrane and the cell wall. Hexoses formed as a result of the activity of the periplasmic form are absorbed by hexose transporters. Melibiose is hydrolysed to glucose and galactose. The reaction products catalysed by melibiose are substrates for hexose-transferring proteins.

Trehalose (α -D-glucopyranosyl-(1, 1)- α -D-glucopyranoside) in *S. cerevisiae* (Kaino *et al.* 2008):

- is a non-reducing glucose disaccharide;
- is an important reserve carbohydrate in the cell;
- it is considered as the most important protective agent against stress (like proline and glycerine; in combination with trehalose, these molecules are effective for providing protection against various types of environmental stresses);
- plays a direct role in stress resistance (high temperature, freezing – thawing), protecting proteins and lipids;
- has cryoprotective activity almost equal to the activity of glycerine or proline (proline increases the stability of proteins and membranes under stress, since it inhibits aggregation during protein refolding and reduces intracellular ATP levels under oxidative stress.

— at the transcription level, the synthesis of glycerine or trehalose, it is induced by many stress conditions. For instance, the synthesis of trehalose is catalysed by a complex of four proteins: Tps1, Tps2, Tps3 and Tsl1.

When yeast cells were subjected to osmotic stress, glycerine was accumulated, and in the presence of ethanol, trehalose accumulated (Kaino *et al.* 2008).

Nwaka noted that two enzymes are capable of hydrolysing trehalose: neutral cytosolic trehalase (Nth1; Nth2), and acidic trehalase (Ath1). Nth1 activity is maximal at pH 6.0-7.0, which plays an important role in protecting cells from heat shock. Ath1 exhibits maximum activity at pH 4.5. The enzyme is localized in vacuoles, but is mainly distributed in the extracellular space and is necessary for cell growth on a medium containing trehalose. Nth1 is responsible for the degradation of intracellular trehalose, and also for the hydrolysis of extracellular trehalose. Jules *et al.* (2008) suggested that Ath1 is involved in the mobilization of intracellular trehalose.

The composition of beet molasses includes water (17-25%), carbohydrates (about 50%), of which sucrose accounts for more than 40% by weight, raffinose (fructose-glucose-galactose) up to 8% and, which, in turn, is hydrolysed by invertase to fructose and melibiose. The latter is not used from-due to the absence of α -galactosidase; polysaccharides (about 25%); some amino acids (lysine, cysteine, arginine, glutamate, serine, etc.); nitrogenous compounds (amino acid nitrogen, nitrates and nitrites, betaine nitrogen, alpha-aminoazote, ammonium and amide nitrogen); vitamins (biotin, pantothenic acid, pyridoxine, riboflavin, thiamine and folic acid); minerals (iron, calcium, magnesium, potassium and sodium; Birke *et al.* 2022). Although molasses contains substances necessary for yeast cultivation, it should be supplemented with vitamins and nitrogen, mainly in the form of ammonium salts. Molasses (cane and beet) also contains harmful substances (excessive content of sulphurous acid, nitrates, volatile acids and colloidal substances) that inhibit yeast growth and baking efficiency, as well as various amounts of suspended solids (oxalic acid, lipids, ash, oxides of silicon, calcium, phosphorus, sulphur, aluminium, iron, magnesium, sulphur, etc.), which should be purified (Mussatto *et al.* 2010). The main loads faced by yeast in the technological regime are temperature, pressure, acidity or alkalinity, osmotic and ionic stress, low oxygen level, ethanol concentration and drying (Table 3). Periodic cultivation is the best way to obtain the maximum cell mass of *S. cerevisiae*. The change in cultivation conditions is accompanied in yeast by the adjustment of adaptation processes (transcription profile and metabolism) to the new situation.

Technology of culture media improvement.

Extracts of cereals (malt of corn and rye grains) were used for the industrial production of baking yeast at the initial stages. By 1920, due to the high cost of malt (a source of nitrogen and enzymes) and the technological need for saccharification of the substrate (the formation of fermenting sugars), molasses replaced cereals (Gélinas *et al.* 2012; Rorke *et al.* 2017). The study of patents showed that in order to reduce costs and improve the technological properties of molasses, the issue of developing suitable nutrient media for baking yeast is of key importance. Technological techniques for improving culture media for *S. cerevisiae* are shown in chronological order (Table 4). As shown in Table 4, the developments were aimed at solving issues related to the replacement of grain media with diluted molasses and the enrichment of molasses with nutrients.

Table 3. Influence of factors on the growth of biomass of baker's yeast *S. cerevisiae* in a culture medium (Levandovsky *et al.* 2018; Kieliszek *et al.* 2019).

| Factor | A feature in the behaviour of baker's yeast <i>S. cerevisiae</i> in a culture medium |
|-------------|--|
| Temperature | It affects the production of cell mass and the rate of yeast metabolism: at 30 °C and 35 °C, a high increase in biomass is noted |
| pH | The largest cell mass was also obtained at a pH of 6.6 |
| Pressure | Affects the yield of yeast biomass: up to 10 MPa increases the rate of biomass growth, 50% of industrial yeast cells die from 39 to 52 MPa |
| Mixing | The amount of total residual reducing sugars in the culture medium depends on the mixing rate: higher mixing rates lead to a lower percentage of residual reducing sugars |
| Aeration | Optimal aeration conditions: 5% dissolved oxygen at pH – 4. Under these conditions, yeast has the best morphological properties. An increase in the intensity of aeration leads to the synthesis of secondary metabolites (glycerine, aldehydes, higher alcohols, volatile acids and esters) |

| | |
|---------------|--|
| Microelements | Presence of trace elements (zinc, copper and iron) promotes biological activation of yeast, increases its survival and productivity. The presence of a high concentration of selenium (10-60 mg Se ⁴⁺ /L) inhibits yeast growth. Cu, Zn-SOD (Cu, Zn-superoxide dismutase) increase resistance to freeze–thaw stress |
| RNase | The content of 0.01 mcg mL ⁻¹ of RNase in molasses stimulates yeast growth in the middle of the exponential phase |
| Stress | They are sensitive to ionic, thermal and oxidative stresses. Compared with laboratory strains, industrial strains have higher survival rates under conditions of oxidative stress and heat shock, they have the same reactions to osmotic and freezing stresses. Stress in yeast cells, due to the lack of protein kinase A activity, leads to G ₁ arrest, causing a protective reaction. Yeast monocultures in the G ₁ phase have increased resistance to heat shock, ultraviolet radiation and stress from hydrogen peroxide compared with asynchronous age cultures |

Notably nowadays, in addition to molasses and starch-based nutrient media, the following nutrient media are used in research: sugar production wastewater, sulphite liquor, corn starch or steep liquor and wood, whey, bread waste, peat, Jerusalem artichoke, seaweed, isopropanol, synthetic media. When using synthetic media (included sugars, vitamins, minerals and inorganic nitrogen) for the cultivation of baker's yeast, a decrease in the fermentation ability in a dough with a high sugar content was observed compared to yeast cultivated in molasses medium.

Table 4. Technological techniques for improving culture media for *S. cerevisiae* (Gélinas *et al.* 2012).

| Time | Research area |
|-------------|--|
| 1915-1929 | waste from the woodworking industry was considered as a cheap alternative to molasses |
| Since 1920 | interest in starch-based media has been displaced by waste from waste fermentation media (brewing, yeast production), cheese production (whey) or wastewater from corn starch or sugar processing |
| 1920 - 1934 | molasses has become a key culture medium for yeast growth, replacing the grain-based medium |
| 1931-1932 | clarification of molasses with the inclusion of various nitrogen sources. To purify yeast molasses, the use of centrifuges was proposed, which made it possible to remove colouring and pollutants from molasses |
| 1935-1939 | To improve the quality of yeast (appearance, smell, yield, preservation), increase the amount of biomass produced and improve gas formation, the addition of inositol (1935), nicotinic acid (1938), vitamins, including thiamine (1939) was proposed. |
| Before 1980 | finding an alternative for molasses |
| 1980-1984 | production of starter cultures (ready-made medium, for existing yeast in the dough; yeast has not been cultivated) beer purified yeast as an alternative to bakery (artisanal methods) |
| 1995-1999 | molasses processing by physical and chemical methods |
| Until now | industrial variants of liquid nutrient media rich in sugar, with and without molasses; development of artificial media |

In the commercial production of baking yeast, a mixture of beet and cane molasses is often used (Mussatto *et al.* 2010). The composition of molasses is variable and depends on the origin. Before use, molasses should be diluted (approximately 3-5 times) and purified from suspended substances (iron, sulphur dioxide, tannins, dextrin, gums, pigments, clay, aluminium, etc. *S. cerevisiae* show a low growth rate in sucrose-based culture media compared to media containing glucose or fructose (Rorke *et al.* 2017). Nowadays, technological methods for enriching beet molasses with nutrients have different solutions. Molasses is enriched with cereals (cottonseed, cereal germs, legumes and peanut fodder cake), plant hormones, specific growth factors, animal products (milk, whey and animal waste), ammonium sulphate, malt (expensive), aqueous ammonia, ammonium salts. The enrichment of molasses with nutrients based on using cereals, glucose syrup obtained from corn, date syrup, whey, cassava, forestry and agricultural waste has commercial potential, since these approaches do not require special processing requirements and provide low production costs (exception: forestry waste). Utilizing inorganic nitrogen salts for the production of baking yeast has limitations, since it is accompanied by the formation of harmful acids (sulfuric acid, etc.) and the need to neutralize them with liquid ammonia (Gélinas *et al.* 2012).

For the cultivation of baker's yeast (Gélinas *et al.* 2012), using:

- starch-containing raw materials are favourable for gas formation, stability and quality of yeast compared to molasses;
- cellulose-containing raw materials require chemical and enzymatic pretreatment to convert starch, lactose or cellulose into monosaccharides;
- whey is accompanied by problems associated with the inability of *S. cerevisiae* to absorb lactose (whey sugar), therefore, for use as a substrate, it should first be hydrolysed with β -galactosidase to glucose and galactose.

CONCLUSION

Nowadays, baker's yeast is produced dry (active, resistant to drying, high sugar concentration and some inhibitors; instant) and wet (pressed with a humidity of 60-75%, creamy with a humidity of 82%). During storage, the enzymatic activity of wet yeast lasts from 15 days at 4 °C and a month at 1 °C (fresh) to 3 months when frozen; while dry from 1 year (active) to 2 years (instant). The energy sources for yeast in the dough are sugars (glucose, fructose, sucrose and maltose) present in flour; sugars added during dough kneading; sugars (maltose, dextrose and sucrose) formed by amylases during amylolytic starch cleavage. Yeast cells use enzymes to convert complex carbohydrates into carbon dioxide and ethanol. Sugar fermentation acts as a baking powder in flour products and leads, due to the gluten matrix and gases, to an increase in the volume of the dough, a change in the structure of the product, the synthesis of organic acids and volatile products that impart flavour and aroma. The resulting carbon dioxide is responsible not only for increasing the volume of the dough (fermentation process) due to the inclusion of gas, but also for a valuable addition of taste and texture. In addition, fermentation products, such as vitamins and amino acids, are responsible for the nutritional properties of bread. Thus, baking yeast produces carbon dioxide (to swell the dough and obtain a light porous texture) and a complex mixture of chemical compounds, which together give the taste of bread, cause the "ripening" of bread.

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