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Yeast physiology – a micro-synopsis

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Abstract. An outline of the main features of yeast physiology is given which focusses mainly – but not exclusively – upon Saccharomyces cerevisiae. The metabolic processes of the cell related to carbon flux, energy production and the formation of reducing equivalents (NADH and NADPH) are discussed for both aerobic and anaerobic conditions. Mechanisms are described by which metabolic processes can be controlled. A brief account of the life cycle of Saccharomyces is given explaining the difference between sporulation and vegetative growth. The Crabtree and Pasteur effects are defined, discussed and explained in relation to glucose metabolism and ethanol formation.

1 Introduction

Microbial physiology is often referred to as microbial biochemistry but strictly it is more than an account of the biochemistry of the cell which could be described as an account of the chemical changes which occur within a cell as it grows and multiplies to become two cells. Physiology does include the chemistry of the cell but extends this beyond the simple account of the flow carbon and the changes which occur to other elements by describing how these processes relate to the whole growth process itself. The chemical changes therefore are to be seen occurring in a three-dimensional array with a fourth dimension of time being added. Not all reactions which are capable of happening will occur; some occur early in the growth of an organism, some may occur during the period of its fastest growth whilst yet others may occur only as the growth rate of the organism is slowing down and entering a period of stasis. Physiology therefore is a complete understanding of the chemical changes within a cell related to its development, growth and life cycle (which then leads into genetics). Where physiology ends and genetics begins will not be explored here!

2 Metabolism

It is a truism to say that a cell grows and uses carbon. A cell obviously uses many other elements which go to make up its final composition: these will include nitrogen, oxygen – which may come from the air if the organism is growing aerobically (otherwise the oxygen must come from a rearrangement of the molecules in which the organism is growing or even water itself) – together with other elements such as K^+, Mg^{2+}, SO_4^{2-}, PO_4^{3-} and an array of minor ions such as Fe^{3+}, Zn^{2+}, Mn^{2+}, etc. The dynamics of the system are set out in Fig. 1. It should be observed that the free enthalpy of the components entering the system is greater than the free enthalpy leaving the system. Thus, although the cell is a more ordered entity than the materials on which it has been growing, it achieves this state of orderedness at the expense of the oxidation of more material than is conserved or is transferred to a higher enthalpy state.

To develop this theme a stage further, we can see in Fig. 2 A that the substrate is degraded by the existing cells to give a number of carbon intermediates as well as providing some reducing power (in the form of the pyridine nucleotides NADH and NADPH) and some energy (in the form of ATP). This degradative process is known as catabolism. Under aerobic conditions, the reducing power is converted to additional energy by the process known as oxidative phosphorylation. This process is the principal one which is responsible for the consumption of oxygen by the cells. The cells then take the carbon intermediates, together with reducing power and the energy that they have produced, to make new cells;

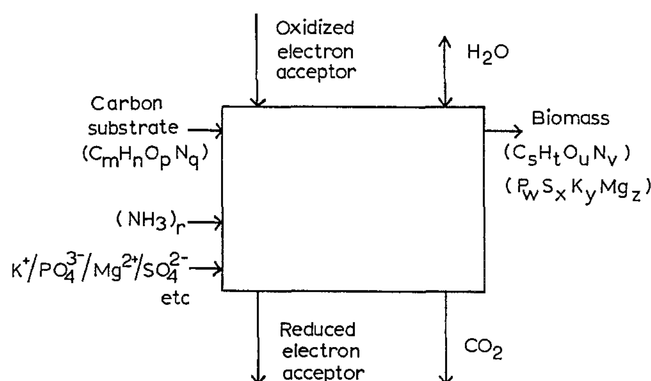


Fig. 1. Thermodynamic balance for a metabolizing cell

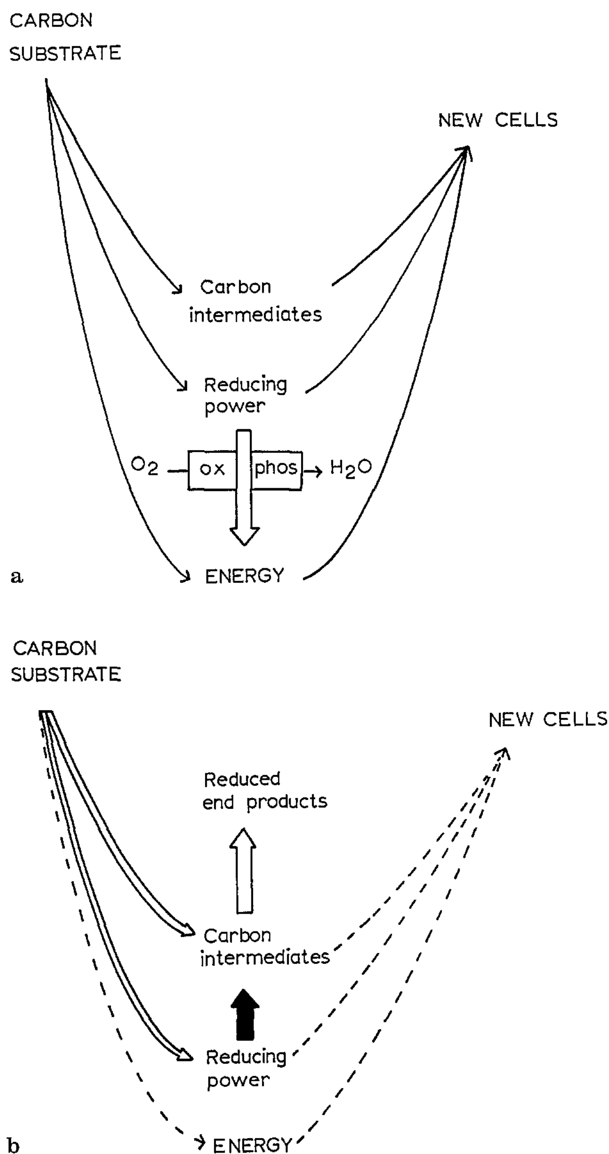
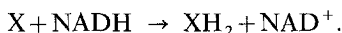


Fig. 2a, b. Processes of catabolism (degradation) and anabolism (biosynthesis) linked to energy production and provision of reducing power. **a** Aerobic metabolism (ox. phos. = oxidative phosphorylation), **b** anaerobic metabolism

these processes thereby involve the making of carbon-to-carbon bonds as well as carbon-to-nitrogen bonds in the synthesis of proteins, nucleic acids and the whole range of other macromolecules which go to make up the new cell composition. The biosynthetic process is referred to as anabolism. The combined processes of catabolism and anabolism are thus known as metabolism.

To balance the supply of carbon being built up into new cells with the reducing power and energy which required for this process, it is necessary for the cell to oxidise a greater amount of carbon to provide the energy than it can subsequently assimilate. The complete degradation of carbon results in the formation of CO_2. The amount of CO_2 being liberated, and thus lost to the cell, will clearly depend upon the amount of energy which is available from the oxidation of the original substrate: some substrates are poor in energy content but high in carbon whereas with others the reverse will apply. Examples of the former would include most of the carbohydrates and examples of the latter would include fats, fatty acids and hydrocarbons. (A fuller discussion of this point is given in the short, seminal review by Linton & Stephenson, [1]).

Under anaerobic conditions the process of oxidative phosphorylation cannot occur and the cell is deprived of its principal way of generating energy. Under such circumstances energy must be provided from the very process of degrading the original substrate. This process, known as substrate level phosphorylation, yields only about 8% of the energy that can be produced under aerobic conditions. This means that to produce a given weight of cells, much more substrate now must be degraded than under aerobic conditions. Consequently, most of the carbon substrate which is degraded to provide energy and the intermediates of metabolism are superfluous to the requirements of the cells to build into new cells. Under anaerobic conditions, the cell is trying its utmost to maximize the formation of energy; the reducing power which is produced during the degradation of the substrate (see Fig. 1) has to be re-circulated for the process to continue as its supply is not endless. The oxidation of the reducing equivalents therefore must be linked to the reduction of some of the carbon intermediates which are accumulating under these conditions:



$$X + NADH \rightarrow XH_2 + NAD^+.$$

Only a little of the reducing power per se is needed for synthesis of new cells. Consequently, the reducing equivalents react with the accumulated carbon intermediates to reduce them in their turn (Fig. 2B). A large formation of reduced carbon compounds is then needed to provide a modicum of energy.

This process of anaerobic catabolism occurs not only in microorganisms, but may occur in higher animals too: an example would be the accumulation of lactic acid during hyper-activity of an athlete in muscle tissue (see later). In microorganisms we can see a whole range of reduced carbon compounds being accumulated by organisms being made to grown anaerobically. Examples may also include lactic acid itself (produced by lactic acid bacteria), but would include short chain fatty acids such as butyric or propionic acids, alcohols such as butanol, ethanol and even propanol. Some organisms may go even still further and produce, as a completely reduced end-product, methane.

These processes of aerobic and anaerobic metabolism of cells are set out in general terms in Fig. 2 A and B. The former process is termed respiration (being linked to O_2), and the latter as fermentation. Unfortunately, 'fermentation' has become a mis-used term over many years and is now often used synonomously (but erroneously) for any biotechnological process (e.g. citric acid fermentation), whether it is anaerobic or aerobic. Strictly, fermentation is metabolism without air.

3 Control of metabolism

Having now established what is the purpose of degradation and how the need for this process relates, in a general sense, to the cells' requirements for intermediates to build into new cells, we can go on to consider in brief form how the processes of catabolism and anabolism are controlled. Control may be by a variety of means. These may be simplified and summarized as follows.

3.1 Compartmentalization

In eukaryotic microorganisms (that is yeasts and moulds which have a defined nucleus compared with bacteria which do not and known as prokaryotic microorganisms) there are various organelles within the cells which have specific functions associated with them. Organelles keep groups of enzymes separated from other enzymes of the cell, so that particular activities will only occur when the organelle receives an appropriate signal or even material for it to act upon from somewhere else in the rest of the cell. A simple example to offer would be the biosynthesis of lipid materials which occurs within the cytoplasm of the cell as opposed to the degradation of lipids which occurs mainly in the peroxisome organelles of a yeast cell. (For a diagram of the main organelles of a yeast cell, please see Fig. 4.)

3.2 Control of enzyme synthesis

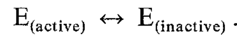
Enzymes, being proteins, are synthesized according to the genetic message which is carried by the cells. Genes can be transcribed or not. If a particular section of DNA (i.e. a gene) is not transcribed then this section of the DNA is said to be repressed, and the enzymes which would have resulted from transcription of that section of DNA will no longer be produced. If, however, that section of the DNA is now so instructed, the gene will be expressed and an enzyme eventually produced. The process is known as induction. (Sometimes this process of induction is also referred to by the term of de-repression.)

3.3 Catabolite repression

Here catabolites – the intermediates of substrate breakdown (see Fig. 2 a) – may build up at a rate too quickly for the cells to handle them effectively and these catabolites then cause the rate of uptake of substrate into the cell to slow down by repressing the genes associated with substrate transport (i.e. uptake). In other words, this process ensures that the cells' rate of uptake of substrate and the breakdown of that substrate match the cells' ability to handle the metabolites which it is producing; if for any reason the system begins to get out of hand, the cell is able to exert primary control over uptake and breakdown by virtue of the accumulating catabolites acting on the key processes either by mechanism 3.2 or 3.3 (see below).

3.4 Modification of enzyme activity

Modification of enzyme activity may occur by two mechanisms. Both may occur at the same time and even to the same enzyme. Enzymes may be modified from one form to another, one form being active and the other inactive or less active:



$$E_{(active)} \leftrightarrow E_{(inactive)} .$$

This process of activating or inactivating an enzyme is carried out by an entirely separate enzyme which has nothing to do with catalysing the reaction that the original enzyme will be involved with. A common way of achieving this reaction is by phosphorylation of the enzyme using a new enzyme – a protein kinase. The activated enzyme may be either the phosphorylated form or its de-phosphorylated form. The de-phosphorylation will be carried out by a phosphatase enzyme. The activity of the protein kinase itself will be obviously controlled by other factors within the cell – see 3.3 above.

The second way in which an enzyme's activity can be controlled is by its response to various effectors. An example is the process known as feedback inhibition. Here, in a sequence of biosynthesis:

$$A \rightarrow B \rightarrow C \rightarrow D \rightarrow E$$

the end product, *E*, may be able to inhibit the first enzyme of the sequence (converting *A* to *B*). This will only occur when sufficient *E* has been produced by the cell for the cells' requirements to be at least temporarily satisfied and there is some *E* left over surplus to requirements. As the cell continuous to grow, it will consume the accumulated *E* and thus diminish the amount of it in circulation. Thus, as *E* is withdrawn for the cells' own needs, the inhibitory effect will be withdrawn and the conversion of *A* to *B* will recommence with the further synthesis of *E* then occurring to match the cells' requirements. This process can, of course, be quite complicated should the pathway not be a simple linear one as depicted above, but be a branching pathway with multiple-end products.

3.5 Degradation of enzymes

Enzymes may be attacked by specific or non-specific proteases (proteolytic enzymes) under certain circumstances. Often under nitrogen-limited conditions, when a cell becomes depleted of nitrogen for further growth, proteases may be activated to degrade surplus copies of enzymes so that the amino acids therein can be scavenged and used for the biosynthesis of enzymes in short supply. Thus, enzymes may be 'turned-over' more rapidly than may occur by simple denaturation.

We thus can see, within a cell, processes of substrate degradation and then build-up of the accumulating intermediates to make materials for the biosynthesis of new cells. All these processes – catabolic and anabolic – occur in a controllable manner. Moreover, different processes, such as the biosyn-

thesis of nucleic acids, proteins, cell wall and membrane, etc., must all be integrated so that none of these processes is out of step with the others. How the cell adjusts the movement of carbon into these respective areas is still poorly understood, although our understanding is much better when it comes to the degradation of small molecular weight materials and their build-up to the small monomers used in the biosynthesis of the polymeric materials of the cell. A very simplified view as to how some of these processes may be linked and controlled in a yeast cell is shown in Fig. 3.

4 Cell composition

The composition of a cell can vary enormously according to how it is grown. This does not only include whether it is grown aerobically or anaerobically, but whether it is grown with a surfeit or a deficit of carbon or indeed any of its other vital nutrients (N, P, K, etc.). Changes in temperature, pH, etc., may also serve to change the cells' composition. A cell therefore responds to its environment by controlling the expression of its genetic information (i.e. the transcription of DNA to RNA and the subsequent translation of that RNA into the biosynthesis of proteins) as well as by controlling the activity of the enzymes themselves within the cell. The above comments can be applied to most microorganisms and indeed to many higher organisms.

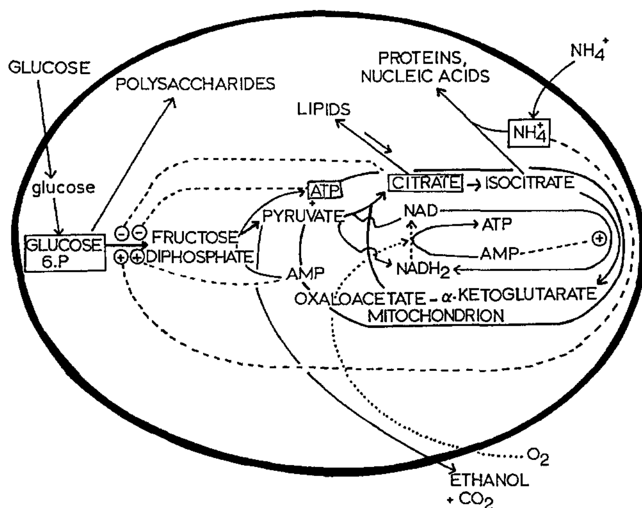


Fig. 3. Simplified view of some of the control mechanisms involved in regulating the flux of glucose to various cell components (adapted from Sols et al. [8]). Dotted lines indicate inhibitions (−) or activations (+) of enzymes

Yeasts are defined as unicellular fungi which reproduce by budding or fission. A simplified drawing of a budding yeast cell is given in Fig. 4. A fuller description of the functions of the various parts of the yeast cell is beyond this simple synoptic account of yeast physiology, but perhaps two organelles, the mitochondrion and the peroxisome, may be mentioned albeit briefly.

Mitochondria are the organelles which are responsible, amongst other things, for the final oxidation of pyruvate arising from glucose metabolism to yield intermediates of the tricarboxylic acid cycle. These intermediates are then used in some abundance for the manufacture of new cell material as well as for the provision of energy, as it is within the mitochondrion that the process of oxidative phosphorylation occurs. Thus it is here that oxygen is consumed and energy is produced at the expense fo pyruvate which yields simultaneously a variety of small carbon fragments.

Peroxisomes are essentially dormant during glucose oxidation and only become activated when cells begin to consume their own stored lipid (in the form of lipid storage bodies) or else to use exogenously supplied lipid where the enzymes associated with lipid degradation are all collected together within the peroxisome. Peroxisomes also are highly important in those yeasts which can grow on methanol as sole carbon source. Here the peroxisome fulfills a unique function of carrying the enzymes associated with methanol oxidation and, in particular, an abundance of methanol oxidase itself which is the first enzyme for the entire process. Under normal, i.e. glucose-using, conditions, peroxisomes are usually difficult to identify in a conventional yeast cell and it is only under the special conditions outlined above will their presence be seen.

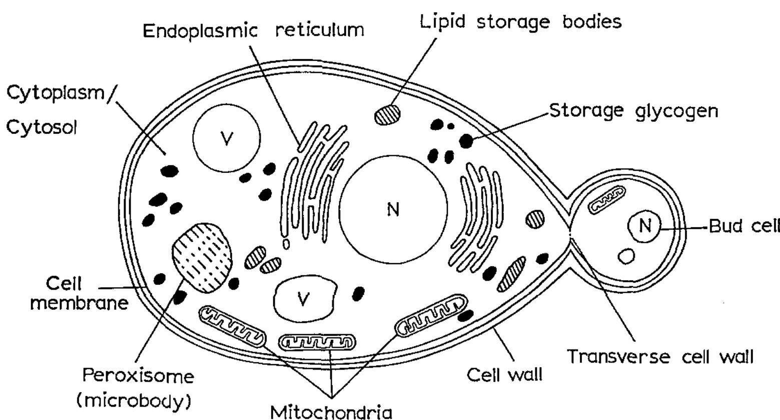


Fig. 4. Idealized representation of a typical yeast cell. N = nucleus, V = vacuole

Table 1. Yeasts

Unicellular fungi reproducing by budding or fission

I *Ascosporogenous yeasts* [Ascomycotina]
(forming ascopores; i.e. spores within as ascus)

33 genera: including

Debaryomyces, Hansenula, Kluyveromyces, Lipomyces, Pachysolen, Pichia, Saccharomyces, Schizosaccharomyces.

II *Basidiosporogenous yeasts* [Basidiomycotina]
(forming basidospores)
Often complex life cycle (diploid, polyploid, aneuploid and haploid)

6 genera

includes *Rhodosporodium*
(also 4 genera of Tremella fungi).

III *Imperfect yeasts* [Deuteromycotina]
(no established life cycle)

17 genera: includes

Candida (196 recognized species), *Phaffia, Yarrowia.*

Major keys to yeast taxonomy: Kreger-van Rij [2] and Barnett et al. [3]

Table 2. Yeasts of biological importance

Saccharomyces cerevisiae (= *S. bayanus, S. carlsbergensis, S. diastaticus, S. sake, S. uvarum* etc.)
baker's yeast – baking
– source of vitamins, enzymes etc.
brewer's yeast – beer, largers, wines, spirits
– industrial ethanol
– autolysed cells for food uses

Kluyveromyces marxianus var. *marxianus* (*K. fragilis, Sacch. fragilis, S. fragi*)
– ethanol from lactose (*ex.* whey)

Kluyveromyces marxianus var. *lactis* (*K. lactis, Sacch. lactis*)
– ethanol from lactose (*ex.* whey)

Candida utilis (*Torula utilis, Torulopsis utilis, Candida guilliermondii, Cryptococcus utilis*)
– fodder yeast/food yeast

Schizosaccharomyces pombe
– rum production

Candida famata (= *C. flaveri*) – riboflavin production

Candida tropicalis – production of long-chain (C_{13}–C_{16}) dicarboxylic acids from alkanes and monocarboxylic acids

Debaryomyces polymorphus – alcohol from inulin (polyfructose, Jerusalem artichokes)

Pichia pastoris – high value biochemicals and enzymes following growth on methanol

Yarrowia lipolytica (= *Candida lipolytica, Saccharomycopsis lipolytica*) and other alkane-utilizing yeasts as sources of SCP in USSR and East Germany*

Candida curvata (*Apiotrichum curvatum*) – potential production of cocoa butter substitute from lactose (whey) [New Zealand, The Netherlands and Canada]

Pichia stipitis/Candida shehatae/Pachysolen tannophilus
– potential for ethanol production from xylose

Phaffia rhodozyma – production of astaxanthin

* The SCP process using C. maltosa finished in 1990

5 Yeasts in general

Yeasts fall into three distinct categories according to the taxonomists [2, 3]. The three groups of yeasts are set out Table 1. Yeasts from all three categories are already being used in biotechnological processes or are being contemplated for such use (see Table 2).

6 Saccharomyces cerevisiae

Saccharomyces cerevisiae itself besides having considerable importance for its use in baking and in brewing is now becoming increasingly important for the expression and production of proteins from the DNA taken from other organisms (i.e. these are termed heterologous proteins to indicate that they are not made by the cells' own DNA) which can be achieved by the insertion of appropriate pieces of DNA from other microorganisms or higher cells into the chromogenetic consitution of Saccharomyces by the technique of genetic engineering.

Saccharomyces is characterized as a yeast which buds multi-laterally (i.e. all over the cell); the cells are described as globose, ellipsoidal or cylindrical. Currently seven species of Saccharomyces are known, these being cerevisiae (of which 86 synonyms have been listed), dairensis, exiguus, kluyveri, servazzi, telluris, and unisporus. (Closely related genera to Saccharomyces are Zygosaccharomyces and Torulaspora).

Of the yeast species, it is of course Saccharomyces cerevisiae which is the most important organism. Its uses in the brewing process are well-described and need not be gone into at this stage. Production of beer can occur in fermenters, which may be up to 1000 m^3, and although mankind has used yeast since time immemorial for the production of alcoholic beverages, it is still obscure in many ways as to the biochemical reasons for ethanol production and indeed the role of oxygen in the process is still poorly understood. Oxygen though is needed specifically for the biosynthesis of sterols and saturated fatty acids and probably nicotinic acid as well. Without oxygen these three materials must be supplied in the culture medium. The process to produce baker's yeast, it must be emphasized, seeks to maximize the yield of yeast biomass, whereas in the brewing process the requirements are to minimize the number of yeast cells being produced but to maximize the amount of the accumulating metabolites, especially ethanol. To produce baker's yeast in large amounts it is necessary to feed the substrate incrementally to limit the amount of ethanol production. Although ethanol can be subsequently used by the cell when the glucose has become exhausted it is more efficient to achieve high cell densities by avoiding ethanol production as much as possible.

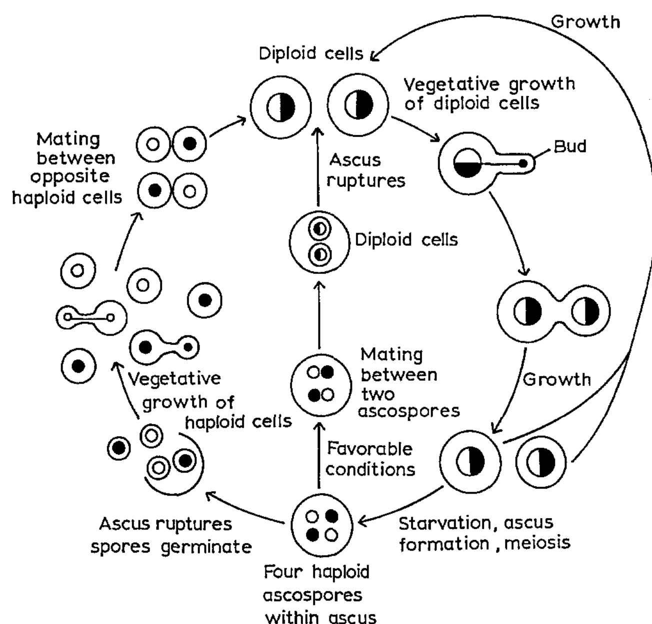


Fig. 5. Life cycle of Saccharomyces cerevisiae (adapted from Darnell et al. [9])

6.1 Life cycle of Saccharomyces

The growth of Saccharomyces, as already indicated, is by budding wherein a bud cell is formed laterally to the main mother cell. This bud increases in size until it is approximately the same size as the mother cell, whereupon it then is separated and breaks away from the parent. The new cell, or daughter cell, along with the mother cell then repeats the process of budding. This part of the cell cycle is known as vegetative growth and is shown on the right-hand side of Fig. 5. Saccharomyces, however, can sporulate to produce ascospores should the conditions become adverse for further vegetative growth. (The ascospores allow the yeast to survive for long periods of dryness, elevated temperatures, etc.). If ascospores are formed, they may or may not be liberated and thus become separated. If separation of the ascospores occurs, there is opportunity to isolate the individual spores and these will be found to contain single copies of the parental chromosomes. Such cells bearing single copies of the chromosome are then described as haploid. Vegetative cells may quickly arise from the spores which germinate under favourable conditions. As there is a distinction between the two chromosomes forming a single pair in the original parent cell, the haploid cells, having just one single copy of the chromosome, are then different. This difference could be described as "male" and "female" but as this terminology has no meaning for a yeast cell they have been given the terms "*a*" and "α". (Equally, they could have been referred to as "α" and, say, "β" or indeed any other simple nomenclature that could have distinguished between two types forming a pair.) Haploid cells of the opposite mating type may then come together and mate whereby the two chromosomes, previously separated in the haploid cell, can now unite together in a single and larger diploid cell. A diploid cell, therefore, contains the pairs of chromosome reunited.

As will also be clear from Fig. 5, the ascospores can under appropriate conditions reunite without separation and dispersion. The spores while still within their containing case – the ascus – then reunite and the chromosomes come together giving once more a diploid cell. The normal status for Saccharomyces is diploid though haploid cells can be grown vegetatively just as easily as the diploid ones. The physiology and behaviour of both types of cells are the same and the difference matters only to geneticists who wish to manipulate the yeast into various activities hitherto denied to that yeast. Diploid cells, because of their double chromosome pairing, are difficult to manipulate as it is virtually impossible to change both chromomoses of a single pair in the identical manner at the same time. A haploid cell having only one chromosome can be genetically manipulated much more easily.

7 Crabtree and Pasteur effects

The physiology of many yeasts is often summarized with respect to their responses to increasing concentrations of glucose or increasing concentrations of oxygen. These effects are referred to as Crabtree and Pasteur effects and are explained with reference to Fig. 6 and 7.

A yeast is regarded as being Crabtree positive if it suffers a repression of its oxidative metabolism when the concentration of glucose is increased in the growth medium. In other words, the effect of increasing glucose beyond a certain critical concentration results in a diminished ability of the yeast to oxidize glucose. As a result, the cell goes into a state of imbalance and reducing equivalents (in the form of NADH) begin to accumulate faster than the cell can reoxidize them by the process of oxidative phosphorylation (Fig. 6 is thus an elaboration of Fig. 2a). The only way that the cell can reoxidize the accumulating NADH is to link up this reaction to the reduction of some accumulating intermediate (cf. Fig. 2b). This, for yeast, takes the form of pyruvate being reduced to ethanol at the same time re-oxidizing the NADH to NAD^+ (Fig. 7). Hence, even in the presence of an adequate supply of oxygen, the efficiency of the oxidative phosphorylation process is limiting in a Crabtree-positive yeast and an increase in the concentration of glucose inevitably causes the accumulation of ethanol.

The degree to which yeasts may show this effect can vary quite considerably; some yeasts may be strongly Crabtree-positive, i.e. will begin to accumulate ethanol even at fairly low concentrations of glucose (at or even below 1 kg glucose/m^3), whereas other yeasts may not show this effect until the glucose has reached a much higher critical value (perhaps 20 to 50 kg glucose/m^3). Yeasts which do not show this effect at all are termed Crabtree-negative and, consequently, can be grown in very high concentrations of glucose without detriment to their oxidative metabolism. Such yeasts will not

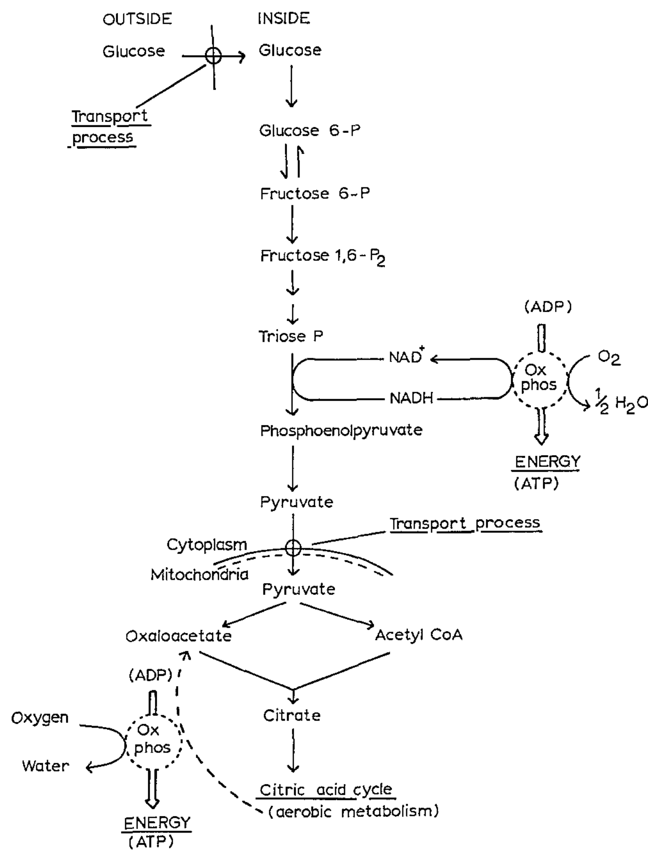


Fig. 6. Metabolism of glucose under aerobic conditions. Glycolysis involves the oxidation of glucose to pyruvate; respiration involves the oxdidation of pyruvate by the reactions of the citric acid cycle (Krebs cycle) in the mitochondrion)

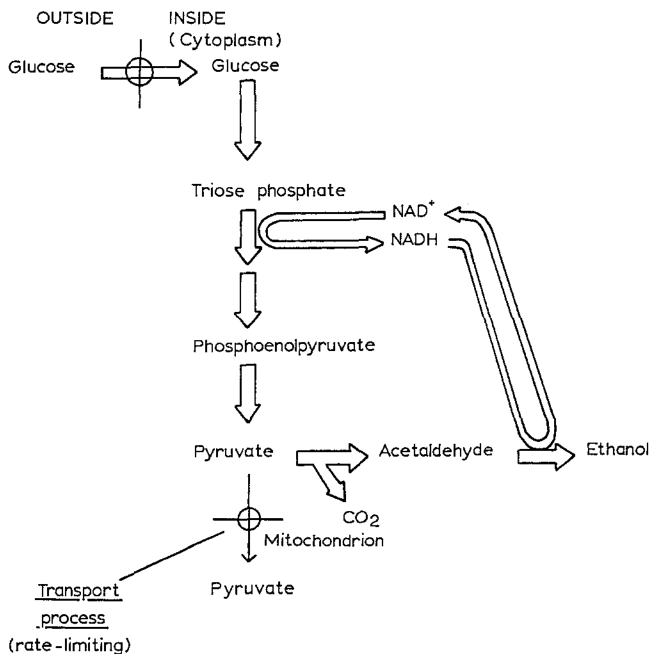


Fig. 7. Metabolism of glucose under anaerobic conditions. Respiration is now minimal (or zero) and reducing equivalents (NADH) generated by glycolysis must now be re-cycled by being linked to the oxidation of some glycolytic intermediate (i.e. in yeasts, the reduction of pyruvate to ethanol)

produce ethanol or indeed any other reduced material, as the cell is able to maintain a balance between the rate of oxidation of glucose, the accumulation of intermediates, including NADH, and the reoxidation of the NADH through oxidative phosphorylation.

The second effect which is applied to yeasts is the Pasteur effect which says, simply, that under aerobic conditions yeasts consume less glucose and the process of fermentation is diminished. As fermentation is mentioned specifically in the definition of the Pasteur effect, this effect can therefore only apply to fermentative yeasts, i.e. those which produce ethanol as an end-product. The Pasteur effect cannot be applied to yeasts which can only grow aerobically – i.e. those which are known obligate aerobes. With the Pasteur effect there can be various degrees of response. The magnitude of the effect will depend on the relative capabilities of the glycolytic pathway (glucose degradation to pyruvate) compared with the respiratory (oxidative) capacity of the cells (the oxidation of pyruvate in the mitochondrion) – see Fig. 6.

Only in situations where respiration is an important pathway of sugar metabolism can a significant Pasteur effect take place [4]. In other words, when a yeast such as Candida tropicalis is placed in an aerated culture it will be able to make more biomass per unit weight of glucose consumed than it would be able to if the oxygen supply was curtailed. This will seem fairly obvious from the remarks which have already been made above. As the oxygen supply is increased to Candida tropicalis, so the organism switches from fermentation (i.e. Fig. 7) towards respiration (Fig. 6) and less ethanol is produced.

With Saccharomyces cerevisiae, however, ethanol can still be produced under aerobic conditions because of the repression of oxidative metabolism in the presence of glucose (i.e. the Crabtree effect is working). Although Saccharomyces cerevisiae is growing aerobically, its fermentative capacity still has to function and thus ethanol is still produced (i.e. the situation is as given in Fig. 7 rather than Fig. 6). Under these circumstances the Pasteur effect is greatly diminished. Examples for the flux of glucose in Candida tropicalis and Saccharomyces cerevisiae are set out in Table 3 to explain the differences in magnitude of these two yeasts, both of which can produce ethanol.

Before moving on to a brief explanation of the Crabtree and Pasteur effects, two other effects which apply to yeasts might also be mentioned briefly. The Kluyver effect is really the Crabtree/Pasteur effect seen with sugars other than glucose. Thus, although a yeast may be fermentative when it grows on glucose, i.e. will produce ethanol, it will not produce ethanol when it is using galactose, maltose, etc. The other effect to mention is the Custers effect which applies to those yeasts that ferment glucose faster aerobically than they can do anaerobically. In other words, the process of converting glucose to ethanol in some yeasts, especially those of the genus Brettanomyces, occurs faster when the culture medium is aerated than when it is unaerated and allowed to go anaerobic. These yeasts usually produce acetic acid as well

Table 3. Crabtree and Pasteur effects in two yeasts

Anaerobically (fermentation only)	Glucose utilization (µmol/min/g cell) in		
	Candida tropicalis	*Saccharomyces cerevisiae*	
	A* 150–200	A* 160	B* 70
TOTAL FLUX:	150–200	160	70
Aerobic fermentation:	5	140	25
Aerobic respiration:	42	7	8
TOTAL FLUX:	47	147	33
O_2 decreases fermentation by:	97%	13%	64%
O_2 decreases respiration by:	73%	8%	53%
Conclusions:	Weakly Crabtree-positive strong Pasteur effect	Strongly Crabtree-positive weak Pasteur effect	

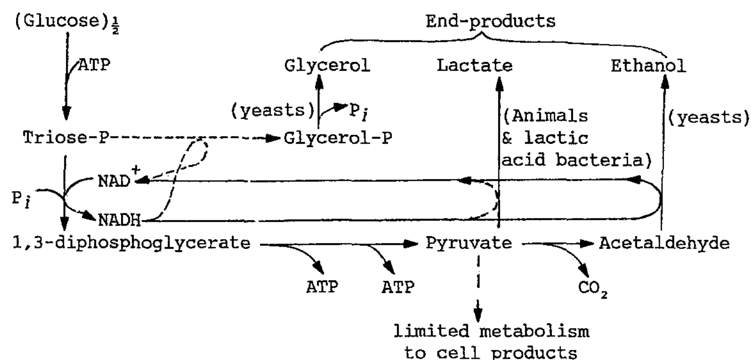
*A: data from De Deken [5];
*B: data from Santa Maria et al. (see [4])

as ethanol as an end product. A full discussion of these effects as well as those of the Crabtree and Pasteur effects is given in the recent review of Gancedo and Serrano [4].

A brief explanation of the Crabtree (and also the Pasteur effect) can be given by an examination of the process of glycolysis, that is the process by which glucose is catabolised. The end-product of glycolysis is pyruvate (see Fig. 6). If ethanol is not being formed, all the pyruvate must be entering the mitochondrion and be converted both to acetyl-CoA and oxaloacetate using the two enzymes pyruvate dehydrogenase (pyruvate → acetyl-CoA) and pyruvate carboxylase (pyruvate → oxaloacetate). The two products from pyruvate combine together to give citrate which is then metabolized through a series of reactions known as the tricarboxylic acid cycle or Krebs' cycle (see Fig. 6). This would represent then the "normal" situation for most cells (yeasts, fungi as well as plants and higher organisms). However, if the oxidation of pyruvate through the tricarboxylic acid cycle is stopped because of a lack of oxygen – which is the main driving force for the operation of this cycle – or alternatively too much pyruvate is being produced from glucose for it to be handled in the mitochondrion, then a surfeit of pyruvate will be built up outside the mitochondrion (Fig. 7). This build-up of pyruvate will therefore have different mechanisms causing its accumulating depending on the yeast cell which is being used. In Saccharomyces cerevisiae it will be the rapid uptake and metabolism of glucose which leads to there being an overproduction of pyruvate beyond the amount that the mitochondria can handle, whereas in other yeasts it will be the absence of oxygen which causes the oxidation of pyruvate to slow down.

Whatever the explanation for the overproduction of pyruvate, this compound must now be effectively dealt with by the cell. The production of pyruvate will coincide with the formation of reducing equivalents (NADH) being produced from the latter stages of glycolysis. These reducing equivalents must be reoxidized, but the very fact that too much pyruvate is being produced for the cell to handle indicates that some mechanism of reoxidizing NADH other than by being linked to oxidative phosphorylation must be found (compare once again Fig. 2A with Fig. 2B). The answer to this is to convert pyruvate to ethanol. This is a reductive process and is linked in the final steps to the reoxidization of NADH (see Fig. 7).

The process of ethanol formation in yeasts serves exactly the same purpose as lactate production by the muscle cells of an athlete running very rapidly. Under these circumstance glucose is used so rapidly that there is insufficient oxygen to oxidize all the pyruvate which is being produced. Simultaneously with this build-up of pyruvate NADH itself accumulates. To reoxidize the NADH in athletes' muscle the pyruvate is then reduced to lactate as a dead-end product. (Like ethanol, lactate can be re-used when normal respiration is restored.) The accumulation of Lactate causes the muscular activity to slowdown, eventually causing seizure or cramp. A yeast which is producing ethanol could be regarded as similarly undergoing respiratory "cramp". These events are summarized in Fig. 8 where the term "respiratory bottleneck" is used to indicate the inability of the cell to handle the vast amount of pyruvate which is being produced under certain circumstances (i.e. too much glucose or too little oxygen). It would be noted from Fig. 8 that besides ethanol being produced there is a small amount of glycerol also produced by yeasts. This is needed to balance the conversion of NADH back to NAD, as any metabolism of pyruvate other than acetaldehyde will deplete the supply of the co-substrate for NADH oxidation. As this always occurs, some glycerol is also always produced as its formation is also linked to NADH oxidation.



A detailed discussion of the regulation of carbon metabolism in Saccharomyces cerevisiae is given in a review by Käppeli [6].

Although this review has concentrated almost exclusively on the use of glucose as a substrate for yeasts to grow on, it should not be forgotten that yeasts themselves accumulate polysaccharides as storage compounds. The usual storage polysaccharide which occurs in yeast is glycogen and this

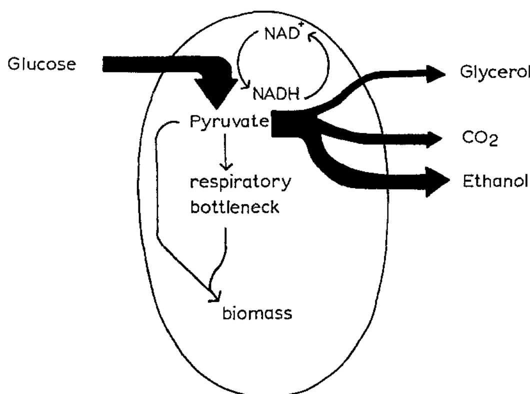


Fig. 8. Schematic diagram to show overflow metabolism of glucose to ethanol when respiration of pyruvate is limited (due either to glucose overload – the Crabtree effect – or due to anaerobic conditions – the Pasteur effect)

often forms a priority substrate for the cell to use before it will begin to consume external glucose. When glycogen which is stored within the cell is used by a yeast it will often be consumed in such a manner so as to exclude the utilization of the external glucose. The breakdown of glycogen will therefore be controlled within the cell, so that its breakdown and formation of pyruvate exactly matches the rate at which the cell can handle the pyruvate. These aspects of the role of glycogen in yeasts and in particular to brewing yeasts has recently been reviewed by Quain [7].

8 Conclusions

In this very brief outline of yeast physiology, I hope I have been able to convey some of the broad features of this very large subject. For readers requiring slightly more detailed information, a wider account of microbial growth and biochemistry was written by the author a few years ago [10]. Detailed descriptions of yeasts used in biotechnology are given in the book edited by Berry et al. [11] and in the all-encompassing multi-volume treatise "The Yeasts" [12].

There are still imperfections in our understanding of ethanol formation: of how the Pasteur and Crabtree effects operate at the molecular level and of how the flux of the major compounds involved in the processes of ethanol formation, i.e. glucose uptake into the cell and pyruvate transport into the mitochondrion, are regulated and controlled. The integration of these processes will no doubt remain subjects which will engage the attention of yeast physiologists for several years, if not many years, to come.

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