Journal of Industrial Microbiology & Biotechnology (1998) 21, 247–253

http://www.stockton-press.co.uk/jim

Initiation of anaerobic growth of *Saccharomyces cerevisiae* by amino acids or nucleic acid bases: ergosterol and unsaturated fatty acids cannot replace oxygen in minimal media

KC Thomas, SH Hynes and WM Ingledew

Department of Applied Microbiology and Food Science, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 5A8

Nine out of ten industrially important strains of *Saccharomyces cerevisiae* did not grow in minimal media under anaerobic conditions even when ergosterol and unsaturated fatty acids were provided. Anaerobiosis was maintained either by flushing the culture flasks with prepurified nitrogen or by incubating the flasks in an anaerobic chamber. Traces of oxygen present in 'prepurified nitrogen gas' were sufficient to initiate yeast growth and on removal of the oxygen by catalytic means the yeasts failed to grow. The yeast grew very well anaerobically if the medium was supplemented with a mixture of amino acids or with a mixture of purines and pyrimidines. The growth initiated by including a mixture of amino acids was further enhanced when the medium was supplemented with ergosterol and an unsaturated fatty acid. Since no oxygen requirement for the synthesis of amino acids or purines and pyrimidines has been demonstrated, growth promotion by these compounds under anaerobic conditions is most likely not by eliminating the need for oxygen for their synthesis. We suggest that the amino acids and the nucleic acid bases yielded, through some hitherto unknown reactions, small amounts of a molecular or usable form of oxygen which allowed key reactions essential for 'anaerobic' growth to proceed.

Keywords: yeast growth; sterols; unsaturated fatty acids; oxygen; anaerobiosis; amino acids; purine and pyrimidine bases; *Saccharomyces cerevisiae*

Introduction

It is generally believed that the yeast *Saccharomyces cerevisiae* cannot grow anaerobically unless the medium is supplemented with certain lipids whose synthesis has an absolute requirement for oxygen. In their pioneering work, Andreasen and Stier [3,4] reported that the nutrient requirements imposed by anaerobic conditions could be satisfied by supplementing the medium with ergosterol and an unsaturated fatty acid, or a source of unsaturated fatty acid such as an oleic acid ester (eg Tween 80). Other steroids such as campesterol, cholesterol, or 7-dehydrocholesterol could fulfill the steroid requirement of *S. cerevisiae* [7,16] and the unsaturated fatty acid requirement could be met with mono- , di-, or tri-unsaturated fatty acids [2,4,7,21].

S. cerevisiae grows very well aerobically in minimal media containing glucose, mineral salts, a few trace elements and very small concentrations of some B-vitamins. Although it is alluded to in the literature that the yeast may grow in the same minimal medium anaerobically if supplemented with ergosterol and an unsaturated fatty acid, this has not been demonstrated under strict anaerobic conditions. Moreover, provision of ergosterol and unsaturated fatty acids does not eliminate the oxygen requirement for growth of some yeasts such as *Pachysolen tannophilus* [13]. In one study [11] where strict anaerobic conditions using the Hungate technique [8] were employed, a strain of *S. cerevisiae* (Montrachet strain of wine yeast) grew in the absence of added ergosterol and unsaturated fatty acid although only after a considerable lag. The media used by these workers [11], however, were not minimal. They contained ingredients which, as the present study shows, were not essential for aerobic growth. The authors concluded that there was no absolute requirement for ergosterol and unsaturated fatty acid for anaerobic growth although the lag phase was considerably reduced by including these compounds in the growth medium. A similar observation was made by Verduyn *et al* [18] who reported that a strain of *S. cerevisiae* grew anaerobically without added unsaturated fatty acids, although the biomass production was considerably reduced. They, however, did not rule out the possibility of traces of oxygen being present in their continuous culture system. Growth media used by most workers [3,5,11] were not minimal and they contained ingredients which were not essential for aerobic growth but apparently had a stimulatory effect on biomass production. The growth media used by Andreasen and Stier [3] and Macy and Miller [11] had amino acids in addition to ammonium sulphate, the main nitrogen source. Other workers used complex media such as wort [6] or included complex nutrients such as yeast extract [2,5,7,9]. Examination of the published work reveals that except for the work reported by Macy and Miller [11], strict anaerobic conditions were not maintained during the cultivation of the yeast. In most studies, fermentors were continuously flushed with ultra pure nitrogen gas which contained traces of oxygen, usually less than 5 ppm [2,7,9,14,18,19]. Although Albers *et al* [1] used nitrogen gas containing less than 1 ppm oxygen, the flushing rate was relatively low (0.25 vol vol^{-1} min^{-1}) and they

Correspondence: KC Thomas, Department of Applied Microbiology and Food Science, University of Saskatchewan, Saskatchewan, Canada S7N 5A8
Received 16 July 1998; accepted 8 October 1998

might not have achieved a dissolved oxygen tension (DOT) low enough to make the medium strictly anaerobic. Visser *et al* [20] showed that with nitrogen containing less than 5 ppm oxygen, a flushing rate of 10 vol vol^{-1} min^{-1} was required to lower DOT to 0.005% of air saturation. At a lower flushing rate the DOT values remained higher. This suggested that the steady state dissolved oxygen level attained is not only a function of the concentration of oxygen in the flushing gas but also on the flushing rate. Even 5 ppm oxygen in nitrogen (0.22 μmoles of oxygen per liter of the commercial gas) may not be too low to permit some of the oxygen-requiring/dependent reactions to proceed. Burke *et al* [5] showed that the yeast strain JM43 (MATα leu 2–3, 112 his4-580 trp1-289 ura3–52) grew very well in a semisynthetic medium in which the oxygen concentration was maintained at 0.44 μM by automatic regulation. When the oxygen supply was cut off (unregulated) the yeast growth was poor. Rogers and Stewart [17] showed that only a very small concentration of oxygen (0.1 μM) was required to increase cellular amounts of unsaturated fatty acids to a great extent and they also reported that potential respiration could be induced at very low oxygen concentrations. A half maximal response in such cases was reached at a concentration of 0.12 μM.

We observed that an industrial brewing strain of *S. cerevisiae* (*uvarum)* NCYC 1324 which grows very well aerobically in a minimal medium, failed to grow anaerobically even if the medium was supplemented with ergosterol and Tween 80. Of nine other yeast strains similarly tested, eight failed to grow anaerobically even though both ergosterol and Tween 80 were included in the medium. Here we present evidence to show that, contrary to the current dogma, ergosterol and unsaturated fatty acids cannot replace oxygen during anaerobic growth of *S. cerevisiae.* Availability of other nutrients is essential for initiation and maintenance of anaerobic growth.

Materials and methods

Yeast

The brewing strain *S. cerevisiae (uvarum)* NCYC 1324 was used in most of the studies. Nine other industrially important strains listed in Table 1 were used in some of the studies. They were kept on Sabouraud's dextrose agar slants at 4°C and subcultured monthly.

Chemicals and prepurified nitrogen

Amino acids, ergosterol and Tween 80 were purchased from Sigma Chemical Co, St Louis, MO, USA. All other chemicals were obtained through local suppliers and were of reagent grade. Prepurified nitrogen containing less than 5 ppm oxygen was supplied by Praxair Products, Mississauga, Ontario, Canada.

Growth media

Yeasts were grown in a modified Wickerham's medium with glucose and ammonium sulfate as carbon and nitrogen sources respectively. The final concentrations of ingredients in the medium were as follows: glucose, 0.56 M; $(NH_4)_2SO_4$, 10 mM; K_2HPO_4, 0.86 mM; KH_2PO_4, 6.25 mM; $MgSO_4$, 2.03 mM; $CaCl_2$, 0.68 mM; NaCl, 1.7 mM; $ZnSO_4$, 100 μM; H_3BO_3, 24.0 μM; KI, 1.8 μM; $MnSO_4$, 20 μM; $CuSO_4$ 10.0 μM; Na_2MoO_4 1.5 μM; $CoCl_2$, 1.5 μM; $FeCl_3$, 1.23 μM. Filter-sterilized vitamins were added to give the following final concentrations per liter of medium: biotin, 20 μg; calcium pantothenate, 2 mg; folic acid, 20 μg; myo-inositol, 10 mg; niacin, 400 μg; *p*-aminobenzoic acid, 200 μg; pyridoxine hydrochloride, 400 μg; riboflavin, 200 μg and thiamine hydrochloride, 200 μg. The medium was prepared in distilled water in two parts, autoclaved, cooled and then mixed. Part A contained $(NH_4)_2SO_4$, $CaCl_2$, NaCl, $MgSO_4$, $FeCl_3$ in 400 ml and part B contained the rest of the ingredients in 600 ml.

A combined stock solution of ergosterol and Tween 80 was prepared as described by Andreasen and Stier [3], filter sterilized and stored at room temperature. Where necessary, a separate ergosterol stock solution was prepared by dissolving 0.2188 g of ergosterol in 30 ml of 95% ethanol, heating to a boil, then cooling and making up to 50 ml with 95% ethanol. A 25% (w/v) stock solution of Tween 80 was prepared in water. Final concentrations of ergosterol and Tween 80 in the medium were 0.004% and 0.4% respectively, the same concentrations used by Andreasen and Stier in their original work [3,4].

Preparation of inoculum

A loopful of yeast culture from agar slants was transferred to 50 ml sterile medium contained in 250-ml Klett flasks, flushed with filter-sterilized carbon dioxide, capped and incubated with shaking at 30°C until the culture reached mid to late exponential phase. The initial pH of the medium was 5.1 but on flushing with CO_2 for 2 min the pH dropped to 4.5.

Growth studies

Depending on the culture density of the inoculum, and the need to minimize carry over of growth-promoting nutrients from the inoculum, different volumes ranging from 0.025 ml to 0.5 ml were used to inoculate 50-ml aliquots of sterile medium contained in Klett flasks. For 'anaerobic growth' the flasks were incubated at 30°C with continuous flushing, at the rate of 50 ml per min, with prepurified nitrogen (< 5 ppm oxygen) which was filter sterilized and humidified. In some studies, anaerobic conditions were maintained by incubating the flasks in an anaerobic chamber (Model 1025, Forma Scientific, Marietta, OH, USA) which had palladium pellets as catalyst and was charged with a gaseous atmosphere consisting of hydrogen, carbon dioxide, and nitrogen in the ratio of 10 :10 : 80. Flasks containing the growth medium and various additives (amino acids, nucleic acid bases, ergosterol, Tween 80, or cell-free culture filtrate) were equilibrated in the anaerobic chamber for 48 h before inoculation with yeast. The growth was monitored at chosen intervals by measuring absorbance with a Klett-Summerson colorimeter (filter No. 66) or by taking samples from flasks in the anaerobic chamber and enumerating cells by direct microscopic count. Other modifications made in this procedure are described in the Results section.

Table 1 Effects of adding mixtures of amino acids, or ergosterol and Tween 80 or a combination thereof on anaerobic growth of ten selected industrial strains of yeasts used in the production of alcohol

S. cerevisiae strain	Type	Aerobic	Anaerobic			
		no additions	no additions	ergosterol + Tween 80	amino acids	amino acids + ergosterol + Tween 80
NCYC 1324	brewing	+++	NG	NG	++	+++
ATCC 26602	distilling	+++	NG	+++	+++	++++
Industrial A	fuel alcohol	+++	NG	NG	+++	++++
Industrial B	brewing	+++	NG	NG	+	++
Industrial C	brewing	+++	NG	NG	++	+++
Industrial D	fuel alcohol	++++	NG	NG	+++	+++
Industrial E	brewing	+++	NG	NG	+	++
Industrial F	brewing	+++	NG	NG	++	+++
Industrial G	brewing	+++	NG	NG	++	+++
Industrial H	brewing	+++	NG	NG	++	+++

NG = no growth up to 7 days, + slight growth, ++ moderate growth, +++ good growth, ++++ very good growth.

Results

The inoculum size determines whether the yeast will grow anaerobically or not

The inoculum size determined the length of the lag phase, and whether the yeast could or could not grow anaerobically (Figure 1). When the minimal medium which did not contain ergosterol and Tween 80 was inoculated with 5.64×10^4 cells per ml (0.1 ml of inoculum per 50 ml of medium), the yeast failed to grow anaerobically but grew aerobically (Figure 1a). At higher inoculum levels the yeast grew under both conditions (Figure 1b, c, d). The lag period was slightly longer under anaerobic incubation and as expected it decreased with increasing levels of inoculum under both conditions of cultivation. Anaerobiosis in these experiments was maintained by flushing the flasks with prepurified nitrogen which contained less than 5 ppm oxygen. No attempt was made to remove traces of oxygen present in the flushing gas by catalytic scrubbing. Growth during 'anaerobic' incubation appeared to be related to two factors. First, as reported in the following section, the presence of traces of oxygen in the flushing gas has an effect on yeast growth. Second, during the growth of the inoculum the yeast secreted some growth-stimulating factor or factors and by adding greater volumes of inoculum, greater amounts of this growth-stimulating factor were provided. The yeast failed to grow in medium incubated in the anaerobic chamber when the inoculum size was reduced to 0.05 ml or less per 50 ml of the medium and in this case inclusion of ergosterol and Tween 80 was not sufficient to meet the nutrient requirement. If, however, a small volume of cell-free filtrate from the inoculum culture (filtered through a membrane filter of 0.22-μm pore diameter) was added, yeast growth was initiated even with an inoculum as low as 0.025 ml per 50 ml medium. In these studies the flasks containing the medium and various amounts of cell-free culture filtrate were kept in the anaerobic chamber for 48 h and then inoculated with a 24-h old culture. As shown in Figure 2, the lag phase was reduced to less than 24 h when 2 ml or more of cell-free culture filtrate was added per 50 ml of the medium. When the volume of the filtrate added was reduced to 1.0 ml the lag period increased to 144 h. No growth occurred even after 30 days of incubation if the quantity of the culture filtrate added was 0.5 ml or less. It is interesting to note that irrespective of the length of the lag period, the maximum growth attained and the doubling times during the exponential phase of growth did not appreciably differ with increasing volumes of culture filtrate added to the medium. For example, the respective doubling times with 1.0, 2.0 and 10.0 ml of cell-free culture filtrate in the medium were 2.5, 2.4, and 2.4 h. To minimize the carry over of this 'growth stimulating factor' and to reduce the number of cells added to the fresh medium, only 0.05 ml inoculum was added per 50 ml medium in most of the following experiments.

Traces of oxygen are sufficient to initiate growth of S. cerevisiae

When the minimal medium was inoculated with either 1.0% or 0.5% of a late exponential culture, and made anaerobic by continuous flushing with prepurified nitrogen (<5 ppm oxygen), the yeast NCYC 1324 grew after a lag of 48 h. The lag period was virtually eliminated by the inclusion of ergosterol and Tween 80 in the medium (Figure 3a) and this might be taken as proof that ergosterol and unsaturated fatty acid can meet the nutritional requirements induced by anaerobiosis. This, however, does not seem to be the case. The yeast failed to grow when traces of oxygen present in the flushing nitrogen gas were removed by passing it through heated (400°C) copper turnings, and under this condition, inclusion of ergosterol and Tween 80 was not sufficient to initiate growth (Figure 3b). The yeast cells remained viable during the anaerobic incubation as indicated by their ability to grow when air was admitted to the flasks (indicated by the arrow in Figure 3b). There was, however, a lag period of 48 h between admitting air to the flasks and manifestation of measurable growth.

A mixture of amino acids or a mixture of purine and pyrimidine bases can promote anaerobic growth of S. cerevisiae

Even though ergosterol and Tween 80 were included in the minimal medium, the yeast NCYC 1324 failed to grow

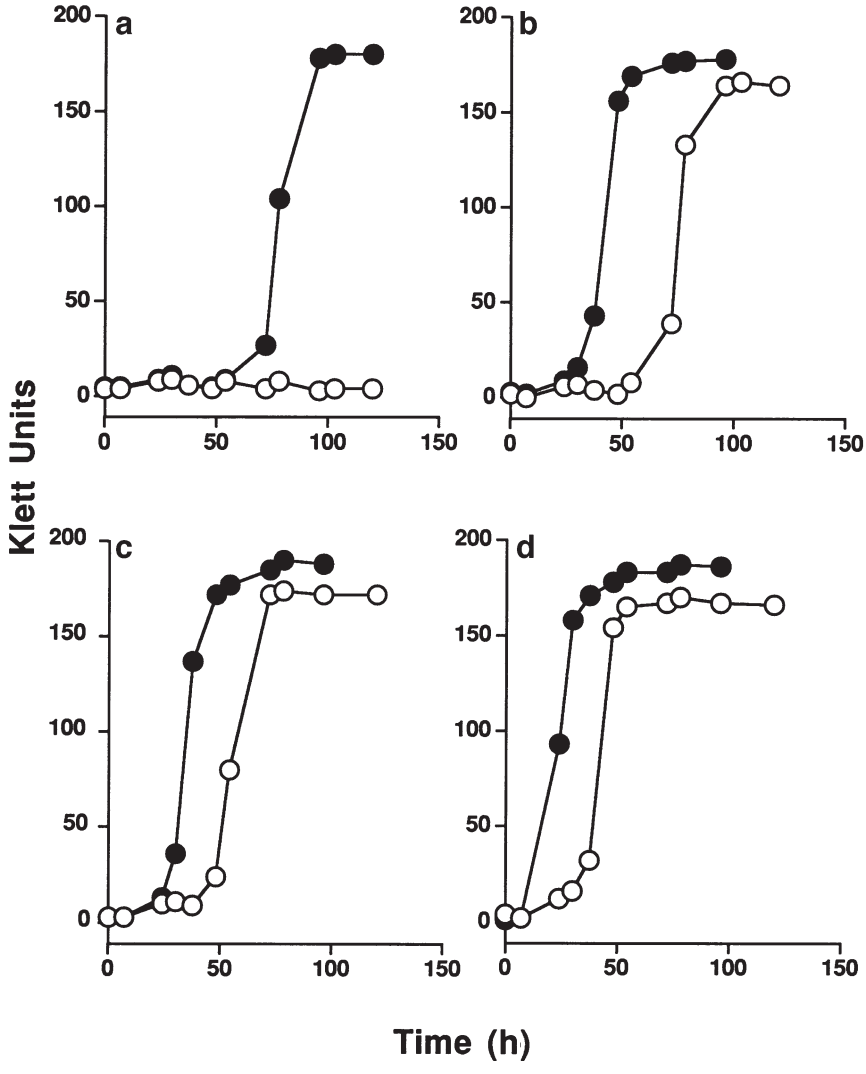

Figure 1 Effect of inoculum size on aerobic (—●—) and anaerobic (nitrogen-flushed) growth (—○—) of *Saccharomyces cerevisiae* NCYC 1324 in minimal medium. The flasks, containing 50 ml medium, were inoculated with a 24-h-old culture at the rate of 0.1 ml (a), 0.25 ml (b), 0.5 ml (c) and 1.0 ml (d). The inoculum contained 2.8×10^7 cells per ml.

anaerobically when the inoculum size was reduced to 0.05 ml per 50 ml medium, corresponding to 2.0×10^4 cells per ml (Figure 4) and when the flasks were continuously flushed with 'oxygen scrubbed' prepurified nitrogen. The yeast started to grow shortly after admitting air into the flasks by flushing them for 5 min with filter-sterilized air at the rate of 50 ml per min (indicated by the arrows in control A and control B in Figure 4). Both controls (A and B) contained ergosterol and Tween 80 but no amino acids or nucleic acid bases. The only difference between them was the time of admission of air into the flasks. If a mixture of 18 amino acids (each amino acid at a concentration of 50 mg per liter) or a mixture of purine and pyrimidine bases (each base at a concentration of 50 mg per liter) was included in the medium, the yeast grew anaerobically and the lag period was only 24 h. Considering the small size of the inoculum, this lag period was not extensive. The following amino acids were included in the mixture: alanine, arginine, aspartic acid, cysteine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine. The nucleic acid base mixture in the study included adenine, guanine, thymine, and uracil. Bases were dissolved in distilled water but complete dissolution of guanine and thymine required addition of a small amount of 1 M KOH. The dissolved mixtures of amino acids and nucleic acids were filter sterilized and added to minimal medium. The flasks containing the medium were then kept in the anaerobic chamber for 48 h or flushed with prepurified nitrogen before inoculation. It was not necessary to include all 18 amino acids to initiate yeast growth although the lag period was the shortest and growth was maximum when all 18 amino acids were present. Separate additions of acidic (glutamic acid and aspartic acid), basic (lysine, arginine and histidine), and a mixture of leucine, isoleucine, methionine, phenylalanine, tryptophan and valine had the same growth-stimulating effect under anaerobic conditions (data not shown). Both nucleic acid bases and amino acids could meet nutrient requirements induced by anaerobic conditions but ergosterol and Tween 80 could not.

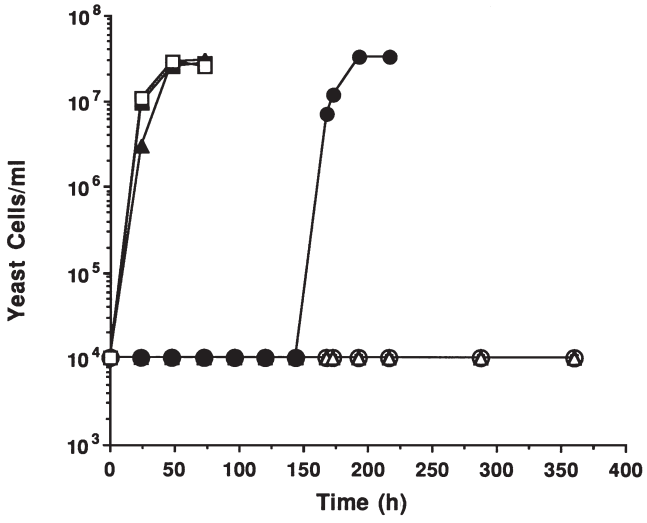

Figure 2 Effect of cell-free culture filtrate on anaerobic growth of the yeast (industrial strain A). Various volumes ((—○—) 0 ml, (—×—) 0.1 ml, (—△—) 0.5 ml, (—●—) 1 ml, (—▽—) 2 ml, (—▲—) 5 ml, (—□—) 10 ml) of cell-free culture filtrate from 24-h-old cultures were added to flasks each containing 50 ml medium. The flasks were equilibrated in the anaerobic chamber for 48 h before inoculating each of them with 0.025 ml of 24-h-old culture.

Availability of ergosterol and unsaturated fatty acid is not sufficient to promote anaerobic growth of most strains of S. cerevisiae in minimal medium

In order to further test whether nutritional requirements induced by anaerobic conditions can be met with ergosterol and Tween 80, three strains of *S. cerevisiae* were inoculated into minimal media with different nutrient additions and incubated at 30°C under strict anaerobic conditions. Fifty-milliliter aliquots of minimal medium containing various additives and the redox indicator resazurin (0.0001% w/v) were distributed in sterile Klett flasks which were then placed in an anaerobic chamber for 48 h. During this time the medium became completely reduced as indicated by the change of color of resazurin from light pink to colorless. Flasks were then inoculated with 0.05 ml of cultures of *S. cerevisiae* NCYC 1324, ATCC 26602 or the industrial strain A and incubated at 30°C in the anaerobic chamber. The results showed that both strain NCYC 1324 and strain A failed to grow anaerobically even though the medium contained both ergosterol and Tween 80 (Figure 5a, c). Strain ATCC 26602, however, started to grow after a lag of 96 h (Figure 5b). Growth of strain NCYC 1324 and strain A was initiated only after the flasks were removed from the anaerobic chamber and air was admitted into the flasks. If a mixture of 18 amino acids was included in the medium all three yeast strains grew anaerobically without much lag. The lag was further reduced and the growth enhanced when ergosterol and Tween 80 were included along with the mixture of amino acids. Seven other brewing, distilling or fuel alcohol strains were similarly tested and none of them grew in minimal media containing ergosterol and Tween 80 while all of them grew if the medium was supplemented with a mixture of amino acids. The results are summarized in Table 1.

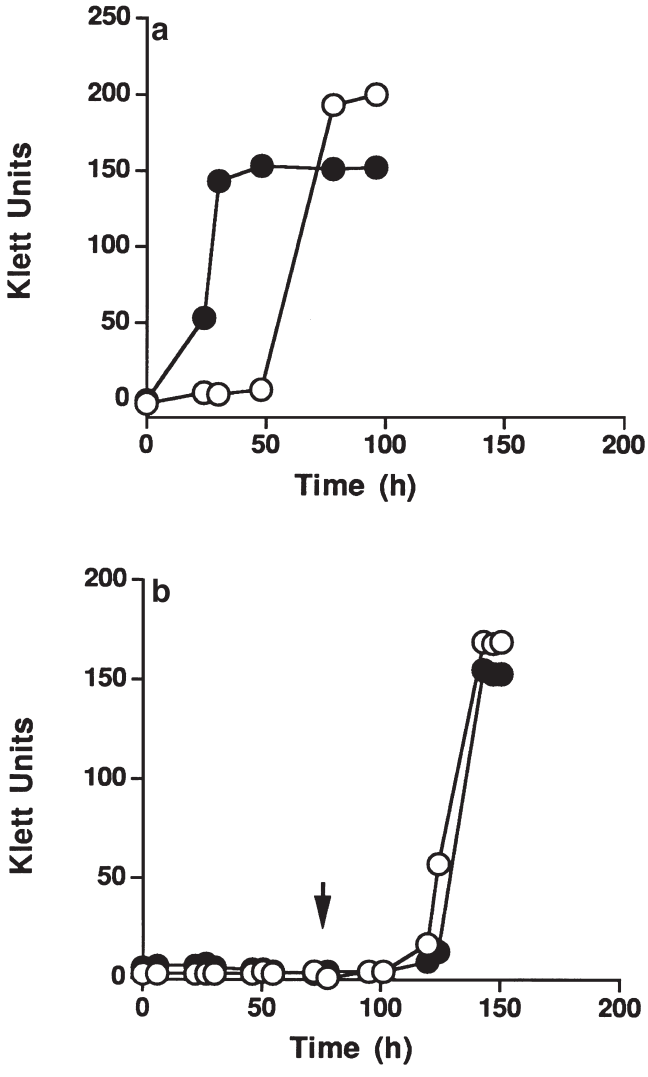

Figure 3 Anaerobic growth of *Saccharomyces cerevisiae* NCYC 1324 in minimal media containing 0.004% ergosterol and 0.4% Tween 80 (—●—). Control flasks (—○—) did not contain ergosterol or Tween 80 as nutrient supplements. The flasks were incubated at 30°C and flushed continuously (a) with commercial prepurified nitrogen gas containing less than 5 ppm oxygen and (b) with nitrogen gas after removing traces of oxygen by passing the gas through heated (400°C) copper turnings. Arrow indicates the time at which air was admitted to the flasks.

Mitochondrial protein synthesis is not involved in amino acid-mediated initiation of anaerobic growth

It is not known whether promitochondria which the yeast can develop under anaerobic conditions [15, 20] play a significant role in the anaerobic growth of *S. cerevisiae*. To test whether added amino acids would initiate growth through mitochondrial development involving protein synthesis, three strains of yeasts (NCYC 1324, ATCC 26602 and Industrial strain A) were inoculated into minimal medium containing a mixture of 18 amino acids and chloramphenicol (2 mg per ml) and incubated at 30°C aerobically or in an anaerobic chamber. Chloramphenicol did not prevent growth of the yeast under either condition of incubation (data not shown) and it can be concluded that initiation of anaerobic growth by amino acids was not through mitochondrial protein synthesis. This is to be

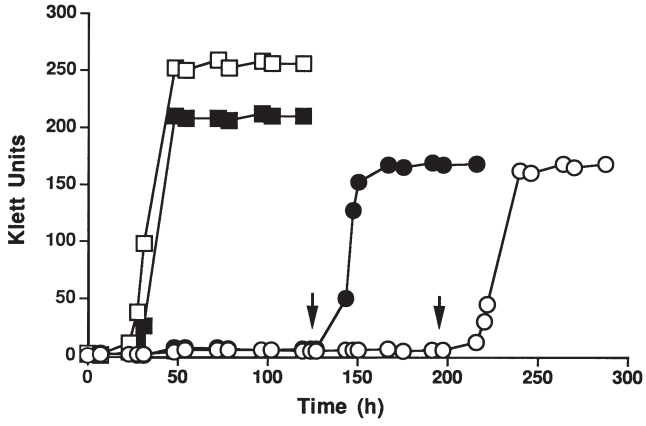

Figure 4 Effects of mixtures of amino acids (—□—) or purine and pyrimidine bases (—■—) on anaerobic growth of *Saccharomyces cerevisiae* NCYC 1324. All flasks contained ergosterol (0.004%), and Tween 80 (0.4%). Air was admitted to the control flasks at the times indicated by the arrows. Control flasks A (—○—) and B (—●—) did not contain amino acids or purine and pyrimidine bases.

expected since anaerobic growth was also initiated by a mixture of purine and pyrimidine bases.

Discussion

Results presented here for several industrially important strains of *S. cerevisiae* suggest that, contrary to what is reported in the literature, nutritional requirements induced by anaerobiosis cannot be fully met by ergosterol and an unsaturated fatty acid . Nine out of ten strains studied failed to grow anaerobically in minimal media even though the media contained both ergosterol and Tween 80. Since all strains grew aerobically in the same minimal medium, the part played by oxygen in the growth of these yeasts is more than that of an essential reactant in the synthesis of ergosterol and unsaturated fatty acids. It must be, however, stressed that there are exceptions as demonstrated by the ability of strain ATCC 26602 to grow anaerobically when the lipid supplements were included in the medium but not when these supplements were absent. The observations made by Andreasen and Stier [4] that the yeast *S. cerevisiae* can grow anaerobically in minimal media if supplemented with ergosterol and unsaturated fatty acids, may be the characteristic of the particular yeast strain used in their studies. They used a haploid strain of *S. cerevisiae* designated as SC-1 (DCL). Our results clearly show that for nine of the 10 strains of *S. cerevisiae*, molecular oxygen cannot be replaced with ergosterol and unsaturated fatty acid, whereas provision of a mixture of amino acids or a mixture of purine and pyrimidine bases allowed anaerobic growth of all strains. Examination of the results of other workers [2,3,7,9,11,14,18,19] indicate that anaerobic growth of *S. cerevisiae* in minimal media occurred only if the medium contained amino acids, or when traces of oxygen present in the flushing gas were not completely removed. It was shown by Visser *et al* [20] that even when the initial DOT was as low as 0.005% of air saturation, *S. cerevisiae* growing in minimal medium was able to reduce its concentration considerably. Anaerobic growth observed by Albers *et al* [1] may be related to the size of inoculum (2.5%) used in

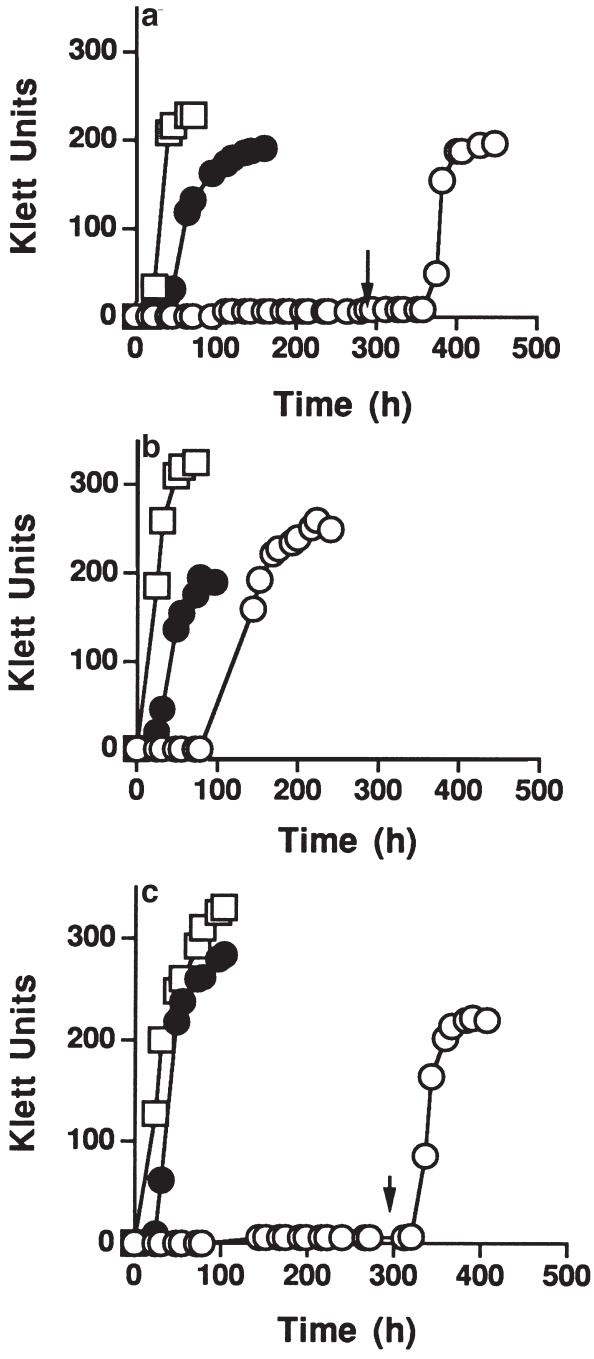

Figure 5 Effects of adding a mixture of amino acids alone (—●—) or a mixture of ergosterol and Tween 80 (—○—) or amino acids with ergosterol (0.004%) and Tween 80 (0.4%) (—□—) on the growth of three industrial strains of *Saccharomyces cerevisiae.* (a) Strain NCYC 1324, (b) strain ATCC 26602, (c) industrial strain A. Arrows indicate the time (300 h) at which air was admitted into flasks in which no growth had taken place.

their study. As we have shown in the present study, growth occurred only when the inoculum size was above a certain threshold value and the lag period decreased with increasing inoculum size or when increasing volumes of cell-free culture filtrate were added to the medium.

The mechanisms by which amino acids and nucleic acid bases promote anaerobic growth is not known. As far as

we know, no oxygen requirement has been demonstrated for the synthesis of these compounds and therefore initiation of growth by these compounds is most likely not through eliminating the need for oxygen for their own synthesis. Since these unrelated classes of compounds (amino acids and nucleic acid bases) had the same positive effect on anaerobic growth of yeast, a derivative rather than the compounds themselves may be involved in the growth promotion. We do not know the nature of this derivative. Urea, a common catabolite of the amino acid arginine and of purines, was not effective in promoting anaerobic growth (data not shown). Although we do not have any direct evidence, it may be worth speculating that the growth-promoting derivative may be oxygen itself produced through biological or chemical decomposition of amino acids or nucleic acid bases. We suggest that the amino acids as well as the nucleic acid bases yielded, through some hitherto unknown reactions, small amounts of molecular or other active form of oxygen which allowed key reactions to proceed. Growth, presumably, cannot occur without these reactions. These essential reactions may be under the control of hypoxic genes which regulate oxygen-dependent functions such as alternate cytochrome subunits and oxidases and desaturases, heme, sterol and fatty acid biosynthesis etc (for a review see reference [22]). Heme, the synthesis of which has an absolute requirement for oxygen [10, 12], has been shown to have a regulatory role in several processes in a wide variety of microorganisms. Coproporphyrinogen III oxidase, the rate-limiting enzyme in heme biosynthesis, has a high affinity for oxygen and its induction at low oxygen concentration results in significant accumulation of heme. No evidence has been presented to suggest that heme does not play a role during the anaerobic growth of *S. cerevisiae* or that the expression of hypoxic genes can occur in the total absence of oxygen. The question that remains to be answered is how do amino acids or nucleic acid bases promote anaerobic growth in spite of the fact that their own syntheses do not have any known oxygen-dependent steps.

Acknowledgements

The authors gratefully acknowledge research support from the Natural Sciences and Engineering Research Council of Canada and the Western Grains Research Foundation.

References

1 Albers E, C Larsson, G Liden, C Niklasson and L Gustafsson. 1996. Influence of the nitrogen source on *Saccharomyces cerevisiae* anaerobic growth and product formation. Appl Environ Microbiol 62: 3187–3195.
2 Alterthum F and AH Rose. 1973. Osmotic lysis of spheroplasts from *Saccharomyces cerevisiae* grown anaerobically in media containing different unsaturated fatty acids. J Gen Microbiol 77: 371–382.
3 Andreasen AA and TJB Stier. 1953. Anaerobic nutrition of *Saccharomyces cerevisiae*. I. Ergosterol requirement for growth in a defined medium. J Cell Comp Physiol 41: 23–26.
4 Andreasen AA and TJB Stier. 1954. Anaerobic nutrition of *Saccharomyces cerevisiae*. II. Unsaturated fatty acid requirement for growth in a defined medium. J Cell Comp Physiol 43: 271–281.
5 Burke PV, KE Kwast, F Everts and RO Poyton. 1998. A fermentor system for regulating oxygen at low concentrations in cultures of *Saccharomyces cerevisiae.* Appl Environ Microbiol 64: 1040–1044.
6 Casey GP, CA Magnus and WM Ingledew. 1984. High-gravity brewing: effects of nutrition on yeast composition, fermentative ability, and alcohol production. Appl Environ Microbiol 48: 639–646.
7 Hossack JA and AH Rose. 1976. Fragility of plasma membranes in *Saccharomyces cerevisiae* enriched with different sterols. J Bacteriol 127: 67–75.
8 Hungate RE. 1969. A roll tube method for cultivation of strict anaerobes. In: Methods in Microbiology, Vol 3B (Norris JR and DE Ribbons, eds), pp 117–132, Academic Press, New York.
9 Keenan MJH, AH Rose and BW Silverman. 1982. Effect of plasma-membrane phospholipid unsaturation on solute transport into *Saccharomyces cerevisiae* NCYC 366. J Gen Microbiol 128: 2547–2556.
10 Labbe-Rois R and P Labbe. 1990. Tetrapyrrole and heme biosynthesis in the yeast *Saccharomyces cerevisiae*. In: Biosynthesis of Heme and Chlorophylls (Dailey HA, ed), pp 235–285, McGraw-Hill, New York.
11 Macy JM and MW Miller. 1983. Anaerobic growth of *Saccharomyces cerevisiae* in the absence of oleic acid and ergosterol? Arch Microbiol 134: 64–67.
12 Mattoon JR, WE Lancashire, HK Saunders, E Carvajal, DR Malamud, GRC Braz and AD Panek. 1979. Oxygen and catabolite regulation of hemoprotein biosynthesis in yeast. In: Biochemical and Clinical Aspects of Oxygen (Caughey WS, ed), pp 421–435, Academic Press, New York.
13 Neirinck LG, R Maleszka and H Scneider. 1984. The requirement of oxygen for incorporation of carbon from d-xylose and d-glucose by *Pachysolen tannophilus*. Arch Biochem Biophys 228: 13–21.
14 Nissen TL, U Schulze, J Nielsen and J Villadsen. 1997. Flux distributions of anaerobic glucose-limited continuous cultures of *Saccharomyces cerevisiae*. Microbiology 143: 203–218.
15 O'Connor-Cox ESC, EJ Lodolo and BC Axcell. 1996. Mitochondrial relevance to yeast fermentative performance: a review. J Inst Brew 102: 19–25.
16 Proudlock JW, LW Wheeldon, DJ Jollow and AW Linnane. 1968. Role of sterols in *Saccharomyces cerevisiae*. Biochim Biophys Acta 152: 434–437.
17 Rogers PJ and PR Stewart. 1973. Mitochondrial and peroxisomal contributions to the energy metabolism of *Saccharomyces cerevisiae* in continuous culture. J Gen Microbiol 79: 205–217.
18 Verduyn C, E Postma, WA Scheffers and JP van Dijken. 1990. Physiology of *Saccharomyces cerevisiae* in anaerobic glucose-limited chemostat cultures. J Gen Microbiol 136: 395–403.
19 Verduyn C, E Postma, WA Scheffers and JP van Dijken. 1990. Energetics of *Saccharomyces cerevisiae* in anaerobic glucose limited chemostat cultures. J Gen Microbiol 136: 405–412.
20 Visser W, WM Batenburg-van der Vegte, WA Scheffers and JP van Dijken. 1990. Oxygen requirements of yeasts. Appl Environ Microbiol 56: 3785–3792.
21 Watson K and AH Rose. 1980. Fatty-acyl composition of the lipids of *Saccharomyces cerevisiae* grown aerobically or anaerobically in media containing different fatty acids. J Gen Microbiol 117: 225–233.
22 Zitomer RS and CV Lowry. 1992. Regulation of gene expression by oxygen in *Saccharomyces cerevisiae.* Microbiol Rev 56: 1–11.