Some controls on oligosaccharide utilization by yeasts:  
The physiological basis of the Kluyver effect *

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1. SUMMARY

Many yeasts can aerobically catabolize exogenously supplied glycosides that are hydrolysed in the cytosol, but few do so anaerobically. This is

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* Names of yeasts are consistent with those given in reference [1]. When the name given here differs from that used by the author whose work is cited, the latter name is given in parentheses. However, the name Kluyveromyces fragilis (Jørgensen) van der Walt has been kept for convenience, although it is now a synonym of Kluyveromyces marxianus (Hansen) van der Walt. Definitions: In view of confusions of terminology, particularly between microbial physiology and molecular biology, the following terms are defined as used in this article: induction, synthesis de novo of an enzyme or transport carrier in response to the presence of a substrate or structurally similar compound; derepression, the converse of catabolite repression, synthesis de novo of an enzyme or transport carrier in response to the removal of a readily metabolizable compound (such as D-glucose); activation, the converse of deactivation, allosteric reactivation of an enzyme already present, by change of substrate affinity. Herein, the term ‘fermentation’ refers to the catabolism of sugars by glycolysis to pyruvate and thence to ethanol and carbon dioxide.

so, even for yeasts that use one or more of the component hexoses anaerobically. The phenomenon, called the Kluyver effect, appears to be brought about by a combination of the following four factors: (i) fast transport of the glycosides into the cells involves proton symport and seems to require aerobiosis, so, under anaerobic conditions, the glycosides enter the cells much more slowly. This is probably because there is less ATP produced anaerobically than aerobically and, consequently, insufficient to supply the proton pump optimally, which is necessary to maintain proton symport; (ii) in addition, anaerobically, the transport carrier may have a lower substrate affinity; (iii) glycosidases generally have low substrate affinities; and (iv) the consequence of (i), (ii) and (iii) is a lowering of glycolytic flux and this deactivates pyruvate decarboxylase.

2. INTRODUCTION: KLUYVER'S ORGINAL OBSERVATIONS

Yeast break down exogenously supplied glycosides by hydrolysing them to liberate their monosaccharide groups, which are usually hex-
Fig. 1. The aerobic and anaerobic pathways of pyruvate catabolism by yeasts. ADH, alcohol dehydrogenase; PDC, pyruvate decarboxylase; PDH, pyruvate dehydrogenase complex.

oses. The hexoses are converted to pyruvate by the Embden-Meyerhof glycolytic pathway [2], and thence, either anaerobically to ethanol and carbon dioxide or, aerobically, by the tricarboxylic acid cycle to carbon dioxide and water (Fig. 1) [3,4]. In 1940, Kluyster confirmed earlier reports that some yeasts could catabolize the component hexoses of certain disaccharides anaerobically, yet could only use those disaccharides aerobically [5]. He held that this effect, later called the Kluyster effect [6], was probably caused by the reversible anaerobic inactivation of some glycoside hydrolases, rather than by problems of cell permeability. However, his observations were made manometrically, with no enzymological investigation. So, up to 1945, it was possible to suggest that the Kluyster effect was consistent with the ‘direct fermentation’ of disaccharides, without preliminary hydrolysis [7]. Subsequent publications [3,8] (also without adequate experimental evidence) mooted that, for some yeasts, the anaerobic utilization of certain glycosides is blocked by the failure of their transport across the plasmalemma into the cytosol. An analogous cessation of trans-

Table 1

<table>
<thead>
<tr>
<th>Yeast</th>
<th>d-Galactose</th>
<th>Maltose</th>
<th>Cellobiose</th>
<th>Lactose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida utilis NCYC 737</td>
<td>-</td>
<td>K</td>
<td>K</td>
<td>-</td>
</tr>
<tr>
<td>Candida viswanathii CBS 4024</td>
<td>+</td>
<td>+</td>
<td>K</td>
<td>-</td>
</tr>
<tr>
<td>Debaryomyces castellii CBS 2923</td>
<td>K</td>
<td>+</td>
<td>+</td>
<td>K</td>
</tr>
<tr>
<td>Debaryomyces polymorphus CBS 4349</td>
<td>K</td>
<td>+</td>
<td>K</td>
<td>K</td>
</tr>
<tr>
<td>Kluyveromyces dolzhanski CBS 2104</td>
<td>+</td>
<td>+</td>
<td>K</td>
<td>-</td>
</tr>
<tr>
<td>Kluyveromyces fragilis NCYC 100</td>
<td>+</td>
<td>-</td>
<td>K</td>
<td>+</td>
</tr>
<tr>
<td>Kluyveromyces marxianus NCYC 111</td>
<td>+</td>
<td>-</td>
<td>K</td>
<td>K</td>
</tr>
<tr>
<td>Kluyveromyces thermotolerans NCYC 144</td>
<td>K</td>
<td>?</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Kluyveromyces wickerhamii CBS 2745</td>
<td>+</td>
<td>-</td>
<td>K</td>
<td>K</td>
</tr>
<tr>
<td>Saccharomyces cerevisiae CBS 1171</td>
<td>?</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Zygosaccharomyces fermentati CBS 4506</td>
<td>+</td>
<td>+</td>
<td>K</td>
<td>-</td>
</tr>
</tbody>
</table>

a Information from reference 6.
b , sugar not utilized; + , sugar used aerobically and anaerobically; K, sugar used aerobically only (i.e. gives the Kluyster effect).
port, on changing to anaerobic conditions, had been described [9] for *Rhodosporidium toruloides* (*Rhodotorula gracilis*). This non-fermenting yeast was reported to transport D-glucose actively under aerobic conditions, but not to take up that sugar anaerobically. In addition, a respiratory deficient mutant of *Saccharomyces pastorianus* (*Saccharomyces carlsbergensis*) was found to have acquired a much reduced rate of maltose uptake [10].

Evidence that a functioning respiratory chain can be necessary for uptake of disaccharides was reported in 1969 for the utilization of maltose, although in this case not by a yeast, but by the filamentous fungus, *Mucor rouxii* [11].

3. OBSERVATIONS BY YEAST TAXONOMISTS

In the 1970s, the late Tony Sims and I were working on aspects of sugar utilization by yeasts, and resolved to try to elucidate the physiological basis of the Kluyver effect. Systematic surveys, by taxonomists, of large numbers of strains of many species (for example, see refs. 1,12,13) show that the effect is widespread amongst the yeasts: out of a total of 229 yeast species that ferment D-glucose anaerobically, it was possible to list 97 yeast species, of which all the strains had been reported to show the Kluyver effect [6]. It was also clear that there was no obvious pattern of occurrence of the Kluyver effect; on the contrary, there was striking individuality amongst the yeasts in their responses to each substrate (see Table 1).

The main difficulty in interpreting the results of these taxonomic surveys arises from the tests not being quantitative [14,15]. Whilst the taxonomic tests for aerobic utilization of sugars are highly sensitive [1], so that exceedingly low rates of growth are recorded as ‘positive’, the tests for anaerobic fermentation, usually involving the use of Durham tubes, are remarkably insensitive [16]: ethanol production may be detected easily in yeasts that taxonomists have reported to be ‘non-fermentative’. Hence, it was necessary first to establish experimentally and unequivocally, for several yeasts, (i) that aerobic utilization occurred at a physiologically significant rate and (ii) that low, but physiologically significant, rates of anaerobic fermentation had not escaped notice.

4. RAPIDITY OF THE KLUYVER EFFECT: ACTIVATION/DEACTIVATION

*Candida utilis* was found to show the Kluyver effect with maltose (4-O-α-D-glucopyranosyl D-glucopyranose) and cellobiose (4-O-β-D-glucopyranosyl β-D-glucopyranose) and *Zygosaccharomyces fermentati* (*Saccharomyces montanus*) did so with cellobiose. Our most striking early observations were made when studying these utilizations with a carbon dioxide electrode: the increased rates of CO₂ output occurred only a few seconds after switching from anaerobic to aerobic conditions (Fig. 2) [6]. There was no corresponding change in activity of the appropriate glycosidase. So, from the start, (i) the rapidity of the
changes was suggestive of activation and deactivation (cf. refs. 17 and 18); (ii) these changes of activity did not apply to the glycoside hydrolases; (iii) there was no indication that the slower process of protein synthesis de novo, involved in induction or derepression, was responsible for the Kluyver effect. Further, the concept of the Kluyver effect was found applicable, not only to the disaccharides, maltose, lactose, cellobiose and \( \alpha, \alpha \)-trehalose, but also to \( \alpha \)-galactose [6] which, like the disaccharides, is converted to \( \alpha \)-glucose 6-phosphate, before glycolysis, without net oxidation [3]. However, most of the subsequent experiments were concerned with the utilization of common disaccharides; since the pathway to \( \alpha \)-glucose 6-phosphate involves hydrolysis and phosphorylation only, disaccharide utilization is peculiarly easy to study. Another relevant observation was that the Kluyver effect seemed to apply only to those glycosides hydrolysed within the confines of the plasmalemma and not to those hydrolysed by external enzymes, such as invertase [19]. This observation was consistent with the notion that transport had a role in the Kluyver effect.

The first direct evidence that the carrier (responsible for the entry of the sugar across the plasmalemma into the cell) was indeed deactivated, came from comparing the uptake of radioactively-labelled, non-metabolized analogues of \( \alpha \)-glucose and \( \alpha \)-galactose [6]. In \textit{Kluyveromyces thermotolerans} (\textit{Torulopsis dattila}), the rate of uptake, in anaerobic conditions, of 2-deoxy-\( \alpha \)-[1-\( ^3 \)H]glucose by glucose-grown cells was 97% of that in aerobic conditions; by contrast, 6% was the corresponding figure for uptake of \( \alpha \)-[1-\( ^3 \)H]fucose (6-deoxy-\( \alpha \)-galactose) by the same yeast grown on \( \alpha \)-galactose (Table 2). One problem with these experiments, however, is that the carrier itself is energy dependent. So, by supplying the yeast with non-metabolizable analogues, we were slowing, or even stopping, transport by depriving the yeast of its energy source [20]. We therefore developed a technique for measuring transport with chromogenic nitrophenolic glycosides [21].

Nonetheless, experiments were done on the transport of the non-metabolized analogue of lactose, methyl 1-thio-\( \beta \)-galactopyranoside (TMG) [22]. This transport was studied for strains of \textit{Debaryomyces polymorphus} and \textit{Kluyveromyces marxianus}, that show the Kluyver effect for lactose, and of \textit{Kluyveromyces fragilis}, that ferments lactose. Uptake under anaerobic conditions differed markedly from that occurring aerobically: TMG did not appear to be concentrated anaerobically, even by these strongly fermenting yeasts. Instead, we suggested that it was transported by

<table>
<thead>
<tr>
<th>Table 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rates of uptake of ( \alpha )-[1-( ^3 )H]fucose and 2-deoxy-( \alpha )-[1-( ^3 )H]glucose under aerobic and anaerobic conditions by \textit{Kluyveromyces thermotolerans} (NCYC 144) (^a)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Carbons source for growth of yeast</th>
<th>Condition</th>
<th>Inhibitor</th>
<th>Rate of uptake (nmol min(^{-1}) (mg dry wt(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \alpha )-Galactose</td>
<td>Aerobic</td>
<td>None</td>
<td>20.7</td>
</tr>
<tr>
<td></td>
<td>Anaerobic</td>
<td>None</td>
<td>4.8</td>
</tr>
<tr>
<td></td>
<td>Aerobic</td>
<td>( \alpha )-Galactose</td>
<td>1.28</td>
</tr>
<tr>
<td></td>
<td>Anaerobic</td>
<td>( \alpha )-Galactose</td>
<td>0.078</td>
</tr>
<tr>
<td>( \alpha )-Glucose</td>
<td>Aerobic</td>
<td>None</td>
<td>1.34</td>
</tr>
<tr>
<td></td>
<td>Anaerobic</td>
<td>None</td>
<td>0.84</td>
</tr>
<tr>
<td>( \alpha )-Glucose</td>
<td>Aerobic</td>
<td>None</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>Anaerobic</td>
<td>None</td>
<td>0.89</td>
</tr>
</tbody>
</table>

\(^a\) From reference 6.
facilitated diffusion (Fig. 3), which could sustain a rate of entry of over half the maximum rate observed aerobically. This difference, between the rates of aerobic and anaerobic transport into yeasts, may apply to glycosides in general: experiments on a strain of *Saccharomyces cerevisiae*, that does not show the Kluyver effect, gave comparable results for its uptake of maltose [22]. Furthermore, *D. polymorphus* transports lactose, and also its analogue, 4-nitrophenyl β-D-galactopyranoside (4NPβGal), at least ten times faster aerobically than anaerobically [23]. In this respect, cells of *D. polymorphus* treated with antitoxin A, an inhibitor of the electron transport chain, behaved like cells made anaerobic. The aerobic rate of transport was restored to such inhibited cells, by adding ascorbate and diamino-durene, indicative that ATP synthesis from glycolysis provided insufficient energy for transporting the β-galactosides [23].

Inhibitors of the proton pump of the plasmalemma [24] each greatly reduced the rates of anaerobic transport of 4-nitrophenyl α-D-glucopyranoside by *S. cerevisiae*, *N,N*-dicyclohexyl-carbodiimide (DCCD) more than 40-fold [21]. This observation was consistent with the involvement of proton symport in anaerobic, as well as aerobic, transport of glycosides, rather than facilitated diffusion, as we had suggested previously [22]. Van den Broek and his colleagues [25] also obtained evidence of anaerobic β-galactoside uptake into *K. marxianus* by proton symport and also of the limitation of the rate of supply of ATP as a critical cause of slow anaerobic transport.

### Table 3

<table>
<thead>
<tr>
<th>Yeast name and strain number</th>
<th>Carbon source for growth</th>
<th>Pyruvate decarboxylase</th>
<th>Alcohol dehydrogenase</th>
<th>Glycosidase</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Candida viswanathii</em> CBS 4024</td>
<td>d-Glucose</td>
<td>0.13</td>
<td>1.04</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Maltose</td>
<td>0.11</td>
<td>0.32</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>Cellobiose</td>
<td>0.039</td>
<td>0.23</td>
<td>0.33</td>
</tr>
<tr>
<td><em>Saccharomyces cerevisiae</em> CBS 1171</td>
<td>d-Glucose</td>
<td>1.62</td>
<td>0.57</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Maltose</td>
<td>0.40</td>
<td>0.64</td>
<td>1.01</td>
</tr>
</tbody>
</table>

*a* Expressed as nmol substrate catalysed min⁻¹ (mg protein)⁻¹.  
*b* Results from reference 26.  
*c* Yeasts grown on maltose were tested for α-glucosidase activity; *C. viswanathii* grown on cellobiose was tested for β-glucosidase activity.  
*d* *C. viswanathii* gives the Kluyver effect with cellobiose.
5. KLUYVER EFFECT NOT EXPLAINED SOLELY BY CHANGES IN TRANSPORT

By 1982, it could no longer be held that the Kluyver effect was explicable simply in terms of failure of transport. Uptake was not abolished, only greatly slowed down. We therefore attempted to approach the problem genetically by making mutants and comparing these physiologically with the wild types. However, all attempts to make the required mutants failed [26]: we could neither (i) make mutants that showed the Kluyver effect from wild-type strains that did not, nor, conversely, could we (ii) make mutants that did not show the effect from strains that did so. Accordingly, it was necessary to use another physiological approach and investigate the possibility of some inhibitory action of glycosides, that produce the Kluyver effect, on the pathway from pyruvate to ethanol.

6. LEVELS OF ENZYMIC ACTIVITY AND THE KLUYVER EFFECT

The pathway from pyruvate to ethanol involves pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH) (Fig. 1), so the activities of these enzymes and the relevant glycosidases were measured [27] for six species of yeast, only one of which (S. cerevisiae) did not show the Kluyver effect. When grown on a glycoside with which it gave the Kluyver effect, each yeast had much less PDC activity than when grown on D-glucose or another glycoside [27]. The example of Candida viswanathii is given in Table 3. There was no consistent corresponding lowering of activity of either ADH, or of the pertinent glycosidase. Hence, we concluded [27], PDC may play a part in producing the Kluyver effect and, accordingly, we investigated this rôle in a strain of C. utilis [28].

7. THE ROLE OF PYRUVATE DECARBOXYLASE

In a second paper published in 1991 [28], we described measurements, made aerobically and anaerobically, of β-glucoside transport and hydrolysis and pyruvate decarboxylase activities of C. utilis. Transport was five-fold faster aerobically, but there was no corresponding difference in β-glucosidase activity. PDC activity varied greatly, being synthesized de novo in response to the presence of D-glucose and anaerobic conditions; the PDC, already shown (for S. cerevisiae) to have allosteric properties [29], was about 50% deactivated on removal of glucose or addition of air (Fig. 4). The activation and deactivation of PDC were rapidly reversible and ethanol production was highly associated with PDC activity.

8. SUMMING UP: CAUSES OF THE KLUYVER EFFECT

Experiments on a very few yeasts now make it possible to make tentative suggestions about the
physiological mechanisms that are responsible for the Kluyver effect.

8.1. Transport

On switching from aerobic to anaerobic conditions, the rate of uptake of sugars may be reduced many times. This has been found for the entry of maltose into *S. cerevisiae* and of β-D-galactopyranosides into *D. polymorphus, K. fragilis* and *K. marxianus* [22,23,25]. Such an alteration may also be accompanied by the uptake system acquiring a lower affinity for the substrate [21].

8.2 Glycosidases

Although there is no evidence of a decline of glycosidase activity when yeasts become anaerobic [27], the substrate affinities of yeast cytosolic glycosidases are notably low, often with half-saturation constants of about 50 mM or more [28], compared with, for example, those of hexokinases, of about 0.1 mM [30]. Hence, in order to maintain glycolytic flux, the concentration of glycoside in the cytosol, achieved by the transport carrier, could be critical.

8.3 Pyruvate decarboxylase

Accordingly, the glycolytic flux may be very low as a result of a combination of (i) the carrier’s response to anaerobiosis, leading to a low concentration of glycoside in the cytosol and (ii) the low affinity of the glycosidase for its substrate. Hence, this diminution of the rate of glycolysis may lead to the rapid deactivation of PDC, as observed on removing glucose from anaerobic *C. utilis*. Such a chain of events may well bring about the Kluyver effect. As well as the slower controls of induction, repression and derepression, providing oxygen to an anaerobic yeast can produce fast changes, such as the deactivation of pyruvate decarboxylase and the provision of an energy supply for transport. Conversely, while switching to anaerobic conditions activates PDC, transport is severely affected by a reduction in the supply of ATP, so PDC activation fails because of the much reduced glycolytic flux.

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REFERENCES