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plenary lecture

The Contribution of Taxonomists to the Understanding of Yeast Nutrition

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Summary

Taxonomists make important and unique contributions to comparative biology by describing large numbers of organisms in detail. Such comparative observations have stimulated research on the physiology and biochemistry of yeast nutrition. Three examples follow. (1) From fermentation tests of Stelling-Dekker, Kluyver concluded that no yeast ferments any sugar unless it ferments glucose and all yeasts that ferment glucose also ferment fructose and mannose. Kluyver made these generalizations in 1931, but the biochemical explanations for them were not fully understood until years later, when the main reactions of phosphorylated sugars and their derivatives in glycolysis were elucidated. (2) Results of aerobic growthtests, by van Uden and Kreger-van Rij, indicated the pathways by which yeasts catabolize pentoses, such as D-*xylose, and* L-*arabinose. (3) Current research on the mechanism of the Kluyver effect, first described in one or two species in 1940, has followed from analysing the results of taxonomists' growth and fermentation tests. These showed the effect to occur in about 20% of yeast species and to apply to glycosides that are hydrolysed in the cytosol and have thrown light on the energy requirements of glycoside uptake.*

Accordingly, research workers concerned with the biology, physiology and biochemistry of yeasts, for academic or commercial reasons, should ensure the continuance and improvement of nutritional testing by yeast taxonomists.

Keywords: Yeast taxonomy and nutrition, sugar catabolism

Introduction

Since taxonomists are some of the few biologists who describe large numbers of different kinds of organism, they make almost unique contributions to comparative biology. This may well be the most important part of taxonomists' work. It is in addition to the business of classification and nomenclature, which is often concerned with legalistic issues (such as priority and validity) rather than scientific problems. However, the methods of molecular biology are becoming more extensively applied to classifying and identifying yeasts, so, in the next few years, phenotypic studies by yeast taxonomists may be much reduced and this would slow down the development of yeast biology.

Three examples are discussed below of taxonomists' work that has stimulated research in the biochemistry of nutritional physiology. These examples concern sugar utilizations. Two derive from the interests of the Dutch microbial biochemist, A. J. Kluyver, and concern hexose and hexoside utilizations. The third example is from studies of pentose catabolism.

Hexose metabolism

In 1928, Guilliermond *(1)* published a major work on yeast taxonomy. The descriptive characteristics he used were cell shape, mode of formation of asci, number of ascospores, formation of filaments, arthrospores, red pigment and pellicles. Three years later, Stelling-Dekker's taxonomic monograph *(2)* described 160 ascospore-forming yeast species. She was working in Delft and, influenced by Kluyver, used the additional criteria of yeasts' ability to ferment (semi-anaerobically) several sugars, namely, D-glucose, D-fructose, D-mannose, D-galactose, sucrose, maltose and lactose.

Kluyver *(3)* made the following comment on Stelling-Dekker's results:[1]

*... la distribution du pouvoir fermentatif des diverses levures visàvis des différents sucres n'est pas tout à fait capricieuse, comme la plupart des auteurs à ce sujet, nous laissent croire.**

He drew two conclusions. First, all yeasts that ferment any sugar, ferment glucose, fructose and mannose. Secondly, no yeast can ferment both maltose and lactose. The significance of the first of these observations was not understood at the time and the need to explain it gave added impetus to subsequent biochemical and physiological findings. It was not until later in the 1930s that the main reactions of phosphorylated sugars in glycolysis were worked out *(4,5)* (Table 1); the specificity of yeast hexokinase for D-glucose, D-fructose and D-mannose was not published until the 1940s *(13)*.

Table 1. Recognition of phosphorylated glycolytic intermediates: approximate dates

Date	Intermediate
1933	D-Glycerate 3-phosphate *(6,7)*
	D-Fructose 1,6-bisphosphate *(6,7)*
	D-Fructose 6-phosphate *(8)*
	D-Glucose 6-phosphate *(8)*
1934	Phosphoenolpyruvate *(9)*
	Dihydroxyacetone phosphate *(9)*
1935	D-Glycerate 2-phosphate *(10)*
1936	D-Glyceraldehyde 3-phosphate *(11)*
1939	D-Glycerate 1,3-bisphosphate *(12)*

Even today, for wild type yeasts, there seems to be no unequivocal exception to Kluyver's statement *(3)* that no yeast can ferment both maltose and lactose; nor does there seem to be any *biochemical or physiological* explanation. Perhaps molecular biology will be able to provide one in the near future.

Two pentoses: L-arabinose and D-xylose

In the 1940s, Siegfried Windisch (in Munich) made many taxonomic studies on yeasts of the Cryptococcaceae that were relevant to the food industry, testing their abilities to grow aerobically on L-arabinose and D-xylose. This work was done before either Wickerham *(14)* or Kudryavtsev *(15)* had published their use of these compounds for discriminating between species. Windisch *(16)* made the remarkable observation that L-arabinose is never used by yeasts that do not also use D-xylose.

This observation was confirmed nearly 20 years later by analysing *(17)* results of tests done, by van Uden and his colleagues at Oeiras, on 43 yeasts for taxonomic purposes. There were no exceptions. Then, in the 1970s, further analysis, this time of Nel Kreger-van Rij's tests, gave only two possible exceptions out of 496 yeasts of many genera *(18)* (Table 2). This analysis gave the first evidence that most yeasts, which catabolize L-arabinose or D-xylose, do so by the routes described for *Penicillium chrysogenum* by Chiang and Knight in 1964 *(19)* (Fig. 1).

Table 2. Abilities of 496 yeast strains of many species to utilize both D-xylose and L-arabinose for aerobic growth *(18)*

		L-arabinose +	L-arabinose –
D-xylose	+	145	143
	–	2	206

+, Pentose utilized; –, pentose not utilized. Figures in each square give numbers of strains; thus, only 2 of the strains that utilized L-arabinose were reported not to utilize D-xylose.

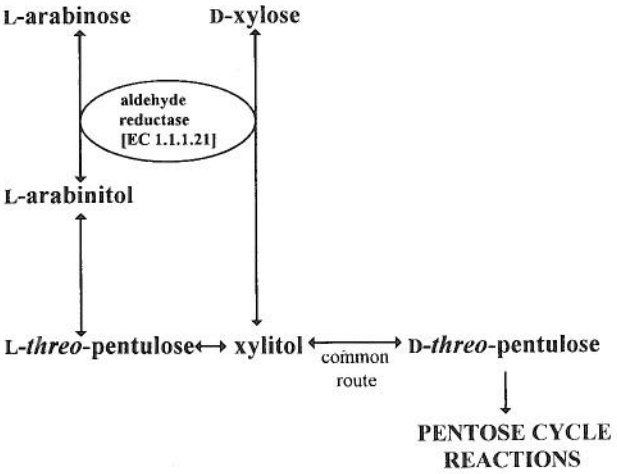


Fig. 1. Routes of L-arabinose and D-xylose catabolism in yeasts

At first sight, the association between the 2 utilizations, of L-arabinose and D-xylose seemed inexplicable. The explanation lay, first, in the wide specificity of the aldehyde reductase [EC 1.1.1.21] responsible for the initial reductions of both L-arabinose (to L-arabinitol) and D-xylose (to xylitol) and, secondly, in the shared catabolic pathway from xylitol through the reactions of the pentose cycle (Fig. 1).

Kluyver effect: anaerobic utilization of disaccharides

In 1940, Kluyver *(20)* confirmed earlier reports that some yeasts could use certain disaccharides aerobically, but not anaerobically, although able to use the component hexoses of those disaccharides anaerobically. One example, shown in Table 3, was that of maltose utilization by *Pichia jadinii (Candida utilis)*.

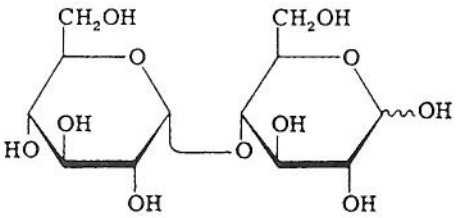
What is remarkable about this phenomenon? Maltose is a glucose-glucose disaccharide (Fig. 2) and the first step in its catabolism is its hydrolysis to two mole-

[1] the distribution of fermentative abilities of the various yeasts with respect to different sugars is not at all capricious, as most authors would have us believe

Table 3. The Kluyver effect shown by *Pichia jadinii* for maltose. The utilization of both substrates by *Saccharomyces cerevisiae*, which does not give the Kluyver effect, is indicated in contrast

	D-Glucose		Maltose	
	Aerobic	Anaerobic	Aerobic	Anaerobic
Saccharomyces cerevisiae	+	+	+	+
Pichia jadinii (Candida utilis)	+	+	+	–

+, sugar utilized; –, sugar not utilized



Maltose
4-O-α-D-Glucopyranosyl-
D-glucopyranose

Fig. 2. The structure of maltose

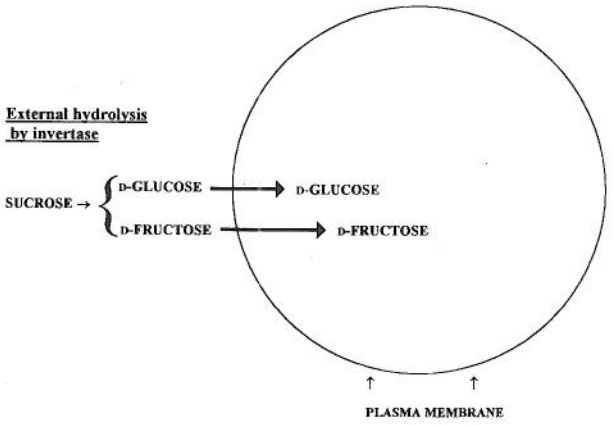
cules of glucose. However, since *P. jadinii* uses glucose anaerobically and maltose hydrolysis does not involve oxidation, there was no obvious reason why the yeast should not use maltose anaerobically too. Furthermore, the same phenomenon was found for other sugars and taxonomists' observations, such as those of Nel Kregervan Rij, showed it to be widespread amongst the yeasts, having been reported for about 20% of all species *(21)*.

It has long been known that certain sugars, such as sucrose, raffinose and melibiose, are hydrolysed externally to the plasma membrane *(22, 23)*. And the taxonomists' findings indicated that yeasts were rarely, if ever, reported to show the Kluyver effect for raffinose or melibiose. However, a number showed the Kluyver effect for sucrose.

Saccharomyces cerevisiae characteristically hydrolyses sucrose, by means of invertase (β-fructosidase), externally to the plasma membrane (in the periplasmic space). However, sucrose is a double glycoside, both a β-fructoside and an α-glucoside and, in certain invertase-negative mutants *(24)*, sucrose is hydrolysed by means of a cytosolic α-glucosidase (Fig. 3). Hence, failure of transport of the substrate into the cell seems to be an important factor in producing the Kluyver effect *(25)*. And, indeed, there is much evidence that this is so. For example, the rate of transport of several sugars into *Debaryomyces polymorphus* is much lower under anaerobic than aerobic conditions *(26)* (Table 4); this is especially so with lactose, for which *D. polymorphus* shows the Kluyver effect. Moreover, both *Debaryomyces yamadae (27)* and *Candida albicans (28)*, which give the Kluyver effect with sucrose, have now been found to hydrolyse sucrose by intracellular α-glucosidases. This effect may be

Table 4. Aerobic and anaerobic transport rates: *Debaryomyces polymorphus*. Results of Schulz and Höfer *(26)*

1 mM Substrate	Uptake rate (nmol/min · mg dry wt yeast)	
	Aerobic	Anaerobic
D-Galactose	44	7.0
D-Glucose	51	5.0
Lactose	1.4	0.1



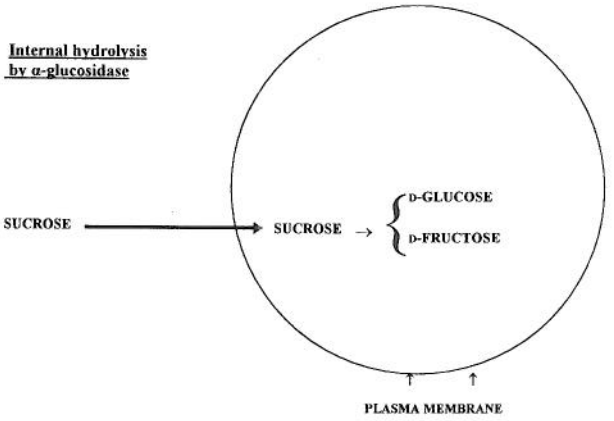


Fig. 3. External hydrolysis of sucrose by invertase and internal hydrolysis by α-glucosidase

explained, at least in part, if the uptake of glycosides usually requires more energy than is supplied by glycolysis alone.

Let us consider only yeasts that ferment D-glucose anaerobically and which use certain glycosides aerobically, hydrolysing them in the cytosol. Results of tests show that only a minority of such yeasts ferment those glycosides anaerobically. For example, lactose (a β-galactoside) is only fermented by about 6% of such yeasts. The corresponding figure is about 25% for melibiose (an α-galactoside), which is usually hydrolysed outside the plasma membrane. The approximate figure for cellobiose (a β-glucoside, usually hydrolysed cytosolically) is 9%; amongst these cellobiose-fermenting species is *Candida wickerhamii*, which is exceptional in its external hydrolysis of cellobiose *(29)*. It would be interesting to see

whether *Debaryomyces castellii*, another cellobiose fermenter, also produces an external β-glucosidase.

Conclusion

The introduction of large numbers nutritional tests for classifying and identifying yeasts, by StellingDekker *(2)*, Lodder *(30)*, Wickerham *(14)* and Kudryavtsev *(15)* between 1930 and 1960, led to major advances in knowledge of yeast physiology and biochemistry. As a consequence, the modes of utilization of various pentoses, hexoses, glycosides and alditols are better understood for many species *(3, 25, 31)*. This improved understanding depended on thousands of tests made by taxonomists.

Moves to diminish the number of nutritional tests are not astonishing, in view of the recent advances in using nucleic acid analyses for classifying and identifying yeasts. Such analyses are already used for assessing evolutionary relationships and for identifying yeasts rapidly *(32)*. Indeed, Kreger-van Rij's 1984 monograph *(33)* already gave results of far fewer tests than were to be found in the previous edition *(34)*. Considerable cost and effort goes into doing between 50 and 100 nutritional tests on each strain.

The tests, themselves, have considerable limitations *(30, 35, 36)*. The fermentation tests are insensitive since, when CO_2 production is slow, the gas may diffuse into the atmosphere, without forming visible bubbles in the medium. (Schwann pointed this out in 1837! *(37)*). So, although positive results are reliable, negative results have little meaning. Conversely, the aerobic growth tests are highly sensitive, so that a positive test result does not distinguish between very fast or insignificant growth rates.

Even using these inadequately designed tests, taxonomists have made major contributions towards understanding yeast biology. So, rather than rejecting the tests, taxonomists should improve them and continue their rôle as important contributors to comparative biology, describing the characteristics of each species as fully as practicable.

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Doprinos taksonoma u razumijevanju ishrane kvasca

Sažetak

Opisujući iscrpno veliki broj organizama, taksonomičari su uvelike pridonijeli komparativnoj biologiji. Ta komparativna opažanja potaknula su istraživanja fiziologije i biokemije ishrane kvasca. Navedena su tri primjera. (1) Na osnovi fermentacijskih testova Stelling-Dekkera, Kluyver je zaključio da nijedan kvasac ne fermentira neki šećer ako ne fermentira glukozu te da svi kvasci koji fermentiraju glukozu fermentiraju fruktozu i manozu. Kluyver je tu postavku objavio 1931. godine, a biokemijsko objašnjenje nađeno je mnogo godina poslije kada su bile otkrivene glavne reakcije fosforiliranih šećera i njihovih derivata u glikolizi. (2) Rezultati aerobnih testova rasta van Udena i Kreger-van Rija upućivali su na biokemijske procese kojima kvasci kataboliziraju pentoze, kao što su D*-ksiloza i* L*- arabinoza. (3) Današnja istraživanja mehanizma Kluyverova učinka, prvotno opisanog s jednom ili dvije vrste tijekom 1940. godine, uslijedila su na osnovi rezultata analiza koje su taksonomi proveli ispitujući rast i fermentaciju. Pokazali su da se Kluyverov učinak pojavljuje u približno 20% vrsta kvasaca, a odnosi se na glikozide što se hidroliziraju u citosolu te upućuje na energetske potrebe pri ugradnji glikozida u stanicu. Stoga istraživači koji se bave biologijom, fiziologijom i biokemijom kvasaca iz akademskih ili praktičnih razloga, trebaju osigurati taksonomima nastavak i unapređenje testiranja ishrane kvasaca.*