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# ENZYMOLOGICAL INTERPRETATION OF UTILIZATION PATTERNS OF SUGARS USED IN YEAST TAXONOMY

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## Introduction

In yeast taxonomy morphological and physiological-biochemical properties are used for characterization of species. As physiological-biochemical properties in the first place assimilation and fermentation of different sugars are taken into consideration (Stelling-Dekker, 1931; Lodder, 1934; Diddens and Lodder, 1942; Lodder and Kreger- van Rij, 1952; Kudriavzev, 1954, 1960; Novák and Zsolt, 1961). According to the general practice assimilation and fermentation of six sugars (diagnostic sugars) are investigated with similar methods. The sugars and the symbols of them used in this paper are the following ones:

| | | |
|---|---|---|
| glucose: D | sucrose: S | lactose: L |
| galactose G | maltose: M | raffinose: R |

The use of symbols and formulas representing physiological-biochemical properties was several times proposed in yeast taxonomy too (e.g. Rieth, 1958; Rieth and Schönfeld, 1959; Novák, 1960 b; Vörös-Felkai and Novák, 1960, Artagaveytia-Allende, 1961).

The diagnostic experiments show presence or absence of assimilation and fermentation of these sugars and these data are considered as properties of the investigated organisms.

Usefulness of this method is evident. Results obtained on the basis of the monographs of the Dutch School are the best proof of it. But at the same time several problems arose. The experimental results are not unambiguous; the same assimilation (growth in the presence, as sole carbon source, of the sugar investigated) and fermentation (gas production in the presence of the sugar investigated) may have a different enzymatic basis. E.g. $^1/_3$ raffinose may be fermented on two ways: 1. $\beta$-h-fructosidase splits off fructose (melibiose remains unfermented) and 2. $\alpha$-galactosidase splits off galactose (sucrose remains untouched). A manometric test shows in both cases fermentation of $^1/_3$ raffinose. It is obvious that to design identically these two cases would be incorrect. Chromatographic control is necessary to determine the type of raffinose splitting (Novák, 1960 a).

Ability to utilize (by assimilation or/and fermentation) a sugar is very important for the organism. It determines the possibility of occurence in a medium and influences competition with other organisms. Kudriavzev (1954, 1960) called emphatically attention to the correlation between the sugars of the substrates and the sugar utilization ability of yeasts occurring in them. Adaptation to the environment is an important factor in evolution. Kudriavzev's comments, as demonstrations of a general connection on special objects, are both general biologically and yeast taxonomically of high value. Naturally, different ways of adaptation to a given sugar are possible. E.g. sucrose splitting may be performed by a $\beta$-h-fructosidase, an $\alpha$-glucosidase etc.

Yeast taxonomists characterize their strains by the results of the diagnostic experiments. Enzymological evaluation of these experiments is in general lacking although positivity or negativity of the diagnostic experiments are only expressions of the enzymes of the organisms; the enzymes are the material basis for the physiological-biochemical properties.

On the basis of utilization of different sugars one may conclude the presence of some enzymes. E.g. the Embden-Meyerhof-Parnas (EMP) way is probably generally occurring in yeast fermentations. This means that fructose fermentation needs ATP ⟶ hexose transphosphatase, fructose and glucose fermentation needs, apart from this, phosphogluco-isomerase too while fructose, glucose and galactose fermentation needs also the enzymes of the galacto-waldenase complex (ATP ⟶ galactose transphosphatase, phosphogalactose-isomerase and glucose- [1 ⟶ 6] -phosphomutase) to say nothing of the permeases.

In this paper authors try to make such evaluations on the basis of literary and own data. They consider worth trying these theoretical deductions because, due to methodical difficulties, direct demonstration of the different enzymes will be not possible still for a long time, at least in case of several hundred species and many thousand strains.

## Biochemical interprepation of the simple sugar utilization spectra

Authors think that the data on utilization of the six diagnostic sugars concerning about 300 species collected in their previous work

TABLE I. Sugar utilization combinations described

| | assimilation | fermentation | | assimilation | fermentation |
|---|---|---|---|---|---|
| D | + | + | $DGSR_3$ | (+)* | + |
| DG | + | + | DGML | + | + |
| DS | + | + | $DGMR_1$ | + | + |
| DM | + | + | $DGLR_1$ | + | — |
| DGS | + | + | $DSM_1R$ | + | + |
| DGM | + | + | $DSMR_2$ | (+)* | + |
| DGL | + | + | DGSML | + | — |
| $DGR_1$** | + | + | $DGSM_1R$ | + | + |
| DSM | + | + | $DGSMR_2$ | (+)* | + |
| $DS_1R$** | + | + | $DGSMR_3$ | + | + |
| DGSM | + | + | $DGSL_1R$ | + | + |
| DGSL | + | — | $DGSML_1R$ | + | + |
| $DGS_1R$ | (+)* | + | $DGSMLR_3$ | + | — |

* Raffinose assimilation was mostly not determined quantitatively and so one can not establish the combination to which the description belongs. In most cases the proportion of utilization could be deduced on the basis of enzymological considerations.

** $_1R$ means raffinose splitting into fructose and melibiose and utilization of fructose; $R_1$ means raffinose splitting into galactose and sucrose and utilization of galactose; $R_2$ means a raffinose splitting into glucose, fructose and galactose and utilization of glucose and fructose; $R_3$ means utilization of the hole raffinose molecule.

(Zsolt and Novák, 1961; Novák and Zsolt, 1961; the nomenclature of these works will be used) may serve a reliable basis for their deductions. The same way will be followed on which authors obtained, by trial and error, their results.

TABLE II. Combinations combinatorically deduced*

| | | | |
|---|---|---|---|
| D | $DSR_1$ | $DGMR_1$ | DGSML |
| DG | $DSR_2$ | $DGMR_2$ | $DGSMR_1$ |
| DS | $DSR_3$ | $DGMR_3$ | $DGSMR_2$ |
| DM | DML | $DGLR_1$ | $DGSMR_3$ |
| DL | $DMR_1$ | $DGLR_2$ | $DGSLR_1$ |
| $DR_1$ | $DMR_2$ | $DGLR_3$ | $DGSLR_2$ |
| $DR_2$ | $DMR_3$ | DSML | $DGSLR_3$ |
| $DR_3$ | $DLR_1$ | $DSMR_1$ | $DGMLR_1$ |
| DGS | $DLR_2$ | $DSMR_2$ | $DGMLR_2$ |
| DGM | $DLR_3$ | $DSMR_3$ | $DGMLR_3$ |
| DGL | DGSM | $DSLR_1$ | $DSMLR_1$ |
| $DGR_1$ | DGSL | $DSLR_2$ | $DSMLR_2$ |
| $DGR_2$ | $DGSR_1$ | $DSLR_3$ | $DSMLR_3$ |
| $DGR_3$ | $DGSR_2$ | $DMLR_1$ | $DGSMLR_1$ |
| DSM | $DGSR_3$ | $DMLR_2$ | $DGSMLR_2$ |
| DSL | DGML | $DMLR_3$ | $DGSMLR_3$ |

* Presupposition was the obligatory presence of glucose in each combination. The possibility that in the case of lacking glucose utilization other sugars may be utilized, here was not taken into consideration because this was not yet observed among yeasts. Between ${}_1R$ and $R_1$ was made no distinction.

TABLE III. Combinations not yet observed

| | | |
|---|---|---|
| DL | $DLR_1$ | $DMLR_1$ |
| $DR_1$ | $DLR_2$ | $DMLR_2$ |
| $DR_2$ | $DLR_3$ | $DMLR_3$ |
| $DR_3$ | $DGSR_2$ | $DGSLR_2$ |
| $DGR_2$ ?* | $DGMR_2$ ?* | $DGSLR_3$ |
| $DGR_3$ ?* | $DGMR_3$ ?* | $DGMLR_1$ |
| DSL | $DGLR_2$ | $DGMLR_2$ |
| $DSR_2$ ?**** | $DGLR_3$ | $DGMLR_3$ |
| $DSR_3$ ?** | DSML | $DSMLR_1$ |
| DML | $DSMR_3$ | $DSMLR_2$ ?**** |
| $DMR_1$ | $DSLR_1$ | $DSMLR_3$ ?** |
| $DMR_2$ | $DSLR_2$ | $DGSMLR_2$ ?*** |
| $DMR_3$ | $DSLR_3$ | |

?*, ?**, ?***, ?****

The combinations for assimilation and fermentation are here summarized; the lack of combinations designated with mark of interrogation may be deduced only logically.

Combinations marked with * can have $R_2$ and $R_3$ only with invertase, but this would involve sucrose utilization too. In combinations marked with ** $R_3$ would be possible only when galactose utilization is also positive. In combinations marked with *** galactose positivity is incompatible with $R_2$; it involves $R_3$! Lack of the combinations marked with **** will be proved later. 1 ${}_1R$ and $R_1$ were not distinguished.

The sugar utilization combinations observed and described till now are collected in Table I, in Table II the sugar utilization combinations which may be deduced with the aid of the theories till now are demonstrated.

Comparing the two Tables it emerges that some of the deduced combinations are not yet described; these are collected in Table III.

One may attempt to give an enzymatical explanation for the lack of these combinations. However, simply supposition absence of „agluconspecificity" cannot solve the problem. In this case only combinations collected in Table IV would remain.

Being unable to obtain this way the described combinations, the following suppositions were chosen as start:

a) Disaccharides and raffinose are split only by hydrolysis to monosaccharides and enter this way the metabolism.

b) The presence of the following hydrolytical enzymes is supposed: lactase ($\beta$-galactosidase), melibiase ($\alpha$-galactosidase), maltase ($\alpha$-glucosidase), and invertase ($\beta$-h-fructosidase).

c) Galactose supplied as such or split off from oligosaccharides is transformed into glucose-6-phosphate by the galactowaldenase complex.

d) Lactose splitting occurs only in yeasts able to perform the galactose $\longrightarrow$ glucose transformation too. In other words: appearance of lactose splitting does not precede glactose $\longrightarrow$ glucose transformation, at most they appear simultaneously. Rogosa (1948) observed that a yeast strain adapted to lactose became adapted at the same time also to galactose. No description is known in which a yeast utilizing lactose does not utilize galactose too. Pardee (1957) observed induction of $\beta$-galactosidase synthesis by lactose and melibiose alike.

e) In case of raffinose the induction of $\alpha$-galactosidase is similarly connected with the synthesis of the enzymes of the galactose $\longrightarrow$ glucose

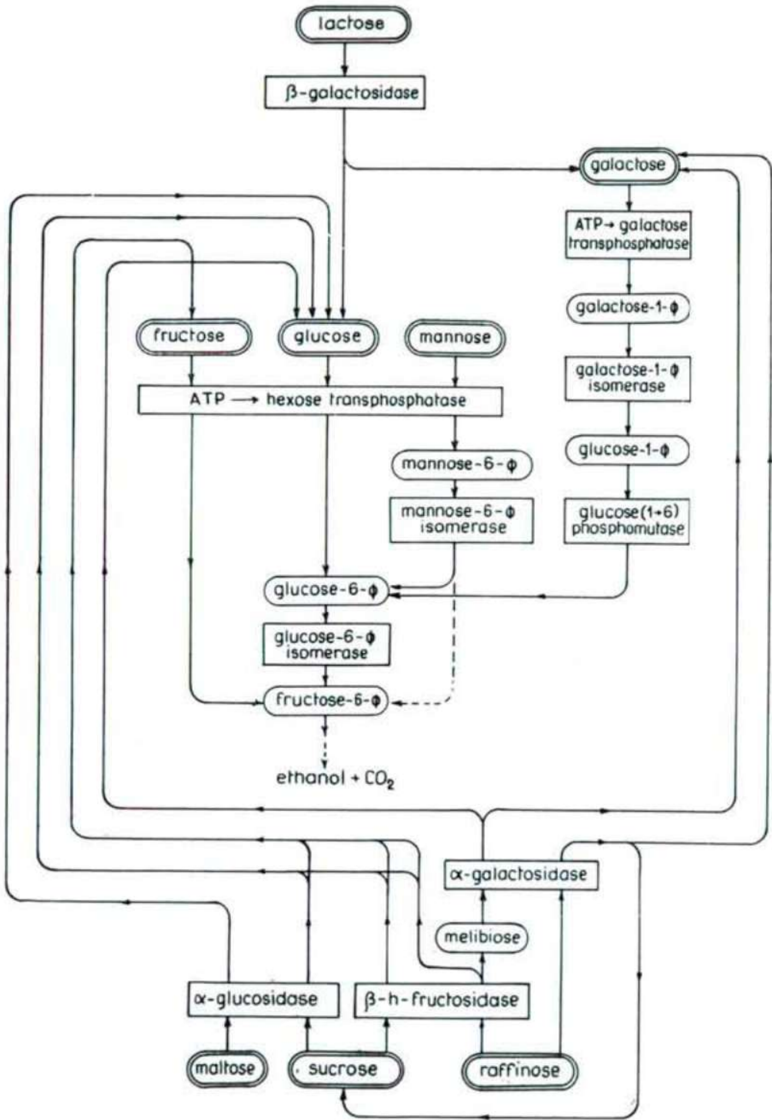
TABLE IV. Combinations remaining when ,,aglucon-aspecific''* glucosidases are supposed

| | |
|---|---|
| D | $DSLR_1$ |
| DG | $DSLR_2$ |
| DL | DGSML |
| DGL | $DGSMR_1$ |
| $DGR_1$ | $DGSMR_3$ |
| $DSR_1$ | $DGSLR_1$ |
| $DSR_2$ | $DGSLR_3$ |
| $DGSR_1$ | $DSMLR_1$ |
| $DGSR_3$ | $DSMLR_2$ |
| $DGLR_1$ | $DGSMLR_1$ |
| DSML | $DGSMLR_3$ |

* „Aglucon aspecificity" relate to $\alpha$-glucosidase (which splits maltose and sucrose alike) and to $\beta$-h-fructosidase (which splits raffinose and sucrose as well). It must be noted thatwhen raffinose is split by melibaise instead of invertase, the combination must contain G because in this case the galactose part of raffinose is utilized ($DGR_1$, $DGLR_1$). ${}_1R$ and $R_1$ are not distinguished.

PLATE I. Metbolic pathways of the diagnostic sugars.

PLATE I



transformation. No yeasts were described with a $^{3}/_{3}$ raffinose fermentation, which do not ferment also the galactose supplied separately.

The processes and enzymes mentioned above are summarized in Plate I.

In authors' first deduction (Figure 1) beginning with the simplest case (utilization of glucose alone) sugar utilization combinations were deduced by adding one by one the enzymes supposed with the restriction that galactosidases must be preceded by the galactowaldenase complex. In this first deduction also raffinose splitting ability of melibiase and invertase was taken into consideration.

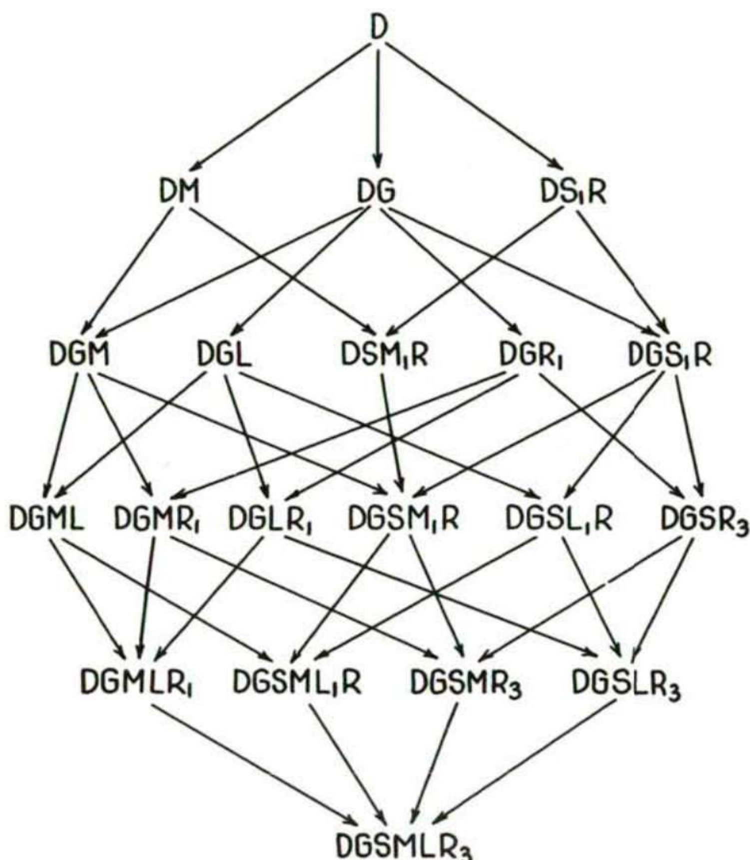



Figure 1. First deduction of the sugar utilization combinatons. Sucrose splitting ability of maltase was here not supposed.

As may be seen, the combinations obtained do not correspond to the combinations observed.

Thereafter it was supposed that maltase ($\alpha$-glucosidase) is not strictly specific and can split sucrose too. This second deduction is seen in Figure 2, and 2 a.

Although the second deduction produced combinations lacking in the first one, some combinations from the first deduction were missing. After all, nor this deduction gave a satisfactory result. (It is remarkable that some combinations present themselves on an enzyme-level earlier. Due to the sucrose splitting of maltose $^{3}/_{3}$ raffinose may be utilized without invertase if splitting begins by melibiase at the galactose end of the molecule.)

Drawing together the two deductions facultativity of sucrose splitting by maltase is supposed. This was suggested by the results of Kosikov et al. (1956) about adaptive sucrose splitting of maltase. Nor this third

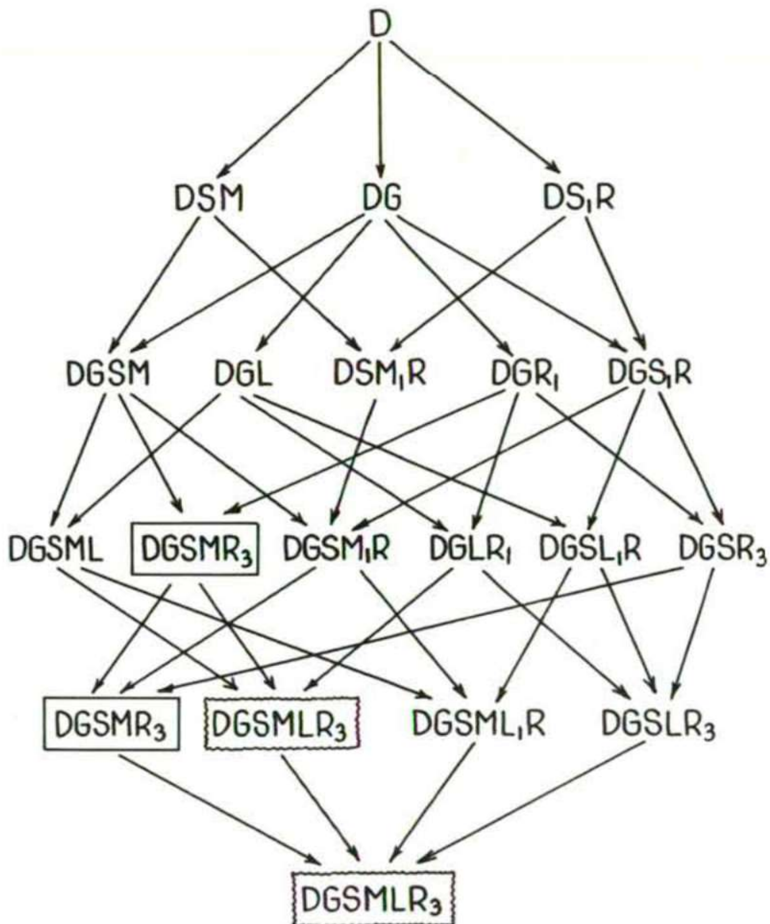



Figure 2. Second deduction of the sugar utilization combinations. Maltase was supposed to split maltose and sucrose alike. Formulas with the same framing emphasize identical utilization; due to sucrose splitting by maltase some combinations appear earlier in the deduction. Adding invertase to these combinations do not change them, only the enzymatical basis will be altered.

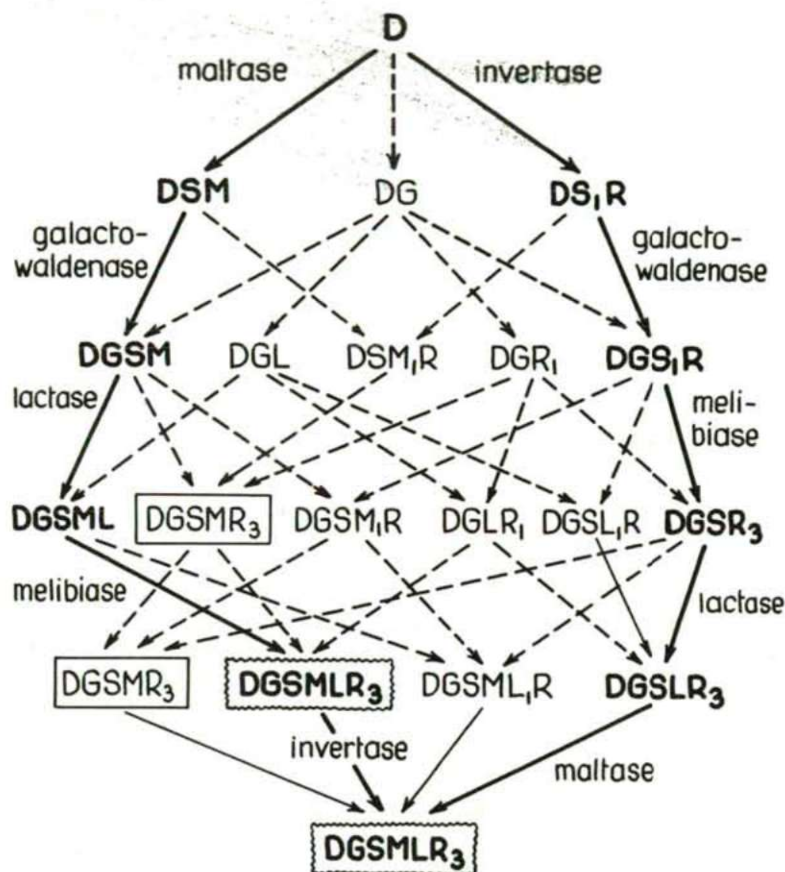



Figure 2/a. An explanation to Figure 3.

deduction represented in Figure 3 is satisfactory. Among others, it does not contain the combination DS.

As a new supposition was introduced the facultativity of raffinose splitting by invertase. This fourth deduction (Figure 4) seems, already conforming to the empirical data. In Table V the combinations of the four deductions are compared with the combinations described. Discrepancies between the combinations of the fourth deduction and the empirical combinations are explained as follows:

1) Reality of combination 18 ($DSMR_2$) is questionable. Only three species were characterized with this combinaton: *Schizosaccharomyces versatilis, Saccharomyces pastorianus, Zymodebaryomyces castellii.* One of the authors (N o v á k, 1959, 1960 b) experimentally demonstrated the $^1/_3$ raffinose fermentation of *Saccharomyces pastorianus.* This fact is supported by other authors too (V a s, 1960; K u d r i a v z e v, 1954, 1960, 1961). In the case of $^1/_3$ raffinose fermentation this combination becomes combination 17. which already corresponds to the suppositions.

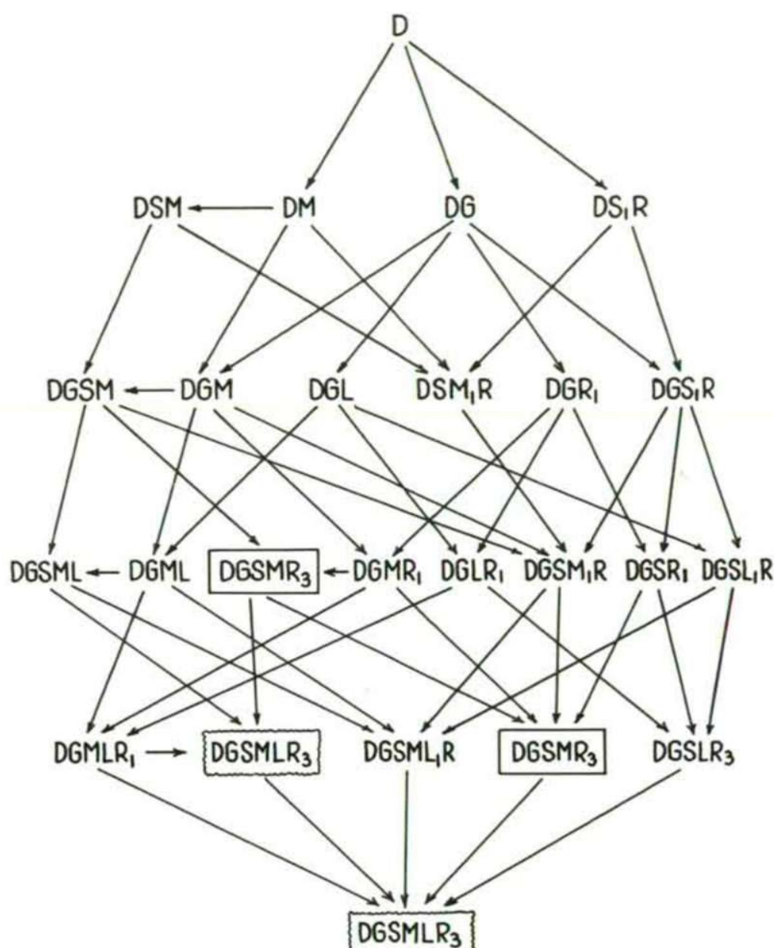



Figure 3. Third deduction of the sugar utilization combinations. Arrows from above downwards mean adding of a new enzyme. Horizontal arrows mean adaption of maltase to sucrose splitting.

2) Reality of combinaton 23 is improbable. It ferments galactose and so one can hardly believe in a $^2/_3$ raffinose fermentation. The melibiase is an exoenzyme (Novák, 1959, 1960 a, b; de la Fuente and Sols, 1958) and no mechanism is known which can distinguish between galactose supplied as such and galactose split off a raffinose molecule. *Candida melibiosi* Lodder et van Rij (1952) characterized with combinaton 23 was found by one of the authors (Novák, 1960 b, 1963) as a $^3/_3$ raffinose fermenter and it must be characterized with combination 24.

3) In the fourth deduction there are two combinations (21. $DGMLR_1$ and 26. $DGSLR_3$) not yet described.

In the first case there is no invertase and raffinose is split by melibiase. This possibility was discovered not long ago (*Saccharomyces oleaceus* Santa Maria, 1958 : $DGR_1$; *Saccharomyces oleaginosus* Santa Maria 1958 : $DGMR_1$; *Saccharomyces italicus* var. *melibiosi* van Uden et Assis-Lopez 1957 : $DGMR_1$; *Paratorulopsis melibiosi* (Shifrine et Phaff 1956) Novák et Zsolt 1961 : $DGLR_1$). The combination $DGMLR_1$ may be very rare and an organism with this spectrum was not yet found.

The lack of combination $DGSLR_3$ may be explained by the fact that it needs lactase and melibiase together, two enzymes rather rare for themselves.

In this way omitting from the list of the observed combinations the two probably wrong combinations and completing it with the two possible but not yet observed combinations a perfect agreement between observation and deduction can be obtained.

Figure 4. Fourth deduction of the sugar utilization combinations. Adaptation of maltase to sucrose splitting and adaptation of invertase to raffinose splitting were supposed. The identical framings mean appearance of a combination earlier.

**Further factors influencing the sugar utilization combinations**

Authors' fourth deduction (Figure 4) was performed supposing only hydrolytical splitting of the oligisaccharides. Other possibilities of splitting and the role of permeases will be taken into consideration in the following.

Phosphorolysis and polymerative cleavage of disaccharides and raffinose (the so-called direct oxydation and fermentation).

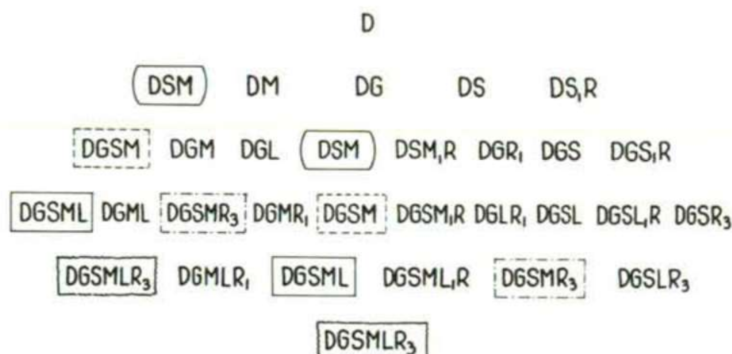



Figure 5. The revaluated deduced combinations.

Sobotka and Holzmann (1934), Willstätter and Bauman (1926), Willstätter and Rohderwald (1937), Willstätter and Steibelt (1920) demonstrated the polymerative splitting of maltose in yeasts with the enzyme amylomaltase. They demonstraed also that the maltase ($\alpha$-glucosidase) content of som yeaests is not enough for the maltose fermentation measured and maltase is inactive below pH 5 but under this condition maltose was well fermented. Leibovitz and Hestrin (1939) demonstrated the pH-optimum of maltose fermentation; they found it between pH 5—6, an intervallum in which maltase is at most slightly active.

In bacteria two ways of non-hydrolytic maltose splitting was demonstrated. Monod and Torriani (1948, 1950), Doudoroff, Hassid, Putman, Potter and Lederberg (1949) demonstrated a polymerative splitting similar to that occuring in yeasts. In this case also the phosphorolytic splitting of the polyose was demonstrated.

Fitting and Sherp (1950, 1951, 1952 a, b), Fitting and Doudoroff (1952 a, b, c) found an enzyme splitting maltose into glucose and $\beta$-D-glucose-1-phosphate.

In *Aspergillus niger* the production of a non-fermentable and non-reducing trisaccharide (panose) was demonstrated from maltose (Pan et al., 1950).

Phosphorolysis of sucrose resulting in $\alpha$-D-glucose-1-phosphate and fructose was demonstrated in bacteria (Doudoroff, 1943, 1945); Doudoroff, Barker and Hassid, 1947 a, b; Doudoroff,

TABLE V

Comparison of the described and the deduced combinations

| combinations | occurrence | | | | deductions | | | |
|---|---|---|---|---|---|---|---|---|
| | assimilation | | fermentation | together | I | II | III | IV |
| 1 D | | 31 | 47 | + | + | + | + | + |
| 2 DG | | 23 | 15 | + | + | + | + | + |
| 3 DS | | 3 | 9 | + | — | — | — | + |
| 4 DM | | 7 | 8 | + | + | — | + | + |
| 5 DGS | | 9 | 6 | + | — | — | — | + |
| 6 DGM | | 9 | 3 | + | + | — | + | + |
| 7 DGL | | — | 1 | + | + | + | + | + |
| 8 DGR$_1$ | | 2 | 1 | + | + | + | + | + |
| 9 DSM | | 20 | 3 | + | — | + | + | + |
| 10 DS$_1$R | | 13 | 18 | + | + | + | + | + |
| 11 DGSM | | 40 | 13 | + | — | + | + | + |
| 12 DGSL | | 3 | — | + | — | — | — | + |
| 13 DGS$_1$R | 12 | | 13 | + | + | + | + | + |
| 14 DGSR$_3$ | | | 5 | + | + | + | + | + |
| 15 DGML | | 3 | 1 | + | + | — | + | + |
| 16 DGMR$_1$ | | 1 | 1 | + | + | — | + | + |
| 17 DSM$_1$R | | | 11 | + | + | + | + | + |
| 18 DSMR$_2$ | | 13 | 3 | + | — | — | — | — |
| 19 DGLR$_1$ | | 1 | — | + | + | + | + | + |
| 20 DGSML | | 23 | — | + | — | + | + | + |
| 21 DGMLR$_1$ | | — | — | — | + | — | + | + |
| 22 DGSM$_1$R | | 1 | 17 | + | + | + | + | + |
| 23 DGSMR$_2$ | 38 | | 1 | + | — | — | — | — |
| 24 DGSMR$_3$ | | 1 | 4 | + | + | + | + | + |
| 25 DGSL$_1$R | 7 | | 5 | + | + | + | + | + |
| 26 DGSLR$_3$ | | | — | —? | + | + | + | + |
| 27 DGSML$_1$R | 17 | 1 | 2 | + | + | + | + | + |
| 28 DGSMLR$_3$ | | | — | + | + | + | + | + |
| number of types | 22 | 26 | 22 | 26 | 20 | 18 | 23 | 26 |

Wianne, and Wolochok, 1949; Doudoroff, Hassid and Barker, 1944, 1947; Kagan et al., 1942).

Phosphorolysis of lactose producing α-D-glucose and galactose was demonstrated in bacteria (Novikova 1956 a, b). In yeasts this way is disputed (Willstätter and Oppenheimer, 1922; Roberts and McFarren, 1953; Kluyver and Custers, 1940; Rogosa, 1948).

Several other authors mention the possibility of splitting disaccharides through phosphorolysis or transglucosidation in bacteria and yeasts (Genevois, 1937; Willstätter and Steibelt, 1921; Doudoroff, 1940).

Several data about the so called direct fermentation belong to the problems of permeation. Mostly there is mentioned only the lack of extracellular splitting or wondering about the observation that a microorganism utilizes a disaccharide faster than its monosaccharide components. Phosphorolysis and polymerative splitting observed till now are intracellular processes but some of the hydrolytical splittings are also intracellular (e.g. maltose and lactose splitting by maltase and lactase respectively).

raffinose splitting in *Pseudomonas saccharophila*. It is performed by three enzymes: invertase, melibiase and sucrose $\longrightarrow$ orthophosphate transglucosidase. All these enzymes are intracellular but the concentration of invertase is very low. Raffinose splitting begins with the aid of melibiase; the sucrose produced in this way is splitted mainly by sucrose-phosphorylase.

Phosphorylases and amylomaltase mentioned above are strictly specific (Willstätter and Rohderwald, 1937; Doudoroff, 1943; Doudoroff, Kaplan and Hassid, 1943).

Consequently supposition of phosphorolysis and polymerative splitting in the deductions means more specific enzymes. In this way sucrose utilization may become independent from maltose and raffinose utilization. This is already realized. although on an other basis, in the fourth deduction, and so it remains valid henceforward. Only the ways of origin of the different combinations must be revaluated. E.g. the combination DS is determined not by a strictly specific (raffinose not splitting) invertase but by sucrose-phosphorylase. The combinaton DM not by a strictly specific (sucrose not splitting) $\alpha$-glucosidase but by a maltose-phosphorylase or amylomaltase. In this case the former cannot be adapted to raffinose splitting and the latter to sucrose splitting. The combination $DS_1R$ supposes a non-specific invertase while DSM a non-specific $\alpha$-glucosidase or the joint presence of sucrose-phosphorylase and maltose-phosphorylase (or amylomaltase). In the case of $DS_1R$ two phosphorolytic enzymes were not supposed because phosphorolytic splitting of raffinose was not yet observed.

## Problems of the permeation of sugars

Differences in the permeation of different sugars into yeasts were observed by Elliot (1949), Berger et al. (1958). Sols (1956) demonstrated that glucose, fructose and mannose were uptaken by a constitutive transportase (permease). According to Sols et al. (1958) the fermented sugars are transported actively without alteration or after extracellular hydrolysis their components are transported through the cell barriere. Fermented sugars have an affinity to the cells, competition was observed between different sugars and inhibition was found by non-fermented analogues. They demonstrated also that baker's yeast splits sucrose with the aid of invertase located in the cell wall and glucose and fructose produced in this way are transported („indirect" fermentation) while maltose is transported as such, is split intracellularly and thereafter fermented („direct" fermentation). Maltose-transportase is strictly specific while the $\alpha$-glucosidase is not (it splits sucrose too). *Dekkeromyces fragilis* ferments lactose „directly", *Saccharomyces carlsbergensis* ferments melibiose „indirectly" (after extracellular hydrolysis). Latter results were confirmed by Novák (1959, 1960 a, b, 1961) too: in *Saccharomyces carlsbergensis* „indirect" raffinose, melibiose and sucrose fermentation, in *Saccharomyces pastorianus* „indirect" (but only $^1/_3$) raffinose fermentation, in *Candida pseudotropicalis* (imperfect form of *Dekkeromyces fragilis)* „direct" lactose fermentation, in *Candida*

*solani* „direct" sucrose fermentation was demonstrated. Avigad (1958) demonstrated a specific sucrose binding and an intracellular permeation barriere in yeasts.

Nelson et al. (1932) found invertase loosely bound in the cell wall (it can be demonstrated in the cell free extract) and therefore its activity is independent of the permeation of sucrose and raffinose.

In connection with trehalose Deere (1939) and Deere et al. (1939) observed that it was hydrolysed by dried yeast cells but not by the living ones.

TABLE VI

Fermentation of sucrose and maltose in the presence of specific transportases

| α-glucosidase | sucrose-permease | maltose-permease | fermentation |
|---|---|---|---|
| + | + | — | ds |
| + | — | + | dm |
| + | + | + | dsm |

As it was already mentioned in the case of phosphorolysis data about faster utilization of an oligosaccharide than its monosaccharide components or other data about the intact uptake of some oligosaccharides belong to the problems of permeability because sugars were uptaken actively with the aid of permeases (transportases) (Wright, 1936; Doudoroff, 1940; Leibovitz and Hestrin, 1942; Pelczar and Doetsch, 1949; Hassid, 1950).

Taking into consideration these data does not make necessary a change in the fourth deduction (Figure 4). Only some revaluations are necessary and these make some combinations more obvious. Viewing the combinations DM and DS and knowing the extracellular location of invertase, the sucrose splitting may be perfomed only by an α-glucosidase (because raffinose utilization is lacking). True enough, α-glucosidase splits sucrose and maltose alike. But sucrose and maltose have different and specific transportases and so lack of maltose-transportase may produce DS while lack of sucrose-transportase produces DM. The precence of both transportases results in DSM (Table VI).

Similarly, on a permeability basis, may be interpreted the sucrose and/or maltose containing combinations completed with galactose and lactose.

Permeability differences cause in the case of sucrose and maltose alternative utilization because here the α-glucosidase is constitutive (if inducible, both sugars may serve as inductor) but the necessary transportases are specific and can be induced only by the specific substrate. Therefore, in the case of organisms utilizing $^1/_3$ raffinose with the aid of melibiase the sucrose remains untouched because of lacking a sucrose-transportase (e.g. *Saccharomyces oleaginosus* Santa Maria, 1958).

Utilization of $^2/_3$ raffinose may hardly be explained supposing impermeability of galactose because correlation of the presence of the galactosidases and the galactose metabolizing enzyme complex (including the specific transportase) seems to be very strong (Losada, 1957).

Accordingly the combinations of the fourth deduction themselves, omitting the connecting arrows and giving other enzymological explanation of their origin, will remain correct (Figure 5).

## Interpretation of the joint combinations of assimilation and fermentation

So far in the deductions assimilation and fermentation were not distinguished; both were considered as „utilization". Now an attempt will be made at the deduction and biochemical interpretation of the joint combinations of assimilation and fermentation.

TABLE VII/1

The deduced joint combinations. Reduction this number to 125 by considering the exo-enzyme nature of invertase and melibiase

| combinations | A | B | C | combinations | A | B | C | combinations | A | B | C |
|---|---|---|---|---|---|---|---|---|---|---|---|
| D- | + | + | + | DGS$_1$R-d | — | + | — | DSM-ds | + | + | + |
| DG- | + | + | + | DGSR$_3$-d | — | — | — | DS$_1$R-ds | — | + | — |
| DS- | + | + | + | DGSL-d | + | + | + | DGS$_1$R-ds | — | + | — |
| DM- | + | + | + | DGML-d | + | + | + | DGSR$_1$-ds | — | + | — |
| DGS- | + | + | + | DGMR$_1$-d | + | — | — | DGSR$_3$-ds | — | — | — |
| DGM- | + | + | + | DGLR$_1$-d | + | — | — | DGSL-ds | + | + | + |
| DGL- | + | + | + | DSM$_1$R-d | — | + | — | DSM$_1$R-ds | — | + | — |
| DGR$_1$- | + | + | + | DGSML-d | + | + | + | DGSML-ds | + | + | + |
| DSM- | + | + | + | DGSM$_1$R-d | — | + | — | DGSM$_1$R-ds | — | + | — |
| DS$_1$R- | + | + | + | DGSMR$_3$-d | + | — | — | DGSMR$_3$-ds | + | — | — |
| DGSM- | + | + | + | DGSL$_1$R-d | — | + | — | DGSL$_1$R-ds | — | + | — |
| DGS$_1$R- | + | + | + | DGSLR$_3$-d | — | — | — | DGSLR$_3$-ds | + | — | — |
| DGSR$_3$- | + | + | + | DGMLR$_1$-d | + | — | — | DGSML$_1$R-ds | — | + | — |
| DGSL- | + | + | + | DGSML$_1$R-d | — | + | — | DGSMLR$_3$-ds | — | — | — |
| DGML- | + | + | + | DGSMLR$_3$-d | + | — | — | DM-dm | — | + | + |
| DGMR$_1$ | + | + | + | DG-dg | + | + | + | DGM-dm | + | + | + |
| DGLR$_1$- | + | + | + | DGS-dg | + | + | + | DSM-dm | + | + | + |
| DSM$_1$R- | + | + | + | DGM-dg | + | + | + | DGSM-dm | + | + | + |
| DGSML- | + | + | + | DGL-dg | + | + | + | DGML-dm | + | + | + |
| DGSM$_1$R- | + | + | + | DGR$_1$-dg | + | — | — | DGMR$_1$-dm | + | — | — |
| DGSMR$_3$- | + | + | + | DGSM-dg | + | + | + | DSM$_1$R-dm | + | + | — |
| DGSL$_1$R- | + | + | + | DGS$_1$R-dg | — | + | — | DGSML-dm | + | + | + |
| DGSLR$_3$ | + | + | + | DGSR$_3$-dg | — | — | — | DGSM$_1$R-dm | — | + | — |
| DGMLR$_1$- | + | + | + | DGSL-dg | + | + | + | DGSMR$_3$-dm | + | — | — |
| DGSML$_1$R- | + | + | + | DGML-dg | + | + | + | DGMLR$_1$-dm | + | — | — |
| DGSMLR$_3$- | + | + | + | DGMR$_1$-dg | + | — | — | DGSML$_1$R-dm | — | + | — |
| D-d | + | + | + | DGLR$_1$-dg | + | — | — | DGSMLR$_3$-dm | + | — | — |
| DG-d | + | + | + | DGSML-dg | + | + | + | DGS-dgs | + | + | + |
| DS-d | + | + | + | DGSM$_1$R-dg | — | + | — | DGSM-dgs | + | + | + |
| DM-d | + | + | + | DGSMR$_3$-dg | + | — | — | DGS$_1$R-dgs | — | + | — |
| DGS-d | + | + | + | DGSL$_1$R-dg | — | + | — | DGSR$_3$-dgs | — | — | — |
| DGM-d | + | + | + | DGSLR$_3$-dg | — | — | — | DGSL-dgs | + | + | + |
| DGL-d | + | + | + | DGMLR$_1$-dg | + | — | — | DGSML-dgs | + | + | + |
| DGR$_1$-d | + | — | — | DGSML$_1$R-dg | — | + | — | DGSM$_1$R-dgs | — | + | — |
| DSM-d | + | + | + | DGSMLR$_3$-dg | + | — | — | DGSMR$_3$-dgs | + | — | — |
| DS$_1$R-d | — | + | — | DS-ds | + | + | + | DGSL$_1$R-dgs | — | + | — |
| DGSM-d | + | + | + | DGS-ds | + | + | + | DGSLR$_3$-dgs | — | — | — |

A = invertase, B = melibiase, C = invertase + melibiase.

The 26 combinations deduced above were combined: 26 assimilation combinations with 27 fermentation combinations (case 27. represents non-fermenters).

(In the followings, assimilation of sugars will be designated with majuscules while fermentation with the corresponding minuscules.)

The restriction that the members of the fermentation combination must be represented in the assimilation combination was taken into consideration. Calculation results in 221 joint combinations (Table VII). But only 71 combinations were observed and described!

It is obvious that fermenting yeasts assimilating raffinose by invertase or melibiase (and at present no other raffinose splitting enzyme is known) will also ferment raffinose. The reason for this is that both invertase and melibiase are exoenzymes. Therefore differences in aerobic and anaerobic permeation, producing DGSM-dm, DGSM-ds etc. combinations in yeasts with the endoenzyme $\alpha$-glucosidase (e.g. N o v á k et al., 1965 a, b, c), are here without any significance. This means that combinations as DS$_1$R-d, DG$_1$R-d, DSM$_1$R-dm etc. are impossible. Data of van U d e n and do C a r m o - S o u s a (1957) and V ö r ö s - F e l k a i and N o v á k (1960) about investigation of 18 species and 268 strains of 41 species respectively support this idea: in all cases raffinose assimilation and raffinose fermentation were strictly correlated.

With the aid of this rule the number of the possible joint combinations became 125 (Table VII).

TABLE VII/2. (continued)

| combinations | A | B | C | combinations | A | B | C | combinations | A | B | C |
|---|---|---|---|---|---|---|---|---|---|---|---|
| DGSML$_1$R-dgs | — | + | — | DGSMLR$_3$-dsm | + | — | — | DGMLR$_1$-dgml | + | — | — |
| DGSMLR$_3$-dgs | + | — | — | DS$_1$R-ds$_1$r | + | + | + | DGSML$_1$R-dgml | — | + | — |
| DGM-dgm | + | + | + | DGS$_1$R-ds$_1$r | + | + | + | DGSMLR$_3$-dgml | + | — | — |
| DGSM-dgm | + | + | + | DGSR$_3$-ds$_1$r | + | — | — | DGMR$_1$-dgmr$_1$ | + | + | + |
| DGML-dgm | + | + | + | DSM$_1$R-ds$_1$r | + | + | + | DGSMR$_3$-dgmr$_1$ | + | + | + |
| DGMR$_1$-dgm | + | — | — | DGSM$_1$R-ds$_1$r | + | + | + | DGMLR$_1$-dgmr$_1$ | + | + | + |
| DGSML-dgm | + | + | + | DGSMR$_3$-ds$_1$r | + | — | — | DGMLR$_3$-dgmr$_1$ | + | + | + |
| DGSM$_1$R-dgm | — | + | — | DGSL$_1$R-ds$_1$r | + | + | + | DGLR$_1$-dglr$_1$ | + | + | + |
| DGSMR$_3$-dgm | + | — | — | DGSLR$_3$-ds$_1$r | + | — | — | DGSLR$_3$-dglr$_1$ | + | + | + |
| DGMLR$_1$-dgm | + | — | — | DGSML$_1$R-ds$_1$r | + | + | + | DGMLR$_1$-dglr$_1$ | + | + | + |
| DGSML$_1$R-dgm | — | + | — | DGSMLR$_3$-ds$_1$r | + | — | — | DGSMLR$_3$-dglr$_1$ | + | + | + |
| DGSMLR$_3$-dgm | + | — | — | DGSM-dgsm | + | + | + | DSM$_1$R-dsm$_1$r | + | + | + |
| DGL-dgl | + | + | + | DGSML-dgsm | + | + | + | DGSM$_1$R-dsm$_1$r | + | + | + |
| DGSL-dgl | + | + | + | DGSM$_1$R-dgsm | — | + | — | DGSMR$_3$-dsm$_1$r | + | — | — |
| DGML-dgl | + | + | + | DGSMR$_3$-dgsm | + | — | — | DGSML$_1$R-dsm$_1$r | + | + | + |
| DGLR$_1$-dgl | + | — | — | DGSML$_1$R-dgsm | — | + | — | DGSMLR$_3$-dsm$_1$r | + | — | — |
| DGSML-dgl | + | + | + | DGSMLR$_3$-dgsm | + | — | — | DGSML-dgsml | + | + | + |
| DGSL$_1$R-dgl | — | + | — | DGS$_1$R-dgs$_1$r | + | + | + | DGSML$_1$R-dgsml | — | + | — |
| DGSLR$_3$-dgl | — | — | — | DGSR$_3$-dgs$_1$r | + | — | — | DGSMLR$_3$-dgsml | + | — | — |
| DGMLR$_1$-dgl | + | — | — | DGSM$_1$R-dgs$_1$r | + | + | + | DGSM$_1$R-dgsm$_1$r | + | + | + |
| DGSML$_1$R-dgl | — | + | — | DGSMR$_3$-dgs$_1$r | + | — | — | DGSMR$_3$-dgsm$_1$r | + | — | — |
| DGSMLR$_3$-dgl | + | — | — | DGSL$_1$R-dgs$_1$r | + | + | + | DGSML$_1$R-dgsm$_1$r | + | + | + |
| DGR$_1$-dgr$_1$ | + | + | + | DGSLR$_3$-dgs$_1$r | + | — | — | DGSMLR$_3$-dgsm$_1$r | + | — | — |
| DGSR$_3$-dgr$_1$ | + | + | + | DGSML$_1$R-dgs$_1$r | + | + | + | DGSMR$_3$-dgsmr$_3$ | + | + | + |
| DGMR$_1$-dgr$_1$ | + | + | + | DGSMLR$_3$-dgs$_1$r | + | + | — | DGSMLR$_3$-dgsmr$_3$ | + | + | + |
| DGLR$_1$-dgr$_1$ | + | + | + | DGSR$_3$-dgsr$_3$ | + | + | + | DGSL$_1$R-dgsl$_1$r | + | + | + |
| DGSMR$_3$-dgr$_1$ | + | + | + | DGSMR$_3$-dgsr$_3$ | + | + | + | DGSLR$_3$-dgsl$_1$r | + | — | — |
| DGSLR$_3$-dgr$_1$ | + | + | + | DGSLR$_3$-dgsr$_3$ | + | + | + | DGSML$_1$R-dgsl$_1$r | + | + | + |
| DGMLR$_1$-dgr$_1$ | + | + | + | DGSMLR$_3$-dgsr$_3$ | + | + | + | DGSMLR$_3$-dgsl$_1$r | + | — | — |
| DGSMLR$_3$-dgr$_1$ | + | + | + | DGSL-dgsl | + | + | + | DGSLR$_3$-dgslr$_3$ | + | + | + |
| DSM-dsm | + | + | + | DGSML-dgsl | + | — | + | DGSMLR$_3$-dgslr$_3$ | + | + | + |
| DGSM-dsm | + | + | + | DGSL$_1$R-dgsl | — | + | — | DGMLR$_1$-dgmlr$_1$ | + | + | + |
| DSM$_1$R-dsm | — | + | — | DGSLR$_3$-dgsl | — | — | — | DGSMLR$_3$-dgmlr$_1$ | + | + | + |
| DGSML-dsm | + | + | + | DGSML$_1$R-dgsl | — | + | — | DGSML$_1$R-dgsml$_1$r | + | + | + |
| DGSM$_1$R-dsm | — | + | — | DGSMLR$_3$-dgsl | + | — | — | DGSMLR$_3$-dgsml$_1$r | + | — | — |
| DGSMR$_3$-dsm | + | — | — | DGML-dgml | + | + | + | DGSMLR$_3$-dgsmlr$_3$ | + | + | + |
| DGSML$_1$R-dsm | — | + | — | DGML-dgml | + | + | + | | | | |

A = invertase, B = melibiase, C = invertase + melibiase.

The 125 deduced and the 71 described joint combinations are compared in Table VIII.

(Originally 73 combinations were described, but three combinations containing $^2/_3$ raffinose utilization were corrected according to the ideas expressed in the foregoings; after this correction two of these combinations turn into already described ones [DGSMN-dgsmr$_2$ ⟶ DGSMR$_3$-dgsmr$_3$; DSMR-dsmr$_2$ ⟶ DSM$_1$R-dsm$_1$r) while the third became a new one (DGSMLR-dsmr$_2$ ⟶ DGSML$_1$R-dsm$_1$r]).

The quantitative and partly the qualitative discrepancies between deduction and observation will be explained in the following.

*Combinations deduced but not yet described*

Table VIII contains only combinations included authors' previous paper (Novák and Zsolt, 1961). Since then many new species were

TABLE VIII/1

Comparison of deduced and described joint combinations

| combinations | | occurrence number of species | deduction | combinations | occurrence number of species | deduction |
|---|---|---|---|---|---|---|
| D- | | 13 | + | DGL-d | — | + |
| DG- | | 9 | + | DSM-d | 9 | + |
| DS- | | 1 | + | DGSM-d | 4 | + |
| DM- | | — | + | DGSL-d | — | + |
| DGS- | | 4 | + | DGML-d | — | + |
| DGM- | | 1 | + | DGSML-d | 2 | + |
| DGL- | | — | + | DG-dg | 6 | + |
| DGR$_1$- | | 1 | + | DGS-dg | — | + |
| DSM- | | 9 | + | DGM-dg | 1 | + |
| DS$_1$R- | | — | + | DGL-dg | — | + |
| DGSM- | | 14 | + | DGSM-dg | 5 | + |
| DGSL- | | — | + | DGSL-dg | 1 | + |
| DGS$_1$R- | 2 | | + | DGML-dg | — | + |
| DGSR$_3$- | | | + | DGSML-dg | 2 | + |
| DGML- | | 2 | + | DS-ds | 1 | + |
| DGMR$_1$- | | — | + | DGS-ds | 3 | + |
| DGLR$_1$- | | 1 | + | DSM-ds | 1 | + |
| DSM$_1$R- | | — | + | DS$_1$R-ds | 2 | — |
| DGSML- | | 15 | + | DGSM-ds | 1 | + |
| DGSM$_1$R- | 8 | 1 | + | DGSL-ds | — | + |
| DGSMR$_3$- | | 1 | + | DGSML-ds | 1 | + |
| DGSL$_1$R- | 1 | | + | DM-dm | 4 | + |
| DGSLR$_3$- | | | + | DGM-dm | 4 | + |
| DGMLR$_1$- | | — | + | DSM-dm | — | + |
| DGSML$_1$R- | 7 | 1 | + | DGSM-dm | — | + |
| DGSMLR$_3$- | | 1 | + | DGML-dm | — | + |
| D-d | | 18 | + | DGSML-dm | — | + |
| DG-d | | 8 | + | DGS-dgs | 2 | + |
| DS-d | | 1 | + | DGSM-dgs | 3 | + |
| DM-d | | 3 | + | DGSL-dgs | 1 | + |
| DGS-d | | — | + | DGSML-dgs | — | + |
| DGM-d | | 2 | + | DGM-dgm | 1 | + |

described, some of them characterized with combinations indicated in Table VIII still as „deduced". E.g.:

1. DM ——. Described in connection with *Trichosporon figueriae* Batista et Silveira 1960.

2. Combination DGSML —— was described by Novák and Vörös-Felkai (1962) *(Rhodotorula slooffii).*

*(Debaryomyces artagaveytiae* Batista, Silveira et Coelho 1961 with the combination DGSL —— could not be considered due to lacking of raffinose tests.)

3. DGS-d was observed by Novák (1961) *(Candida requinyii* Szép et Novák 1962).

4. DGS-dg was described by Batista, Campos et Coelho (1960) *(Endomycopsis dermatensis).*

5. DSM-dm was described as characteristic to *Procandida grubyi* Novák, Vitéz et Marton 1961.

TABLE VIII/2. (continued)

| combinations | occurrence number of species | deduction | combinations | occurrence number of species | deduction |
|---|---|---|---|---|---|
| DGSM-dgm | 2 | + | DGSMR$_3$-dgsr$_3$ | 3 | + |
| DGML-dgm | — | + | DGSLR$_3$-dgsr$_3$ | — | + |
| DGSML-dgm | — | + | DGSMLR$_3$-dgsr$_3$ | — | + |
| DGL-dgl | — | + | DGSL-dgsl | — | + |
| DGSL-dgl | 1 | + | DGSML-dgsl | — | + |
| DGML-dgl | — | + | DGML-dgml | 1 | + |
| DGSML-dgl | — | + | DGSML-dgml | — | + |
| DGR$_1$-dgr$_1$ | 1 | + | DGMR$_1$-dgmr$_1$ | 1 | + |
| DGMR$_1$-dgr$_1$ | — | + | DGSMR$_3$-dgmr$_1$ | — | + |
| DGSR$_3$-dgr$_1$ | — | + | DGMLR$_1$-dgmr$_1$ | — | + |
| DGLR$_1$-dgr$_1$ | — | + | DGSMLR$_3$-dgmr$_1$ | — | + |
| DGSLR$_3$-dgr$_1$ | — | + | DGLR$_1$-dglr$_1$ | — | + |
| DGSMR$_3$-dgr$_1$ | — | + | DGSLR$_3$-dglr$_1$ | — | + |
| DGMLR$_1$-dgr$_1$ | — | + | DGMLR$_1$-dglr$_1$ | — | + |
| DGSMLR$_3$-dgr$_1$ | — | + | DGSMLR$_3$-dglr$_1$ | — | + |
| DSM-dsm | 1 | + | DSM$_1$R-dsm$_1$r | 11 | + |
| DGSM-dsm | 2 | + | DGSM$_1$R-dsm$_1$r | 2 | + |
| DGSML-dsm | — | + | DGSML$_1$R-dsm$_1$r | 1 | + |
| DS$_1$R-ds$_1$r | 11 | + | DGSML-dgsml | — | + |
| DGS$_1$R-ds$_1$r | 1 | + | DGSM$_1$R-dgsm$_1$r | 14 | + |
| DSM$_1$R-ds$_1$r | 2 | + | DGSML$_1$R-dgsm$_1$r | 3 | + |
| DGSM$_1$R-ds$_1$r | 4 | + | DGSMR$_3$-dgsmr$_3$ | 4 | + |
| DGSL$_1$R-ds$_1$r | — | + | DGSMLR$_3$-dgsmr$_3$ | 1 | + |
| DGSML$_1$R-ds$_1$r | — | + | DGSL$_1$R-dgsl$_1$r | 3 | + |
| DGSM-dgsm | 9 | + | DGSML$_1$R-dgsl$_1$r | 2 | + |
| DGSML-dgsm | 3 | + | DGSLR$_3$-dgslr$_3$ | — | + |
| DSML(R)-dgsm | 1 | — | DGSMLR$_3$-dgslr$_3$ | — | + |
| DGS$_1$R-dgs$_1$r | 7 | + | DGMLR$_1$-dgmlr$_1$ | — | + |
| DGSM$_1$R-dgs$_1$r | 3 | + | DGSMLR$_3$-dgmlr$_1$ | — | + |
| DGSL$_1$R-dgs$_1$r | 3 | + | DGSML$_1$R-dgsml$_1$r | 2 | + |
| DGSML$_1$R-dgs$_1$r | — | + | DGSMLR$_3$-dgsmlr$_3$ | — | + |
| DGSR$_3$-dgsr$_3$ | 2 | + | | | |

6. DGSM-dm characterizes *Kloeckera faecalis* Batista et Silveira 1959.

7. DGSML-dgs was observed on *Endomycopsis interdigitalis* Batista et Coelho 1960.

8. $DGSR_3$-$dgr_1$ was observed on some atypical strains of *Saccharomyces oleaceus* by Santa Maria (1958).

9. DGSML-dsm was observed by Lodder and Kreger van Rij (1952) on some strains of *Debaryomyces subglobosus.*

10. $DGSL_1R$-$ds_1r$ was observed by Vörös-Felkai and Novák (1962) on a *Torulopsis strain* (No. 58/316 OKI).

11. $DGSML_1R$-$ds_1r$ was observed by Lodder and Kreger van Rij (1952) on some *Saccharomyces polymorphus* strains.

12. $DGSML_1R$-$dgs_1r$ was described by Capriotti (1961) as characteristic to *Debaryomyces cantarellii.*

13. $DGSMR_3$-$dgmr_1$ was observed by Santa Maria (1958) on some atypical *Saccharomyces oleaginosus* strains.

With these 13 combinations the number of the observed ones became 84.

## *Insufficiencies in the investigation of raffinose utilization*

Several descriptions contain no data about raffinose assimilation, sometimes also data about raffinose fermentation are lacking. Performing additionally the raffinose tests it may be expected that some species will be recognized as belonging to not yet described combinations. E.g.:

1. *Sporobolomyces odorus* described as DS —— may become $DS_1R$ ——. (Combinations DS —— will not be eliminated through this; atypical strains of *Prosporobolomyces salmonicolor* showing DS —— will replace it.)

2. The combinations $DGS_1R$ —— and $DGSR_3$ —— may be emerged as characteristic to *Paratorulopsis apis* and *Rhodotorula minuta* described both with the combination DGSR ——. The same may be expected about *Endomyces ovetensis, Prosporobolomyces salmonicolor, Sporobolomyces odorus, Rhodotorula graminis, Dioszegia hungarica.*

3. Similarly some of the species described with the combination DSM —— *(Endomycopsis bispora, Azymohansenula canadensis, Prosporobolomyces holsaticus, Sporobolomyces boleticolus, Bullera grandispora, Trichosporon cutaneum, Azymocandida japonica, Azymoprocandida mesenterica)* may have the combination $DSM_1R$.

*Rhodotorula texensis* (DGSLR ——) was considered as $DGSL_1R$; transferring it to $DGSLR_3$ —— would give no use.

4. As $DGSMR_3$-$dgr_1$ may be expected, after additional performing of the raffinose tests, *Fermentotrichon hellenicum* described as DGSM-dg.

5. Similarly *Fermentotrichon intermedium* (DGSML-dg) may reveal itself as $DGSMLR_3$-$dgr_1$.

In several other cases completion of the raffinose tests may improve the qualitative picture without any gain, however, from the quantitative point of view. E.g.:

*Cryptococcus gastricus* (DGM ——) may be in reality $DGMR_1$ —— but hereby DGM —— will be lost.

Two species described as DGML —— *(Trichosporon infestans* and *Cryptococcus terreus)* may be in reality $DGMLR_1$ ——; this possibility will not be taken into consideration being combination $DGMLR_1$ not yet observed (see later!).

*Brettanomyces anomalus* (DGSL-dgl) has perhaps the combination $DGSLR_3$-$dglr_1$; but hereby DGSL-dgl will be lost.

With these the number of the possible joint combinations became 89.

### *Explanation of further discrepancies*

It was supposed that the occurence of many combinations has a very low probability: this is the cause that they are not yet discovered.

### Joint combinations containing combinations not yet observed

These are the following 12 joint combinations containing the combinations $DGSLR_3$ and $DGMLR_1$ not yet observed:

$DGSLR_3$ ——, $DGMLR_1$ ——, $DGSLR_3$-$dgr_1$, $DGMLR_1$-$dgr_1$, $DGSLR_3$-$dgsr_3$, $DGMLR_1$-$dgmr_1$, $DGSLR_3$-$dglr_1$, $DGMLR_3$-$dglr_1$ $DGSLR_3$-$dgslr_3$, $DGSMLR_3$-$dgslr_3$, $DGMLR_1$-$dgmlr_1$, and $DGSMLR_3$-$dgmlr_1$.

Nothing could be said against the possibility of these joint combinations. They contain two very rare properties (lactose and melibiose splitting) the joint occurrence of which has only an extremly low probability.

With these the number of possibly occurring cases became 101.

### Lactose assimilation and fermentation

The well-known rare occurrence of the two properties may explain the lack of further 24 joint combinations:

DGL-dgl, DGML-dgl, DGSML-dgl, DGSL-dgsl, DGSML-dgsl, DGSML-dgml, $DGLR_1$-$dglr_1$, $DGSMLR_3$-$dglr_1$, DGSML-dgsml, and $DGSMLR_3$-$dgslr_3$ with lactose fermentation and DGL ——, DGL-d, DGSL ——, DGSL-d, DGL-dg, DGML-dg, DGSL-ds, DGML-dm, DGSML-dm, DGML-dgm, DGSML-dgm, $DGLR_1$-$dgr_1$, $DGSMLR_3$-$dgsr_3$, and $DGSMLR_3$-$dgmr_1$ with lactose assimilation.

With these the number rises to 125.

### Rarity of the combination $DGMR_1$

This combination was only once observed *(Saccharomyces oleaginosus* Santa Maria, 1958). This makes comprehensible that combinations $DGMR_1$ —— and $DGMR_1$-$dgr_1$ are not yet observed.

With these the possibility of 127 joint combinations may be expected.

### About combinations incongruent with the deduction

According to the foregoings the number of described and probably occuring combinations is 127. This is higher than the number of the deduced combinations (125). This discrepancy is caused by two combinations ($DS_lR$-ds and DGSMLR-dgsm) the reality of which is doubtful due to their raffinose assimilation without raffinose fermentation. Only one species was described with the latter combination *(Candida pseudotumoralis)* with a comment on week raffinose assimilation. With the other combination only two species were characterized *(Torulopsis apicola* and *Zymodebaryomyces globosus).*

## Conclusions

The deduction of the sugar utilization combinations was based on data known about the enzymes participating in the metabolism of the diagnostic sugars. The fact, that a larger part of them are not yet observed has no enzymological basis. Rare occurrence of lactose and melibiose splitting must have a phylogenetical explanation in connection with the rare occurrence of the sugars in question.

Authors' outlined deduction seems to be a rather useful working hypothesis. Most of the data published after accomplishing this article can be fitted in without any difficulty; the deduced combinations, so to say, predicted future observations.

Naturally, no theoretical deductions can replace observation and experimentation. Some of authors' investigations demonstrated that reality is more complicated than the theoretical scheme. E.g.: separate endoenzymes for maltose and sucrose splitting were found in *Procandida albicans* (Novák and Zsolt 1963). Maltose splitting exoenzyme was also demonstrated (Novák et al., 1966).

A promising possibility for discovering new sugar splitting enzymes are offered by organisms which show utilization patterns incongruent with authors' deduction. E.g. *Saccharomyces inusitatus* with its $^2/_3$ raffinose fermentation (van der Walt, 1965) or *Saccharomyces hienipiensis* Santa Maria (1962) and *Saccharomyces norbensis* Santa Maria (1963) utilizing melibiose but not raffinose etc.

## Summary

Utilization (assimilation and fermentation) combinations of the six diagnostic sugars generally used in yeast taxonomy were interpreted on the basis of present-day enzymological data.

26 combinations of assimilation and 27 combinations of fermentation and from these 125 joint combinations were deduced.

Agreement between the deduction and the observed data was demonstrated and discrepancies were explained. The deduction may be considered as a useful working hypothesis in yeast taxonomy and enzymology alike.

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