INVESTIGATIONS ON THE OLIGOSACCHARIDE DECOMPOSITION BY PICHIA WICKERHAMII (VAN DER WALT) VAN RIJ

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(Received September 30, 1968)

The pathways of the carbohydrate utilization of yeasts are important both from taxonomical and from industrial points of view and also in respect of the fermentation industry. As on the basis of theoretical deductions (Novák and Zsolt, 1961; 1962; 1964) the possibility of more ways of sucrose and maltose utilization seemed to exist, a series of experiments, dealing with uptake and cleavage of these oligosaccharides were started.

As to sucrose splitting, only the role of invertase was earlier accepted by taxonomists (Lodder and Kreger-van Rij, 1952), but later on some data were published demonstrating a possibility of sucrose splitting by maltase (group-specific α-glucosidase) in invertase-less yeasts (Kosikov, Gelyman and Raevskaya, 1956). According to Halvorson (1961), sucrose was split by all of the so-called yeast-maltase preparates too. Lindegren and Lindegren (1949), however, demonstrated in baker's yeast also an endo-enzyme splitting sucrose which differred from invertase not only by its localization but by its inactivity against raffinose as well. This enzyme showed sensitivity against lipid solvents and did not split maltose.

A sucrose splitting enzyme differring from invertase was observed by us first in *Candida solani* (Novák, 1963) and it was also demostrated that it was not identical with the "yeast-maltase". On the basis of its lipid solvent sensitivity, also a similar enzyme was later isolated by us (Novák and Zsolt, 1963a) from *Procandida albicans* — syn. *Candida albicans* (Novák and Zsolt, 1961) — on the basis of the behaviour of this enzyme against inhibitors it was established that it was not identical with the enzyme described by Lindegren et al. (1949) mentioned above (Novák and Zsolt, 1963b). Since then a similar enzyme was demonstrated by us in some other yeasts, too: *Candida requinyii*, *Procandida stellatoidea* and *Procandida grubyi* (Novák, Kevei, Oláh and Zsolt, 1965a,b,c).

Investigating the general occurrence of this enzyme in yeasts, first of all some species utilizing sucrose but not raffinose were tested.

In our present work the results of the investigation of splitting of sucrose, maltose and raffinose by *Pichia wickerhamii* are reported. This species assimilates glucose, sucrose and maltose but it ferments only glucose. Besides, an interesting comparison is given by the fact that *Procandida grubyi* investigated earlier (Novák, Kevei, Oláh and Zsolt, 1965c) shows a sugar assimilation pattern identical with that of *Pichia wickerhamii* but it ferments beside glucose maltose too.

Materials and methods

For the experiments a strain of *Pichia wickerhamii* received from van der Walt was used. It was cultivated on Csillag's molasses agar (Csillag, 1950) in Roux-bottles. The method of experiments, performed with intact and acetone treated cells and cell-free extracts, as well as the method of paper-chromatography were published earlier (Novák, 1960, 1961; Novák, Kevei, Oláh and Zsolt, 1965a).

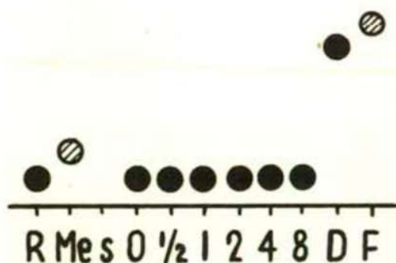

Fig. 1. Raffinose utilization by intact *P. wickerhamii* cells. 750 mg live wet cells and 60 mg raffinose in pH 7,2 M/30 phosphate buffer in 3 ml volume. Copy of the chromatogram. Left raffinose, melibiose and suspension, right glucose and fructose controls. Numbers under start line represent the sampling intervals in hours.

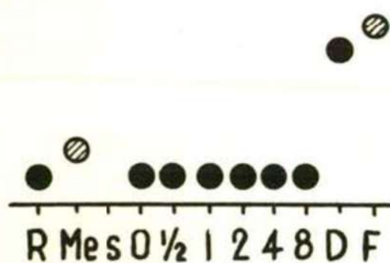

Fig. 2. Raffinose utilization by acetone treated *P. wickerhamii* cells. Acetone treated 750 mg live wet cells; others as indicated in Fig. 1.

Results

Raffinose splitting. Neither cleavage nor uptake of raffinose was demostrated by any preparates (Figs. 1—2).

Maltose splitting. The living and acetone treated cells neither splitted nor took up this sugar (Figs. 4 and 5), while in the cell-free extract maltose splitting was demonstrated (Fig. 6).

Sucrose splitting. The living and the acetone treated cells

neither splitted nor took up sucrose (Figs. 7 and 8), but in the cell-free extract sucrose splitting was found (Fig. 9).

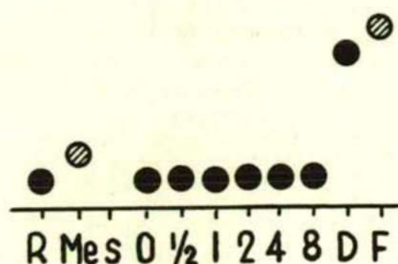

Fig. 3. Raffinose utilization by cell-free extract of *P. wickerhamii* cells. Cell-free extract of 500 mg live wet cells desintegrated with quarz sand and 40 mg raffinose in pH 7,2 M/30 phosphate buffer in 2 ml volume; others as indicated in Fig. 1.

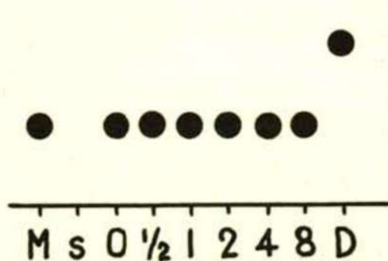

Fig. 4. Maltose utilization by intact *P. wickerhamii* cells. 750 mg live wet cells and 60 mg maltose in pH 7,2 M/30 phosphate buffer in 3 ml volume. Copy of the chromatogram. Left maltose and suspension, right glucose controls. Numbers under the start line represent the sampling intervals in hours.

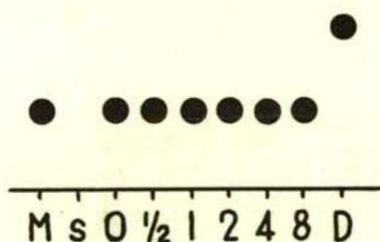

Fig. 5. Maltose utilization by acetone treated *P. wickerhamii cells.* Acetone treated 750 mg live wet cells; others as indicated in Fig. 4.

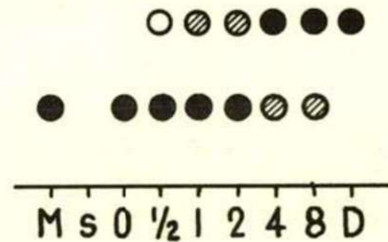

Fig. 6. Maltose utilization by cell-free extract of *P. wickerhamii* cells. Cell-free extract of 500 mg live wet cells desintegrated with quarz sand and 40 mg maltose in pH 7,2 M/30 phosphate buffer in 2 ml volume; others as indicated in Fig. 4.

Discussion

From incubations made with sucrose and raffinose it is seen that the sucrose splitting enzyme of *Pichia wickerhamii* is not of invertase type, because it does not cleave raffinose. The acetone sensitivity of it refers to its similarity (or identity) to the enzyme isolated by us from some other yeasts (Novák, 163; Novák and Zsolt, 1963a,b; Novák, Kevei, Oláh and Zsolt, 1965a,b,c).

The maltose splitting enzyme of *Pichia wickerhamii* differs considerably from those isolated by us from other yeasts (Novák 1961, 1963;

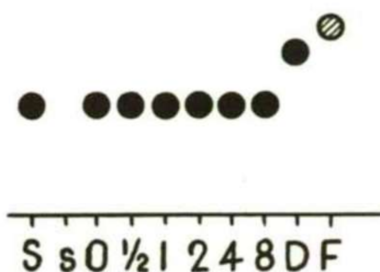

Fig. 7. Sucrose utilization by intact *P. wickerhamii* cells. 750 mg live wet cells and 60 mg sucrose in pH 7,2 M/30 phosphate buffer in 3 ml volume Copy of the chromatogram. Left sucrose and suspension, right glucose and fructose controls. Numbers under the start line represent the sampling intervals in hours.

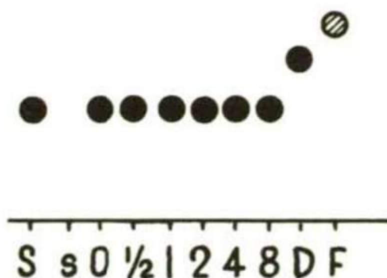

Fig. 8. Succrose utilization by acetone treated 750 mg live wet cells; others as indicated in Fig. 7.

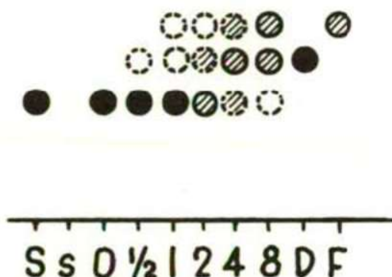

Fig. 9. Sucrose utilization by cell-free extract of *P. wickerhamii* cells. Cell-free extract of 500 mg live wet cells desintegrated with quarz sand and 40 mg raffinose in pH 7,2 M/30 phosphate buffer in 2 ml volume; others as indicated in Fig. 7.

Novák and Zsolt, 1963a,b; Novák, Kevei, Oláh and Zsolt, 1965b,c), because the former is acetone sensitive while the others are not. On the basis of these it is to be supposed that *Pichia wickerhamii* has an other type of maltose splitting enzyme than the species investigated by us earlier.

Comparing the results obtained on the living cells of *Pichia wickerhamii* with the same preparate of *Procandida grubyi* (Novák, Kevei, Oláh and Zsolt, 1965c), it can be established that no maltose consumption was observed even in suspensions of a high density (750 mg wet cells in 3 ml) i.e. the living cells did not take up maltose. In contrast to this, in the case of *Procandida grubyi* a maltose uptake was demonstrated in suspensions of a lower density (560 mg in 3 ml), too (Novák, Kevei, Oláh and Zsolt, 1965c).

Accordingly, in our incubation system during the investigation period, from the two maltose assimilating species only the maltose fermenting one *(Procandida grubyi)* took up this sugar in a demonstrable amount. Taking into consideration that our incubation method provides rather anaerobic conditions, it is plausible that only the species having anaerobic maltose metabolism take up this sugar and thus these results confirm in the case of maltose utilization the existence of two types (anaerobic and aerobic) of transportase systems and enzymes respectively.

Summary

Investigating the oligosaccharide decomposition of the yeast *Pichia wickerhamii*, no raffinose splitting enzyme was demonstrated. The sucrose splitting enzyme found in this species proved to be similar (or identical) to those isolated by us from other yeasts, too. The maltose cleaving enzyme of the investigated species, contrary to the other ones isolated from other yeasts, showed an acetone sensitivity.

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