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Mid-Infrared Spectroscopic Analysis on Kinetic Sugar Uptake Phenomena of Monosaccharide and Disaccharide by Suspension TBY-2 Cells

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Abstract

On the basis of mid-infrared information of liquid culture medium suspending plant cells acquired by a Fourier transform infrared spectrometer equipped with an attenuate total reflection accessory, sugar metabolic kinetics of the plant cells were studied. The sugars as carbon sources in the media have received our attention. We performed plant cell cultivation with *Nicotiana tabacum* cv. Bright Yellow No.2 (TBY-2) cells, which were sub-cultured in the Murashige-Skoog medium containing sucrose. The TBY-2 cells were cultured in **glucose**, **fructose**, **mannose**, **galactose**, **sucrose**, **trehalose**, **maltose**, or **lactose** medium. So, as the results, the lag time from starting of cultivation to consuming sugar was different from that for each cultivation. In particular, during **sucrose**, **glucose** or **fructose** cultivation, the cultivation times were much shorter than those during **mannose**, **galactose**, **trehalose** or **maltose** cultivation. Additionally, the cells could not consume **lactose**. Furthermore, by analyzing the kinetic metabolism using the non-dimensional cultivation time for cell growth behavior, it was suggested that the TBY-2 cells consumed sugar before cell growth and that ethanol content in culture media increased just after cell growth.

Experiments

Materials

Test plant cells: *Nicotiana tabacum* cv. Bright Yellow No.2 (TBY-2)
Liquid media: MS (Murashige-Skoog) medium base
Sugar: glucose, fructose, mannose and galactose (monosaccharide)
sucrose, trehalose, maltose and lactose (disaccharide)

Plant Cell Cultivation

Sub-cultivation period: 7 d
Cultivation period: longer than 14 d, maximum 180 d

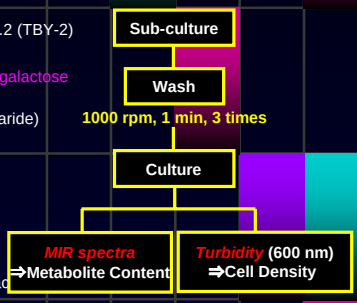
MIR Spectral Measurement

System

FT-IR system: Magna 750, Nicolet
ATR accessory: Specaclamp ATR 11080, Grasby Specac
(IRE : ZnSe, 6 reflections, 45 °)

Measurement conditions

64 scans, 4 cm^{-1} resolution



Experimental Results

MIR Spectrum of Metabolite

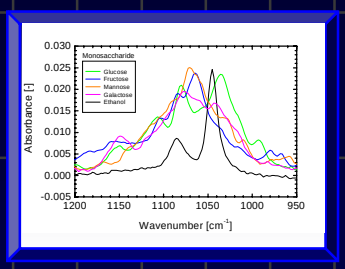


Fig. 1a MIR spectra of monosaccharide in the MS media

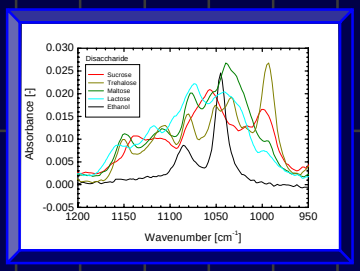
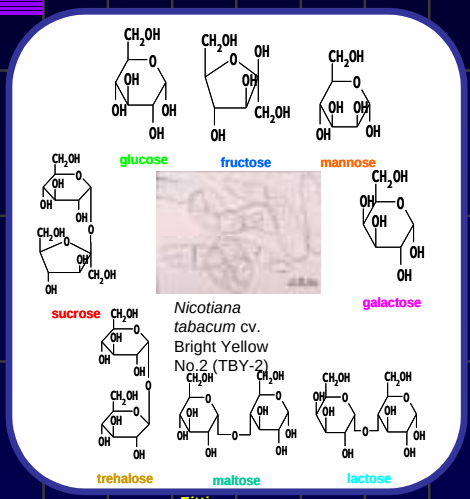


Fig. 1b MIR spectra of disaccharide in the MS media



Fitting

Amount of sugar uptake:

$$U_{sugar} = C_{sugar,ini} + \frac{C_{di\text{-}sac,ini} - C_{di\text{-}sac}}{M_{di\text{-}sac}/M_{sugar}} - C_{sugar}$$

Cell density:

$$x = \frac{x_{ini} - x_{fin}}{1 - e^{\frac{t - t_{0,x}}{x_x}}} + x_{fin}$$

Standardization

Vertical axis : U_s/X_s
Sugar uptake efficiency that is the ration of the standardized sugar uptake amount to the standardized cell density. The both are calculated using the parameters of the logistic functions.

Horizontal axis : $(t - t_0)/w_x$
Non-dimensional cultivation time standing for the cell growth behavior.

Metabolite contents determination

During cultivation, the absorbance decreased almost over the finger print region, especially, a sharp peak identifying glycosidic linkage around 993 cm^{-1} decreased.

The spectral patterns had gradually changed from **trehalose** to **glucose**.

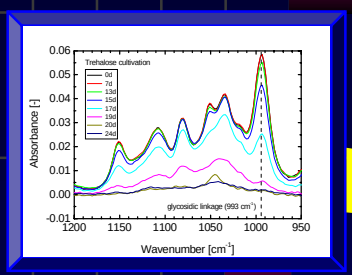


Fig. 2 Time behavior of ATR spectra of medium component during trehalose cultivation

The **trehalose** content in the medium decreased with cultivation time, and then the **glucose** content increased gradually.

Then, **trehalose** was hydrolyzed to **glucose**, which was consumed by TBY-2 cells.

Additionally, the ethanol content increased just after the dry cell weight.

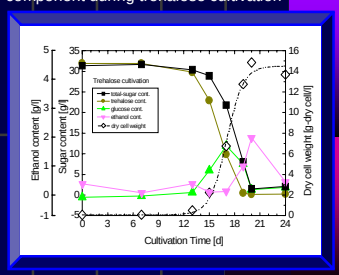


Fig. 3 Time courses of sugar content, ethanol content and dry cell weight during trehalose cultivation

Influence of sugar spices on sugar uptake kinetics

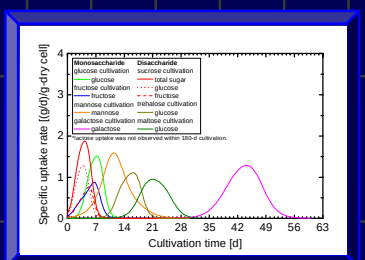


Fig. 4 Time courses for specific uptake rates of sugar during cultivation

The peak time of the specific uptake rate was different from each sugar cultivation.

Interestingly, sugar uptake on **trehalose**, **maltose**, **mannose** and **galactose** required a longer lag period than on **sucrose**, **glucose** and **fructose**, which are usually used as the carbon sources.

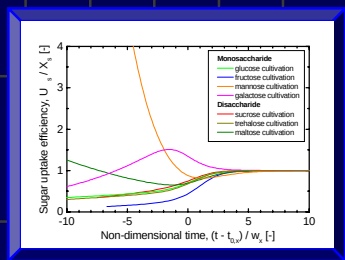


Fig. 5 Non-dimensional time courses for sugar uptake efficiency, *Us/Xs*, during cultivation.

Sugar spices affected the kinetic sugar uptake phenomena of the TBY-2 cells cultures in the medium.

In particular, **mannose**, **galactose** and **maltose** cultivation were different from each sugar cultivation.

Conclusion

The significant influences of the mono / di-saccharide spices on the sugar uptake kinetics of the TBY-2 cells were accurately, rapidly and continuously observed by using FT-IR/ATR techniques.

References

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