

NMR INVESTIGATION OF THE SIMULTANEOUS FERMENTATION OF XYLOSE AND GLUCOSE BY A SELECTED STRAIN OF *KLEBSIELLA PLANTICOLA* (G11).

C.Rossi*, A.Lepri*, M.P.Picchi*, S.Bastianoni*, D.Medaglini°,
M.Vanassina° and E.Cresta*.

*Department of Chemistry, University of Siena, Pian dei Mantellini
44, 53100 Siena, ITALY.

°Department of Molecular Biology, University of Siena, 53100
Siena, ITALY

1. INTRODUCTION

The hydrolysis of hemicellulose yields a mixture of sugars of which D-xylose and D-glucose are the major constituents.⁽¹⁾ This sugar mixture is an excellent substrate for growing microorganisms and yields high energy products such as ethanol^(2,3). In developing this project two main problems have to be analyzed in detail: i) the isolation and identification of microorganisms whose metabolism can be sustained by the hemicellulose-derived sugar mixture, ii) the characterization of the metabolism, and the selection of specific metabolic pathways of microorganisms growing on sugar mixtures. It has already been shown that the use of selectively carbon-13 enriched substrates enables the "in vivo" metabolization process of microorganisms and tissues^(4,5) to be studied by NMR. This technique was applied to the study of the simultaneous fermentation of xylose and glucose by a newly isolated *Klebsiella planticola* (G 11) strain.

In the present investigation [2-¹³C]-glucose and [1-¹³C]-xylose were used as carbon-13 enriched substrates. Isotopic enrichment in different positions of the sugar chain enabled us to: i) separate the xylose from the glucose signals in the carbon spectrum and ii) calculate the contribution of each sugar to end-product yield.

2. EXPERIMENTAL

Klebsiella planticola G11 was isolated from the soil of a corn field and selected for its capacity of growing on a mixed sugar substrate⁽⁶⁾. Identification of the bacterium was performed on the basis of taxonomic characters and biochemical behaviour. The microorganism was cultivated in a nitrogen atmosphere at pH 7.5 and 35°C on a mineral medium containing 0.2 g/l of yeast extract. The metabolism of the bacterium was investigated by "in vivo" NMR spectroscopy, using selectively

carbon-13 enriched sugar substrates. The cell culture used for microbatch NMR experiments had an initial Optical Density (O D) of 0.5. The microorganism was grown on a NMR coaxial tube containing D₂O as lock signal on the external section and positioned permanently in the magnetic cavity during fermentation [1-¹³C]-xylose and [2-¹³C]-glucose obtained from Cambridge Isotope Laboratories were used as enriched substrates.

The sugar metabolic process was followed by recording carbon spectra at 30 minute intervals until the end fermentation.

3. RESULTS AND DISCUSSION

The NMR spectra of the enriched sugar and the end-products of sugar fermentation are shown in Figure 1. The metabolization of xylose and glucose followed two different and

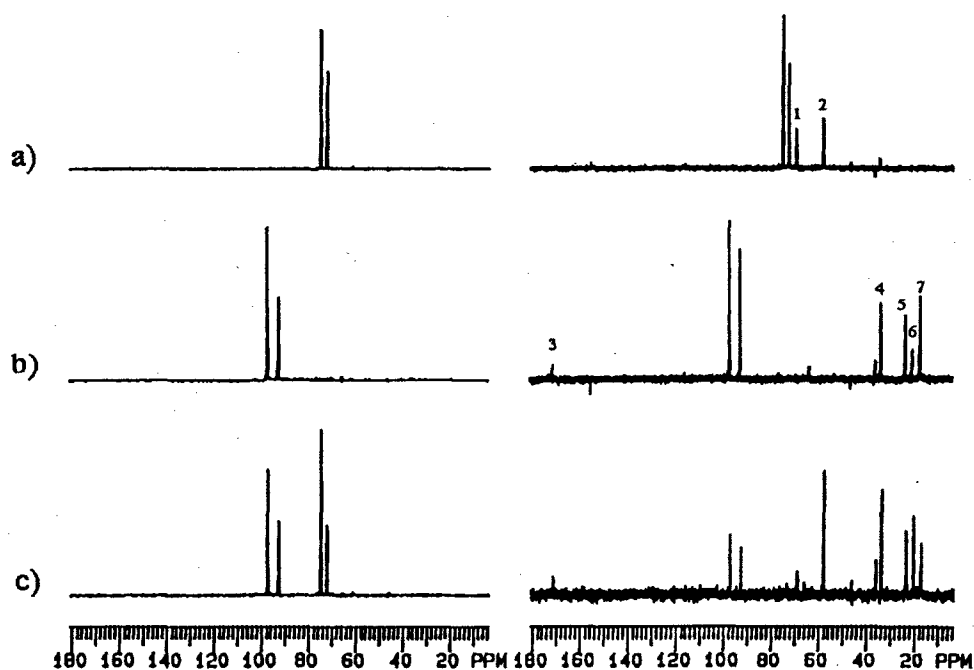


Figure 1 - ¹³C-NMR spectra obtained at the beginning (left) and at the end (right) of fermentation by *K. planticola* G11. Total sugar 10g/l; pH 7.5; Temperature 35 °C. a) [2-¹³C]-glucose fermentation; b) [1-¹³C]-xylose fermentation and c) simultaneous [1-¹³C]-xylose and [2-¹³C]-glucose fermentation. End-product signals detected by ¹³C-NMR were: 1) [2-¹³C]-lactic acid, 2) [2-¹³C]-ethanol, 3) formic acid, 4) [2-¹³C]-succinic acid 5) [1-¹³C]-lactic acid 6) [1-¹³C]-acetic acid and 7) [1-¹³C]-ethanol.

The end-products of fermentation were identified on the basis of the NMR chemical shifts. Analysis of the dependence of chemical shift on pH enabled the preliminary identification of carboxylic and non carboxylic end-products.

independent pathways: "ethanol and mixed acids" for xylose⁽⁷⁾ and "Emboden-Meyerhof" for glucose. Diagrams of the sugar pathways are shown in Figure 2. Glucose metabolism was also analyzed using [1-¹³C] enrichment. The same end-

products as for [2-¹³C]-glucose enrichment (Figure 1A) are detected.

biomass have been detected. The increase in uptake rate with increasing xylose concentration in

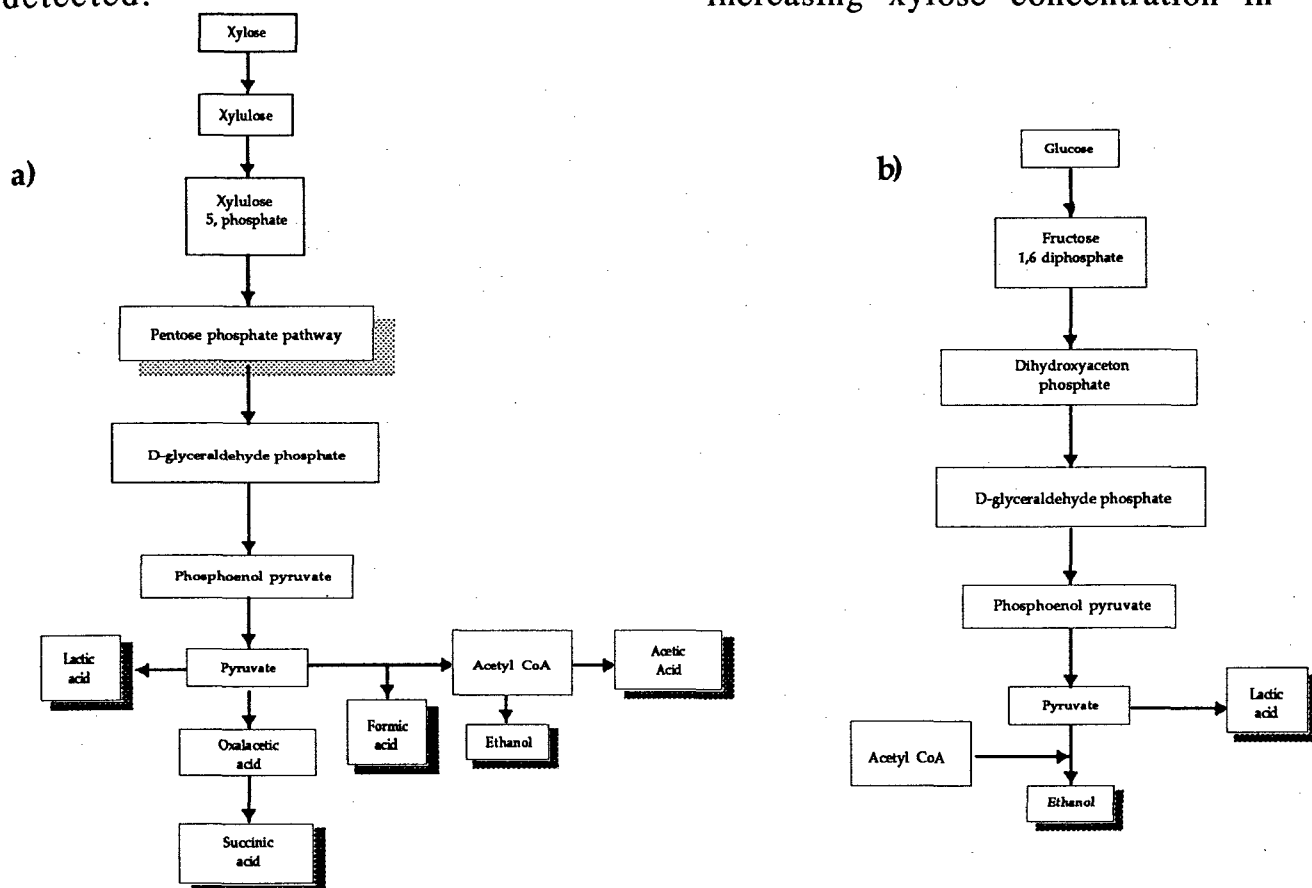


Figure 2 - Metabolic pathways of xylose (a) and glucose (b) fermentation by *Klebsiella planticola* G11.

The metabolization of xylose by *Klebsiella planticola* G11 was interesting from the view points of the metabolization rate and the end-products of the process. Following the xylose metabolization process by NMR in a range of sugar concentration from 5 to 100 g/l, the uptake rate can be calculated. This parameter it is very important because is correlated with the efficiency of the process of sugar transport through the cell membrane. Table 1 shows the xylose uptake rate during the first three hours of fermentation. Sugar consumption rates for *Klebsiella planticola* ATCC 33531, in the range of 0.8-1.6 g.l⁻¹.h⁻¹ per gram of

our case is indicative of a "low affinity" mechanism (not previously detected in *Klebsiella* species) similar to the "facilitated diffusion" transport process described in *Candida sheabetae*.⁽⁹⁾

Figure 3A shows the effect of glucose on the metabolization of xylose. The NMR spectra show that glucose does not interfere with xylose metabolism and its uptake rate. Figure 3B reports the results of a similar experiment, in which glucose was added after two hours of xylose fermentation. The addition of glucose did not affect xylose fermentation. It is very unusual for two substrates to be metabolized at

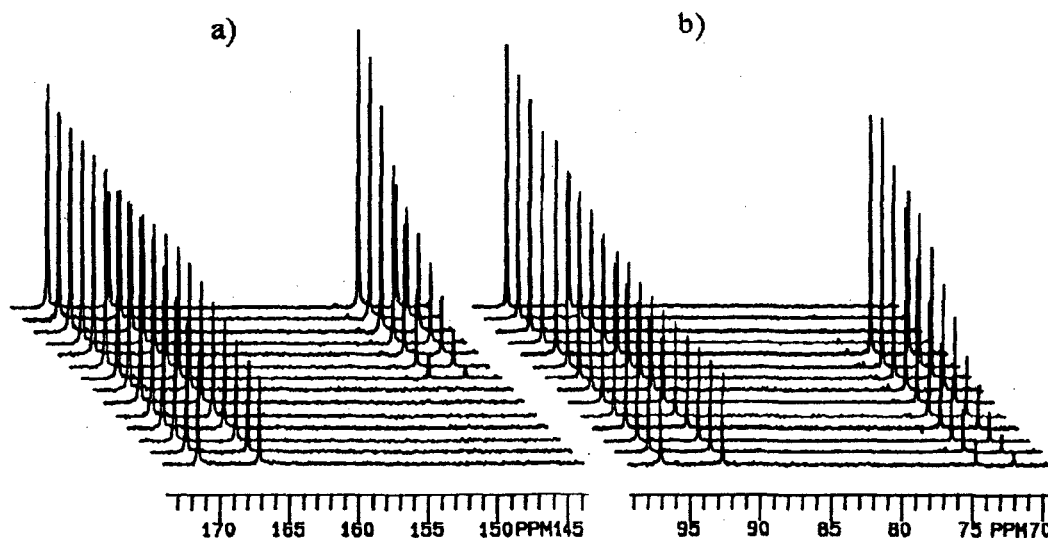


Figure 3)- ^{13}C NMR spectra recorded at different stages of sugar fermentation. a) Simultaneous fermentation of xylose and glucose b) The effect of addition of glucose after 2 hours of xylose metabolization. Spectra were recorded every 30 minutes.

TABLE 1

Xylose uptake rate calculated from NMR spectra during the first three hours of fermentation.

Xylose concentration g/l	Uptake rate ^a g.l ⁻¹ .h ⁻¹
5	1
10	2.1
20	3.5
40	4.6
50	5.1
80	6.2
100	7.0

a) per gram of dry weight biomass

the same time. The phenomenon has been hypothesized before but has only ever been demonstrated in *Candida sheabetae*. The non diauxic growth of *Klebsiella planticola* G11 is very important because it could be used to ferment sugar mixtures like that obtained from the hydrolysis of hemicellulose.

If the property of good xylose uptake can be combined with good end-product yields by selection or genetic engineering^(10,11), it will constitute a further advance in the development of bioethanol production.

4. REFERENCES

- 1) T.E.Timell; Adv. Carbohydr. Chem. 19, 247 (1964).
- 2) K.Skoog and B.Hahn-Hgerdal; Enz. Microb. Technol., 10, 66 (1988).
- 3) H.Shneider; Critical Review in Biotechnology, 2, 18 (1989).
- 4) K.Ugurbil, T.R.Brown, J.A.Den Hollander, P.Glynn and R.Shulman; Proc. Acad. Sci. USA, 75, 3742 (1978).
- 5) J.A.Den Hollander, T.R.Brown, K.Ugurbil and R.Shulman; Proc. Acad. Sci. USA, 76, 6096 (1979).
- 6) E.Cresta, S.Jez, A.Lepri A.Pisani, C.Rossi and G.Sabatini; "NMR investigation of xylose bioconversion by *Klebsiella* sp. strain isolated from soil". In "Biomass for Energy and Industry" G.Grassi, G.Gosse and G.dos Santos eds., Elsevier Applied Sciences, 2, 253 (1990).
- 7) C.S.Gong, L.F.Chen, M.C.Flikingier and G.T.Tsao; Adv. Biochem. Eng., 20,93 (1981).
- 8) J.Tolan and R.K. Finn; Appl. Environ. Microbiol., 53, 2039 (1987).
- 9) C.Lucas and N.van Uden; Appl. Microbiol. Botechnol., 23, 491 (1986).
- 10) F.Alterthun and L.O.Ingram; Appl. Environ. Microbiol., 55 1943 (1989).
- 11) J.S.Tolan and R.K.Finn; Appl. Environ. Microbiol., 53 2039 (1987).