ZBORNÍK PRÍSPEVKOV
3. KONFERENCIE
CENTRA EXCELENTNOSTI

Aplikácia OMICS nástrojov v štúdiu vzniku chorôb a ich prevencie

Chemický ústav SAV, v. v. i., Dúbravská cesta 9, Bratislava

30. november 2022

Recenzent

RNDr. Jana Bellová, PhD. chemjbel@savba.sk

Editor

Mgr. Mária Šedivá, PhD. chemsedm@savba.sk
Ing. Mária Kopáčová chemmari@savba.sk

ISBN 978 – 80 – 971665 – 4 - 0

In vivo NMR monitoring of [1-^{13}C]-glucose biotransformation by *Bacillus* sp. PDD-3b-6, bacterium isolated from cloud water

Mária Matulová[1], Martine Sancelme[2] Mounir Traikia[2] and Anne-Marie Delort[2]

[1]*Institite of Chemistry, Slovak Academy of Sciences, Dúbravská cesta 9, 845 38 Bratislava, Slovakia*
chemmatu@savba.sk
[2] *Institut de Chimie de Clermont-Ferrand (ICCF), UMR6096 CNRS-UCA-Sigma, Clermont-Ferrand, France*

Introduction

Bacillus sp. PDD-3b-6, bacterium isolated in cloud water, was a subject of our study for many years. Particularly, a biotransformation of sugars and other substances, usually encountered in the atmosphere by this microorganism. We have shown that on high glucose concentration this bacterium produces exo-polymeric compounds (Matulova, 2014). Here we evaluate results of *in situ* and *in vivo* incubations of this bacterium on low concentration of [1-^{12}C]-D-glucose and [1-^{13}C]-D-glucose, respectively.

Material and methods

In vivo ^{13}C NMR: Bacterial suspension was diluted with Volvic water (with 10% of D_2O) to obtain final volume 150ml. Suspension was placed into a tempered water bath (27°) with oxygen perfusion. Equipment for *in vivo* NMR was set up (Fig. 1). Into 10 mm NMR sample tube a sealed capillary with benzene (external standard for chemical shift calibration) was inserted. After a perfusion stabilisation [1-^{13}C]-Glucose (300 mg) was added into bacterial suspension and the first spectrum was registered. Subsequently a series of ^{13}C NMR spectra were measured (each with 1 000 scans, overnight, 19.5h). Samples of bacterial suspension $\mathbf{t_3}$ and $\mathbf{t_{24}}$ were taken after 3 h and 24 h of incubation and centrifuged (3 min, 12 500 rpm). Supernatants and sediments containing cells were stored at -40°C until their detailed NMR analyses.

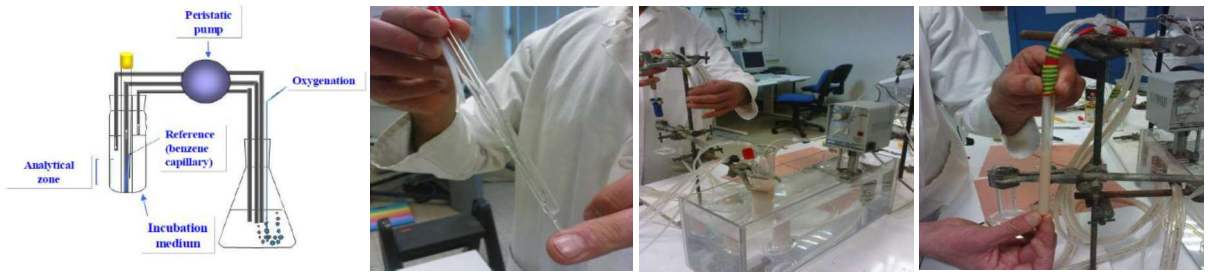

Fig. 1 Perfusion system for *in vivo* NMR described by Chorao et al. 2009.

Perchloric acid extract preparation: PCA extracts were prepared according Chorao et al. 2009. Briefly, cells (10 g wet weight) were quickly frozen in liquid nitrogen and thawed several times to ensure disruption of cells. Then 1 mL of 70 % (v/v) perchloric acid was added to the solution. Suspension was centrifuged (15 000 g, 15 min, 4 °C) to remove matter; supernatant pH was adjusted to 5.0 using $KHCO_3$, followed by centrifugation (10 000 g, 10 min, 4 °C) to remove $KClO_4$ and resulting supernatant was frozen in liquid nitrogen and freeze-dried. Freeze-dried material, containing non-volatile compounds, was re-dissolved in 2.5 ml of water containing 10 % (v/v) D_2O, neutralized

to pH 7.5 with 5 M KOH and buffered with 50 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid). Divalent cations (particularly Mn2+ and Mg2+) were chelated by addition of enough amounts of CDTA ranging from 50 to 100 mM depending on the sample. Chelating of paramagnetic cations is a prerequisite for obtaining sharp resonance signals during the NMR experiments. The main advantage of this technique is that during the cell disruption the proteins are eliminated from the sample and thus it is less complicated to observe the intracellular metabolites of sugar origin.

Results and Discussion

Fig. 2 Shows ^{13}C NMR spectra of the *in vivo* monitoring of [^{13}C-1]-glucose consumption by *Bacillus* sp. PDD-3b-6. Detailed analyses of *in vivo* ^{13}C NMR spectra from kinetic of the [^{13}C-1]-glucose degradation and those of the supernatant samples $\mathbf{t_3}$ and $\mathbf{t_{24}}$ have shown that succinate, lactate and acetate were the most abundant metabolites. In the sample taken $\mathbf{t_3}$ the ^{13}C label was incorporated only into lactate and acetate with an enrichment 29% and 16%, respectively. Succinate was not enriched (spectra not shown). However, in the ^{1}H NMR spectra (Fig. 3A) of samples of cell extracts $\mathbf{t_3}$ and $\mathbf{t_{24}}$ prepared from broken cells in sediments, the diagnostic anomeric H1 NMR signal at δ 5.167 ppm due to not labelled MBA is present. MBA was the dominant metabolite in the $\mathbf{t_{24}}$ sample. In cells MBA was found not ^{13}C enriched. This fact suggests that its production is linked with internal processes of bacterial metabolism (or internal deposits of glucose) and its formation seems to be substrate independent. Fig. 3B shows a comparison of ^{1}H NMR spectra of samples from incubation media on [^{13}C-1]-glucose (96h - ^{13}C Glc t96 and 89h- ^{13}C Glc t89), and not labelled [^{12}C-1]-glucose (39h - ^{12}C Glc t39 and 63h - ^{12}C Glc t63). In samples ^{12}C Glc t39 and ^{12}C Glc t63 the presence of the diagnostic MBA H1 singlet signal at δ_{H1} 5.167 ppm is evident, while in samples ^{13}C Glc t96 and ^{13}C Glc t89 this signal is absent/disappearing in noise. It means that no unlabelled MBA was excreted from cells. However, instead of the anomeric signal at δ_{H1} 5.167 a doublet signal, with chemical shift corresponding to δ_{H1} 5.167 of MBA. It was split due to an interaction with $^{13}C1$ ($^{1}J_{H1}$-^{13}C 170.2 Hz) due to [^{13}C-1]-glucose confirm the presence of ^{13}C labelled MBA. We can deduce that in this case MBA should be synthetized extracellularly.

Production of MBA by this bacterium was later discovered in incubation media on other sugar substrates – on fructose, maltose, sucrose, turanose and trehalose. In all cases, the most important quantity of MBA was produced in time interval 39 – 48 hours, in the case of turanose at 72 h of incubation. It was further degraded within 5 to 8 hours without its accumulation in incubation medium.

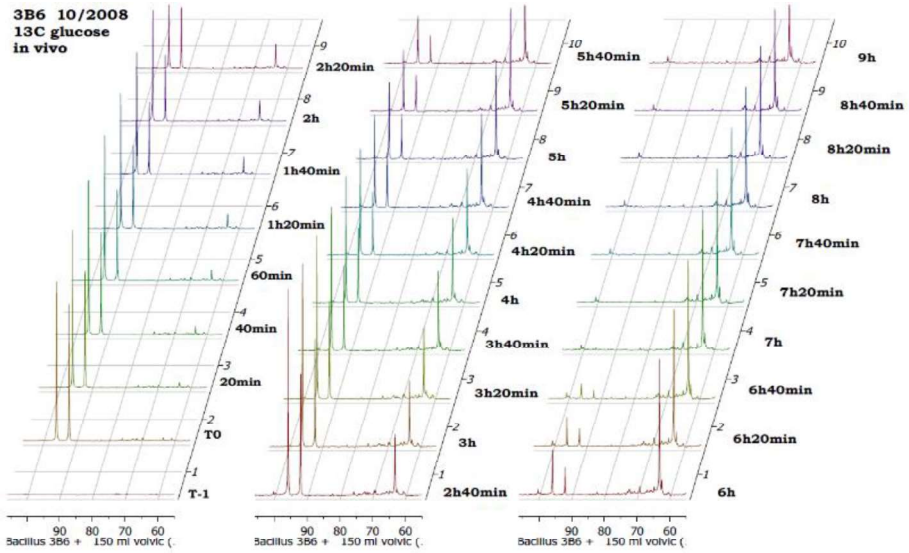

Fig. 2 *In vivo* ^{13}C NMR kinetic of [^{13}C-1]-glucose degradation by *Bacillus* sp. PDD-3b-6.

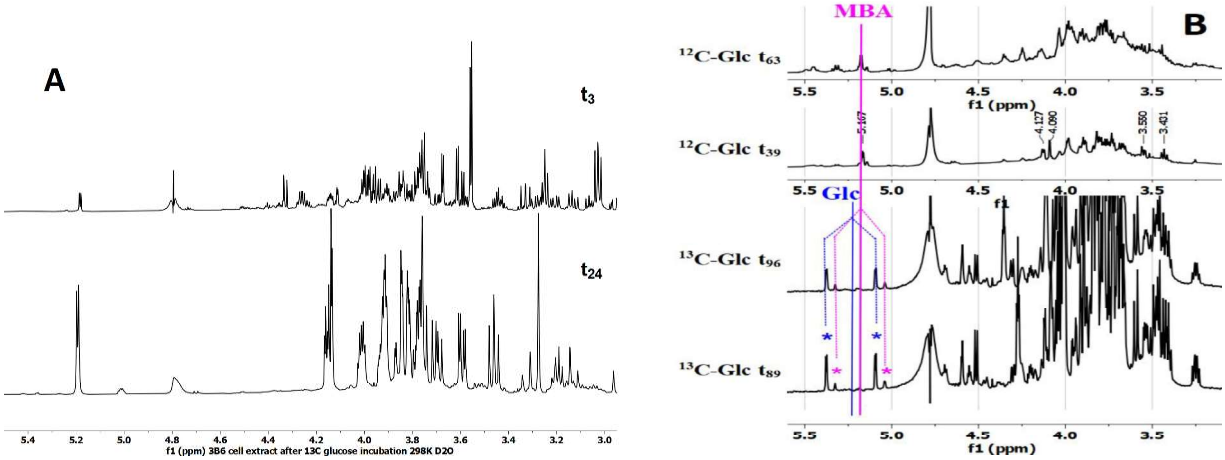

Fig. 3A ^{1}H NMR spectra of *Bacillus* sp. PDD-3b-6 cell extracts taken from the incubation medium with [^{13}C-1]-glucose at 3 and 24h of incubation. Fig. 3B ^{1}H NMR spectra of samples taken from incubation media of *Bacillus* sp. PDD-3b-6 on [^{13}C-1]-glucose (96h - ^{13}C Glc t96 and 89h- ^{13}C Glc t89), and [^{12}C-1]-glucose (39h - ^{12}C Glc t39 and 63h - ^{12}C Glc t63). * - anomeric H1 signal of glucose is split into a doublet due to $^{1}J_{H\text{-}C}$: blue from the substrate - labelled [^{13}C-1]-glucose; pink – from the product [^{13}C-1]-glucose in MBA. Region of carbohydrates is shown only.

Acknowledgements

This publication was created with the support of the Operational Program Integrated Infrastructure for the project: Study of structural changes of complex glycoconjugates in the process of inherited metabolic and civilization diseases, ITMS: 313021Y920, and project: Integration of multiomics study

results and biotechnological production of biologically important substances, ITMS 313011T560, co-financed by the European Regional Development Fund.

References

M. Matulová, S. Husárová, P. Capek, M. Sancelme, and A.-M. Delort. *Environ. Sci. Technol.* 2014, 48, 14238−14247. Doi: 10.1021/es501350s

C. Chorao, M. Traikia, P. Besse-Hogan, M. Sancelme, R. Bligny, E. Gout, G. Mailhot and A.-M. Delort. *J. Appl. Microbiol.* 108 (2010) 1733-1743. doi:10.1111/j.1365-2672.2009.04577.x