AN HPTLC-OPLC APPROACH FOR UNDERSTANDING THE MICROBIAL METABOLISM IN THE PRESENCE OF DIFFERENT COMPLEX CARBOHYDRATES MIXTURES: THE CASE OF *Bifidobacterium adolescentis MB 239.*

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SUMMARY:
Bifidobacteria are one of the most predominating microflora in the human gut, being among the first colonizers of the sterile gastrointestinal tract of newborns. They are consi dered to exert a range of beneficial activities related to host health and, as a consequence, are increasingly entering the marketplace in the form of nutritional supplements and functional foods, such as yoghurt, cheese or infant food. The intestinal microflora as *Bifidobacteria* has adapted for utilization as a source of energy of complex carbohydrates that escape hydrolysis by human digestive enzymes, as fructo-oligosaccharides (FOS). The ability of intestinal microflora to utilize carbohydrates in complex mixtures is of particular interest, even thought few data have been reported. This poster presents the results obtained using an innovative analytical approach based on instrumental HPTLC-OPLC for investigating the metabolic behavior of *Bifidobacterium adolescentis MB 239* as a case study. Raffinose , FOS (sucrose, 1- Kestose, nystose, fructosil-nystose), lactose and thier monomeric moieties glucose, galactose, and fructose, were simultaneously present as carbon sources in the solution to be fermented by the bacterium. The method proposed have permitted to quantitatively monitor the sugars concentrations during the entire time-course of the processes, having set up the separation of the complex sugars mixtures with acetronitile-water (85/15) eluent on silica layers, derivatized with lead (IV) acetate-dichlorofluorescein reagent. The matrix effect of the fermentation solution on the calibration performances in OPLC have been also investigated. All the results will be presented and widely discussed.

FERMENTER

Batch cultures with controlled pH were carried out with a working volume of 2 L. The fermenter was inoculated (10 % v/v) with exponential phase pre-cultures grown in the same medium. The culture pH was continuously measured and regulated at 6.5 by automatic addition of 4 M NaOH. Growth was monitored by following changes in OD600 and biomass dry weight. To determine the kinetics of sugar uptake, samples were periodically collected for carbohydrate analysis.

FEEDING SOLUTION CONTAINED:

- Fructosil-nystose
- Nystose
- 1-Kestose
- Raffinose
- Lactose
- Sucrose
- Glucose
- Galactose
- Fructose

Bifidobacterium Adolescentis **MB 239**

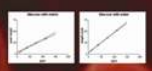
CALIBRATION:

The matrix effect is particularly significant in samples deriving from fermentation broth because they contain several other soluble components, such as salts, free aminoacids, peptides and proteins. Therefore, we have studied standard lines for every analyte using a pure standard dissolved in distilled water and in an artificial matrix containing the same real matrix but without the presence of the analyte of interest.

The method proposed have permitted to monitor the sugars concentrations during the entire time-course of the processes, having optimized the separation of the complex sugars mixtures in OPLC.

SEPARATION CONDITIONS

CHROMATOGRAPHIC DEVELOPMENT: OPLC 50 (BIONISIS)
STATIONARY PHASE: silica layers (BIONISIS, HTSorb Fine silica gel, size: 20*20 cm)
ELUENT: Acetonitril/water 85/15, FLR E: 300 ml, Vol. R: 300 ml, Vol. E: 10000 ml, elution time: 2010 sec.
DERIVATIZATION: mix 5 ml $Pb(CH_3COOH)_4$ (2% in glacial acetic acid solution) and 5 ml 2,7 dichlorofluorescein (0,2% ethanol solution) and make up to 200 ml with toluene



So we have used standard lines with matrix for quantifing sugars content during the entire fermentation

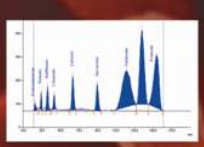
Sugars	R^2	Working range (ppm)	LOD (ppm)	$S_{y/x}$
Lactose	0,9993	10-200	18	0,68
1-kestose	0,9995	10-80	4	0,75
Glucose	0,9991	10-200	14	1,39
Fructose	0,9996	10-200	16	0,70
Galactose	0,9961	10-200	28	1,61
Sucrose	0,9950	10-200	22	1,32
Nystose	0,9904	10-80	17	4,23
Raffinose	0,9996	10-180	2	0,63
Fructosil- nystose	0,9986	10-30	6	0,47

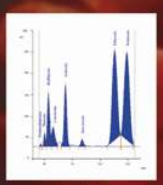
RESULTS:

A densitogram of a standard solution shows that all the sugars are perfectly separated in the elution conditions described.

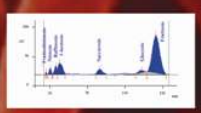
An example of some samples collected at different times with different sugars concentrations.

Thanks to quantitative results it has been possible to understand the behaviour of microrganism with a complex mixture of nutrients.

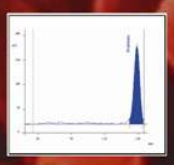




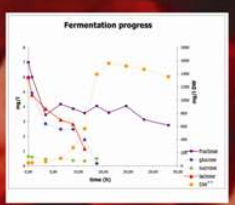
T_0= 45'

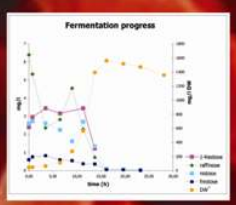


T_1= 13 h 45'



T_2= 28 h 09'





CONCLUSIONS:

HPTLC technique have many advantages: simplicity of operation, the availability of sensitive and selective reagents for detection and confirmation without interference of the mobile phase, the ability to repeat detection and quantification at any time with changed paramenters because fractions representing the entire samples are stored on the plate.
OPLC technique represents an interesting alternative to the analytical techniques traditionally employed for the monitoring of fermentation time course, because it allows reliable results, obtained in real-time.
From the microbiological point of view, it becomes possible to understand the metabolic behaviour and the uptake of the different sugars during the various phases of the *Bifidobacterium* growth: lag-phase, log-phase and stationary phase.