

EXPERIMENTAL STUDIES REGARDING THE BACTERIAL METABOLISM OF SUGARS

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Abstract

In this paperwork was studied the metabolism of sugars like glucose, lactose and sucrose through various microorganisms (bacteria, yeasts, molds). Both oxidative and fermentative metabolism of the sugars for the studied microorganisms will be evaluated by using an adequate culture base. In concordance with the type of sugar that is available for the metabolism, the selected microorganisms will transform the sugars by various means of metabolism (glycolysis, pentoso-phosphate cycle, alcoholic fermentation, acetic fermentation, lactic fermentation, etc.). The metabolic path used by a certain microorganism will be determined by analyzing the final metabolic products (acids, CO₂, aldehydes, etc.) by the evolution of each microorganism and by the reaction of the initial and final culture base.

Keywords: *oxidative metabolism, fermentative metabolism, sugars, aerobic-anaerobic microorganisms*

Introduction

Generally metabolism mechanisms are studied by examining its components, being known the fact that each type of metabolism is formed of metabolically sequences and each enzyme can have a regulating or catalytically way of working (Banu, 1999).

The present paperwork although is based on catabolic reactions of sugars, reactions that are well known for several sugars that are used as energetically substrates (Ikura, 1987) for several species of microorganisms (bacteria, yeasts, moulds), brings furthermore another argument to confirm that certain metabolic path (Bullok, 1987; Cuesta, 1988) are common to the microbial cells, with specific modifications according to each case (Durand, 1970; Faulkner, 1982; Richlik, 2000).

Thus was studied the oxidative and fermentative metabolism for several sugars like glucose, saccharose and lactose (Luttinger, 1996) by various microorganism while using an adequate culture medium.

Experimental

The following species of microorganisms were used in experiments:

- *Escherichia coli*
- *Bacillus subtilis*
- *Proteus vulgaris*
- *Saccharomyces cerevisiae*
- *Penicillium expansum*

These microorganisms were seeded on the Hugh-Leifson culture medium (Livanova, 2002), semi-solid and which has the following composition: Triptone 2 g; NaCl 5 g; K₂HPO₄ 0,3 g; agar 2,5 g; and brome-thymol blue, aqueous solution 1% 3 ml.

The components are brought in 1000 ml distilled water, are homogenized, the pH is corrected to 7.1 and then is autoclaved for 15 minutes at 121°C. 5 ml of base medium is transferred in sterile vials then right away is incorporated 1 ml of each sterile sugar (1% concentration) and two vials of each sugar are inseminated with the respective tested microorganism. One sample of each sugar is covered with 1 ml sterile mineral oil. Thus are obtained 30 samples, 15 samples for testing the oxidative metabolism and 15 vials for testing the fermentative metabolism (Jurcoane, 2000; 2004; Motoc, 1962).

The samples are placed in a thermostat at +37°C for 48 hours after which the following analyses are made:

- a) Identification of lactic acid by color reactions;
- b) Identification of acetic acid by color reactions;
- c) Establishing the medium reaction with the help of an electronic pH-meter.

Results and Discussions

The results obtained by degradation of the sugar base under the influence of the tested microorganisms by identifying the two metabolites (lactic and acetic acid) are presented in table 1.

Table 1. Identification of lactic and acetic acid resulted after metabolisation of the tested sugars

Tested microorganism	Sugar base	Identified metabolite			
		Lactic acid		Acetic acid	
		aerobe	anaerobe	aerobe	anaerobe
Escherichia coli	Glucose	+	+	-	-
Escherichia coli	Lactose	+	+	-	-
Escherichia coli	Saccharose	+	-	-	-
Bacillus subtilis	Glucose	-	-	-	-
Bacillus subtilis	Lactose	-	-	-	-
Bacillus subtilis	Saccharose	+	-	-	-
Proteus vulgaris	Glucose	+	+	-	-
Proteus vulgaris	Lactose	-	-	-	-
Proteus vulgaris	Saccharose	+	-	-	-
Saccharomyces cerevisiae	Glucose	-	-	+	-
Saccharomyces cerevisiae	Lactose	-	-	-	-
Saccharomyces cerevisiae	Saccharose	+	-	+	-
Penicillium expansum	Glucose	-	-	-	-
Penicillium expansum	Lactose	-	-	-	-
Penicillium expansum	Saccharose	-	-	-	-

Legend : (+) – present ; (-) – absent ;

By analyzing the results from table 1 one can see that sugars metabolizing up to lactic or acetic acid is done mainly by bacteria and especially by *Escherichia coli* (Larpen, 1993).

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The results obtained after establishing the pH of the sample (after metabolizing the sugar base) are presented in table 2. The measuring of the pH for the samples where the degradation of the sugars was made by fermentative metabolism (F) was made after previously heating the samples on water bath, necessary step in order to bring the samples to a liquid consistency that allows the measuring of the environment reaction in optimal conditions.

Table 2. The pH values obtained for the tested microorganisms according to the metabolism type

Tested microorganism	Sugar base	Work temperature (°C)		Medium reaction (pH)	
		O	F	O	F
Escherichia coli	Glucose	28.9	49.8	5.99	7.51
Escherichia coli	Lactose	25.2	51.3	7.87	7.64
Escherichia coli	Saccharose	26.3	48.4	7.47	7.12
Bacillus subtilis	Glucose	23.2	44.7	6.92	7.98
Bacillus subtilis	Lactose	22.9	52.6	7.96	7.63
Bacillus subtilis	Saccharose	22.7	47.4	7.76	8.01
Proteus vulgaris	Glucose	23.9	53.3	8.28	8.05
Proteus vulgaris	Lactose	24.9	53.8	8.22	7.73
Proteus vulgaris	Saccharose	23.5	52.6	8.01	7.92
Saccharomyces cerevisiae	Glucose	28.0	52.5	6.53	6.26
Saccharomyces cerevisiae	Lactose	28.1	46.8	4.72	5.63
Saccharomyces cerevisiae	Saccharose	26.2	58.2	7.13	5.92
Penicillium expansum	Glucose	24.5	51.3	8.32	7.66
Penicillium expansum	Lactose	23.2	52.8	8.22	7.91
Penicillium expansum	Saccharose	23.4	54.5	7.75	7.77

Legend. Type of metabolism: O – oxidative ; F – fermentative ;

Conclusions

Taking into consideration the results from table 1 and 2 the followings can be concluded:

- a) the samples with *Escherichia coli* metabolize all sugar types used (glucose, lactose and saccharose), with an exception for saccharose, which is degraded only by means of oxidation. Glucose can be metabolized by the path of anaerobe glycolysis, by the path of hexose's-mono-phosphates (HMP) or by the Entner-Doudoroff path (ED). Lactose can be metabolized due to the fact that *Escherichia coli* possesses β -galactosidase;
- b) metabolization of saccharose is made by *Bacillus subtilis*, *Proteus vulgaris* and *Saccharomyces cerevisiae* because invertase, an enzyme that hydrolyses saccharose to glucose and fructose is present within most of the yeasts varieties (*Candida utilis*, *Saccharomyces cerevisiae*), fungi (*Aspergillus niger*, *Penicillium chrysogenum*) and various bacteria. Saccharose is hydrolyzed at the exterior of the yeasts and fungi cells while in the case of bacteria (*Bacillus subtilis*) saccharose is transported to the interior of the cells under the form of saccharose-P and then is hydrolyzed;
- c) it is very probable that the samples with *Penicillium expansum* (mostly the sample with glucose) degrade glucose to gluconic acid without a previous phosphorylation, like in the case of microorganisms that use the Entner-Doudoroff path. Glucose is transformed in glucolactone, the reaction being catalyzed by glucosoxidase (having as coenzyme vitamin B₂) and then glucolactone is hydrated to gluconic acid.

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