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Studies of the Bacterial Surfaces by XPS and SEM Methods

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This work presents the results of studies on bacteria *Bacillus subtilis* which was grown at different temperatures. The experiments were carried out using X-ray photoelectron spectroscopy and scanning electron microscope equipped with "cryo-chamber", which gives the possibility of testing biological samples in a frozen state. These methods enabled receiving images of surface topography of the bacteria and demarcated their chemical composition. After deconvolution of the lines C 1*s* and O 1*s* information concerning the intensity of the various functional groups in the bindings presented in the cell walls surface of tested strain and reallocation in relation to the temperature of incubation was obtained. These changes are visible in the form of an additional peak in both the C 1*s* and O 1*s* in bacteria cultured in suboptimum temperature.

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1. Introduction

The outer surface of the bacterial cells is very important, as it mediates many crucial interactions of the cells with the surrounding environment. One of the environmental factors which significantly affect the metabolism and the construction of the bacterial cell structures is temperature. It can influence the growth rate, development, enzymes activity and in particular the chemical composition of the bacterial cells. Each species of microorganisms characterized by three cardinal temperatures for growth: minimum, optimum and maximum (optimum temperature for *Bacillus subtilis* is 37 °C) [1]. Understanding these complex interactions requires detailed knowledge of the elemental, molecular, and structural composition of the cell surface, which is obviously different from the bulk of the cell wall [2, 3]. Therefore an object of the study was to monitor chemical changes in the surface of the cell walls of the bacteria by means of modern physical techniques [4].

2. Experimental

The gram-positive bacterium *Bacillus subtilis* was used in the present examinations. They were cultured on agar plates for 48 h in the temperature 37 °C and 20 °C. This bacterial species may change the composition of the outer rigid peptidoglycan layer in response to different growth conditions and external factors.

The chemical composition of the surface layer of the bacteria and morphological changes in the cell wall due to the different incubation temperatures were investigated using multi photoelectron spectrometer PHI 5700/660 of Physical Electronics. In comparison with other surface techniques, X-ray photoelectron spectroscopy (XPS) has the advantage of being nondestructive and highly surface sensitive. Moreover, it gives high information content and yields quantitative data on elemental composition and chemical bonding.

The morphological changes were based on the results obtained from measurements carried out using the scanning electron microscope (FE-SEM) from JEOL 7600F field department, which is equipped with an adapter "Cryo-SEM", allowing to study biological samples in a frozen state. It has also allowed obtaining images of surface topography bacteria and thanks to a special knife it was possible to cut the wall and observe the cell's interior.

The above mentioned methods enabled receiving images of surface morphology of the bacteria, analyzing their chemical composition and clarify the change in the chemical bonding.

3. Results and discussion

XPS gives high information content and yields quantitative data on elemental composition and chemical bonding of the surface layer of *Bacillus subtilis*. Based on the registered inspection XPS spectra (Fig. 1), in the energy 0–1400 eV, average atom concentration elements on the surface of the tested microorganisms was determined. The calculations were performed using the Physical Electronics Multipak which includes only those elements for which the characteristic lines are clearly visible in the spectrum. When interpreting the results the special attention was put on the determination of major elements (C, O, N, P) and remaining (K, Cl, S, Mg).

Elemental analysis of the bacterial surfaces was made. Figure 1 shows two examples of XPS spectra of *Bacillus subtilis* in the temperature 37 °C and 20 °C. Characteristic peaks can be observed for carbon, oxygen, nitrogen, and phosphorus for both the test samples. Elemental atomic concentrations was calculated from the XPS peak areas. The C 1*s* peak at 285.0 eV was used as an

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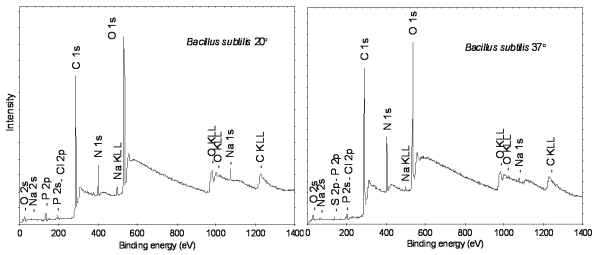

Fig. 1. Examples of the XPS spectrum for the gram-positive bacteria *Bacillus subtilis* samples.

internal standard to correct the peak positions. Data fitting was carried out by a least square routine supplied by the instrument manufacturer, using mixed Gaussian-Lorentzian peaks. The intensities of the peaks of the various elements have been normalized with respect to the intensity of the carbon peak and converted into atomic concentration ratios (O/C, N/C, and P/C) collected in Table I. The atomic ratios O/C are higher for the bacteria grown at suboptimal temperature and can be related to the presence of a slime layer surrounding the cell wall. These ratios are consistent with the literature data [5].

TABLE I

Surface chemical composition determined by XPS.

Sample	C 1*s* [%]	O 1*s* [%]	N 1*s* [%]	P 2*p* [%]	O/C	N/C	P/C
B.subtilis 20 °C	61.59	27.74	7.19	1.96	0.45	0.12	0.03
B.subtilis 37 °C	63.93	21.95	12.41	0.63	0.34	0.19	0.01

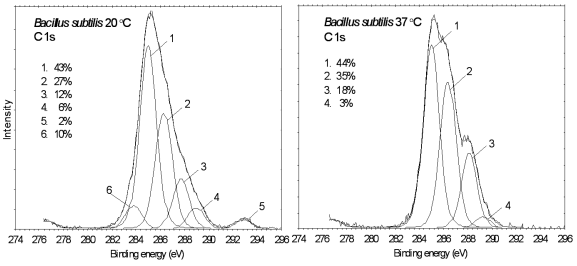

Fig. 2. C 1*s* photoelectron line deconvolution for *Bacillus subtilis* samples.

Also the fitted lines of carbon (C 1*s*) and oxygen (O 1*s*) of the bacterial samples tested for both temperatures (20 °C and 37 °C) using SimPeak. Analysis of this main line allowed the identification of various functional groups participating in the biomaterial of the bacterial surface. The XPS lines related to the optimal temperature are narrower and the functional groups are better distinguishable. This indicates the better quality of the cell wall surface. The carbon peaks for *Bacillus subtilis* cultured in both temperatures were generally decomposed into four components, attributed to carbon bound to carbon and hydrogen C–(C, H) at a binding energy of

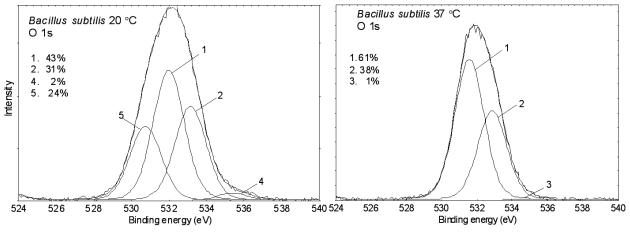

Fig. 3. O 1*s* photoelectron line deconvolution for *Bacillus subtilis* samples.

285.0 eV; to carbon singly bound to oxygen or nitrogen C–(O, N) including ether, alcohol, amine, and amide, at a binding energy of 286.4 ± 0.5 eV; and to carbon making one double bond or two single bonds with oxygen, C=O including amide, carbonyl, carboxylate, ester, acetal, and hemiacetal, at a binding energy of 288.1 ± 0.4 eV [6]. A weak component attributable to carboxylic functions was found at 289.3 ± 0.4 eV. Additionally at bacteria cultured in temperature 20 °C has appeared on peak from potassium and the line at lower binding energy associated with suboptimal temperature (Fig. 2).

The oxygen peak was tentatively decomposed into three components. The first one was attributed to oxygen singly bound to carbon or nitrogen O–(C, N), at a binding energy around 532.7 ± 0.3 eV, the second one was attributed O=C oxygen double bound carbon at a binding energy around 533.0 ± 0.4 eV, and the third to the group carboxylate (534.1 ± 0.4 eV) or waters (535.4 ± 0.3 eV). In the bacteria grown at 20 °C an additional peak (530.8 eV) was observed. It was also associated with the suboptimal growth temperature (Fig. 3). The line of carbon and oxygen are divided into different functional groups which is shown in Figs. 2 and 3. In Tables II and III the contributions of the functional groups in the total content of carbon and oxygen are calculated. The contribution of the individual functional groups changed with temperature culture.

TABLE II

Content of individual functional groups in the C 1*s* cell wall [%]. The numbers in brackets are related to the functional groups indicated in Fig. 2.

Sample	(1) C–(C, H)	(2) C–(O, N)	(3) C=O	(4) COOH	(6) Adline
B.subtilis 20 °C	26.48	16.63	7.39	3.69	6.16
B.subtilis 37 °C	28.13	22.38	11.51	1.92	–

TABLE III

Content of individual functional groups in the O 1*s* cell wall [%]. The numbers in brackets are related to the functional groups indicated in Fig. 3.

Sample	(1) O–(C, N)	(2) O=C	(3) COOH	(4) H_2O	(5) Adline
B.subtilis 20 °C	11.93	8.60	–	0.55	6.66
B.subtilis 37 °C	13.39	8.34	0.22	–	–

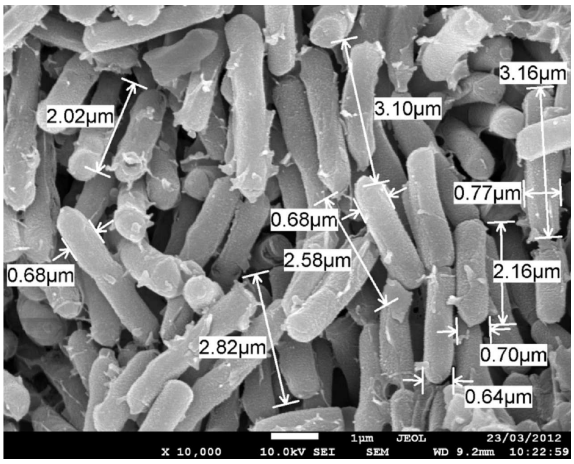

Fig. 4. Scanning electron micrograph of *Bacillus subtilis* in the optimum temperature 37 °C (used magnification ×10 000).

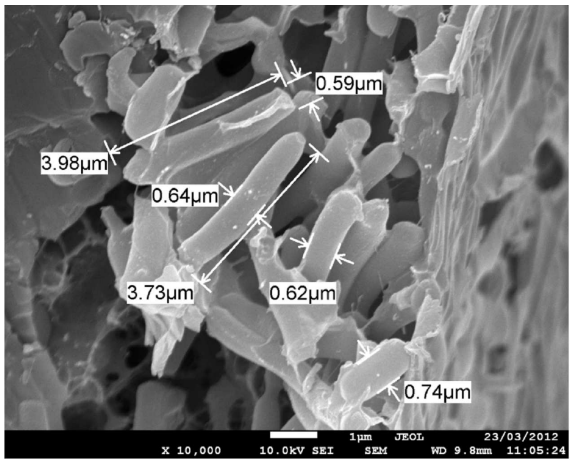

Fig. 5. Scanning electron micrograph of *Bacillus subtilis* in the temperature 20 °C (used magnification ×10 000).

Observations of the frozen bacteria in Cryo-SEM allowed to gain information about their surface structure, both at low and high magnifications and to determine their size. The image showing the bacteria grown at 37°C, that is the optimal temperature for *Bacillus subtilis* can be seen that the surface is smooth, as they are capsular strains when grown on solid medium. The length of these bacteria is 3.8 μm while the thickness of about 0.6 μm (Fig. 4). In the bacteria cultured for 48 h at 20 °C (Fig. 5) this area of the cells is rougher and the length of cells is reduced by about 1 μm (the average length of 3 μm and thickness 0.9 μm). Measurement uncertainty for the length determination is 0.05 μm.

4. Conclusion

In this paper we discussed the problem of the surface morphological and chemical changes in cells of *Bacillus subtilis* under the influence of different temperatures incubations. The length, thickness and smoothness of the surface of the bacteria grown in the optimal temperature are different than at a sub-optimal for that species of bacteria. Also, the electronic structure is different, and the deconvolution lines C 1*s* and O 1*s* in bacteria grown at temperature 20 °C revealed an additional peak. Its intensity was about 6%. This is due to immaturity of the cell wall surface. Presence of the additional lines indicates that the temperature is one of environment of the main factors affecting the chemical composition of the bacterial cell wall surface [7].

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