PROTEIN AND POLYSACCHARIDE DISTRIBUTION IN P. PESTIS GROWN AT 28° AND 37°

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A study of plague microbe cells, incubated on Hottinger agar at 28 and 37°, showed that they differ strongly in the amount of polysaccharide and contained approximately the same amount of protein (Bakhrakh et al., 1962). This was unexpected, since it is known that the capsular substance, formed by the plague microbe mainly at 37°, consists primarily of specific protein.

In the present work a determination was made of the content of protein and polysaccharide components in cells of the plague microbe, incubated at 28 and 37°, and in the capsular extracts obtained from them.

The plague microbes were incubated on Hottinger agar (pH 7.2) for 48 hours at 28° and [illegible] hours at 37°. A more prolonged incubation time for the plague microbe at 37° causes its slower growth under these conditions.

The plague microbe cells were washed from the agar with a physiological solution of sodium chloride, the microbial suspension was filtered through several layers of gauze, precipitated with cold acetone and dried in a vacuum dryer.

The dry cells were extracted with physiological solution. For this purpose, 1 g of cells was infused in 50 ml of physiological solution for 24 hours. Cells of the plague microbe were extracted by a similar method for obtaining soluble specific protein components of the cell -- fraction I, toxin (Baker et al., 1947, 1952). The suspension of cells in a physiological solution, buffered with a phosphate buffer (0.01 M) at pH 7.4, was shaken in a shaking machine for an hour. According to the data of Zhukov-Verezhnikov and Lipatova (1933), during the stated method of processing the cells of the plague microbe only the capsular antigens pass over into the solution. The suspension of cells was heated for an hour at 100° (in a boiling water bath). During this the thermostable antigens passed over into the solution.

For simplicity, in the future the stated extracts will be designated correspondingly 1, 2 and 3.

Following extraction the cell residue was washed off with acetone and dried in a vacuum-dryer. In the cells and extracts from them, the protein was determined by the intensity of the biuret reaction (Stickland, 1951), and the polysaccharide by the reaction with anthrone reagent (Gary et al., 1957). The monosaccharides were determined by the method of unidimensional ascending paper chromatography. For this purpose the perparations being investigated were hydrolyzed with a 1 N solution of sulfuric acid for five hours in sealed test tubes in a water bath. The hydrolyzates were passed through an SBS ion exchanging resin. The sugars were carefully washed from the column by water into an evaporating dish, evaporated to dryness in a water bath at 60--80°, diluted in a specific amount of water and applied to paper. The solvent was a mixture consisting of pyridine, benzene, butanol and water (3:1:5:3), and the developer was aniline phthalate in water saturated butanol.

The serological specificity of the cells and extracts were tested with the help of the polysaccharide hemagglutination reaction (in Sokolov's modification, 1945) and the precipitation reaction on agar plates (in Akimovich's modification, 1962). In the first case consideration was given to the maximum dilution of hapten, causing a distinct hemagglutination reaction on a slide, and in the second case -- the time for the appearance on the agar plate of a sharp line, corresponding to the line of precipitation formed by fraction I.

The results of the analysis of whole cells of the plague microbe support the earlier results: Cells incubated at 28° contain considerably less polysaccharide than cells incubated at 37° (table 1). A certain decrease in the amount of protein in cells grown at 37° was the result of an increase in these cells of polysaccharide. It is easy to be convinced of this if, during the calculation of the percentage content of protein, the amount of polysaccharide is taken into consideration.

The data obtained shows that during the extraction of cells of the plague microbe, incubated at 28 and 37°, a different amount of protein components passed into the solution. During extraction in the cold (extracts 1 and 2) of cells incubated at 37°, 36% of the protein contained in the cell passed into solution, while from the cells incubated at 28° half as much protein was extracted (18%). Though extraction under heating (extract 3) led to a considerable denaturing of the protein, the relationship between the amount of protein passing into solution in the cultures of the plague microbe, incubated at 37 and 28°, was preserved (27.1 and 11.9% correspondingly).

Apparently, in the cells of the plague microbe incubated at 37°, and forming a capsule under these conditions, a different localization of protein components is observed than in the cells incubated at 28°. The cultivation of the plague microbe at 37° leads to an increase in the amount of protein components located on the surface of the cell, while at the same time the total amount of protein in cells incubated at 28 and 37° remains constant.

The results obtained support the point of view expressed by a number of investigators, according to which the formation of a capsule by the plague microbe cultivated at 37° is explained, not by the strengthened production of this antigen, but by the loss, under these conditions, of some enzyme systems, leading to an accumulation on the surface of the cell of the products of the incomplete synthesis of protein substances (Burrows, 1960).

However, the increase of protein extraction, observed in cultures of the plague microbe incubated at 37°, may also be caused by other reasons, particularly by a change in the stability and penetrability of the surface layers of the cell. It is not ruled out that under the stated conditions of cultivation, in the cell the ratio of proteins with a various solubility changes.

Below the results are presented of the precipitation reaction in standard agar plates, from which, as it should be expected, it is apparent that the serological specificity (based on the presence of fraction I) of cultures of the plague microbe incubated at 37°, and the extracts from them, is considerably higher than the corresponding cultures, incubated at 28°, and the extracts from these cultures (table 2). It should be noted that the heating of cells of plague microbe at 100° for an hour did not lead to a marked lowering of the serological activity of fraction I.

As regards the polysaccharide components, their total amount in the cell increased sharply in cultures incubated at 37°; accordingly their amount increased in the extracts of these cultures (see table 1).

During an analysis of the results obtained it must be taken into consideration that in the given case we were dealing with the unwashed cells of the plague microbe, due to which, unconditionally in the cells there was a specifically increased amount of polysaccharide. However, an analogous ratio of polysaccharide components in cultures of the plague microbe incubated at 28 and 37° was also observed during an analysis of washed cells (Bakhrakh et al.).

The difference in the quantitative composition of monosugars in cells of the plague microbe, incubated at 28 and 37°, is clearly seen in figure 1 (upon obtaining a chromatogram, all the quantitative relationships were observed as far as possible).

However, the percentage of polysaccharides passing into solution during extraction of the cells remained approximately the same, regardless of at which temperature (28 or 37°) the cells of the plague microbe were incubated. In the case of extraction in the cold it comprised 24--26, and during heating - 56--60. In this manner it can be considered that a change of cultivation temperature does not speak for the localization of polysaccharide components in the cell.

However, in the tests it was noted that, regardless of the temperature

at which the culture was incubated, the polysaccharide which contained arabinose passed into the extract considerably easier, and the polysaccharide in which glucose was detected -- with more difficulty. This is clearly seen in figure 2 which represents the chromatograms of monosugars, detected after hydrolysis in the extracts and residues of cells incubated at 37°, following extraction in the cold (extract 1) and during heating (extract 3). Following extraction in the cold almost all the arabinose was detected in the extract, but glucose -- in the residue of the cells after extraction; even as a result of prolonged heating of the cells it wasn't possible to transfer all the glucose into the extract.

The data obtained correspond with the results of the investigation of Seal (1951), who detected arabinose in the polysaccharide of a plague microbe, isolated from the supernatant fraction of a broth culture, and also of Davies (1956) and our data (Bakhrakh et al., 1958), which verified that the basic component of the somatic polysaccharide of the plague microbe is glucose.

Thus, the assumption expressed by us in the previously cited work confirms the existence in the plague microbe of two specific carbohydrate components: The capsular, which includes arabinose, and the somatic, containing glucose. As regards galactose, xylose and ribose, which are detected in large quantities in cells of the plague microbe, then the presence of the latter is explained by the presence of nucleoproteides in the cell, and the presence of the first two is the result of incubation of the plague microbe on an agar medium, the main components of which are galactose and xylose. The amount of galactose in a culture of the plague microbe incubated at 37° is increased as a result of the accumulation of this sugar by the plague microbe at the stated temperature conditions, which are less favorable for its growth than 28°.

The nonspecificity of galactose is testified to by the results of the hemagglutination reaction with hapten, which was isolated from the plague microbe by hydrolysis with a strong alkali under heating (see table 2). The serological specificity of this reaction, as is known, is determined by the concentration of specific polysaccharide contained in the cells. The data obtained by us showed that cells of the plague microbe, incubated at 37° and containing large quantities of galactose, and the extracts from them, had a considerably lower titer of hemagglutination than the cells of the plague microbe incubated at 28° and the extracts obtained from them. It must be pointed out that in cells of the plague microbe incubated at 28°, there was approximately twice as much galactose as in the corresponding cells incubated at 37°.

Conclusions

1. The cultivation of the plague microbe on an agar medium at 37°, characterized by the increased formation of capsule under these conditions, led to an increase in the amount of easily extracted protein components, while at the same time the total content of protein in cells incubated at

28 and 37° remained constant. A change of incubation temperature did not show up in the extraction ability of the polysaccharide components of the cell.

2. Regardless of the temperature of incubation of the culture, the polysaccharide which contained arabinose passed into the extract with considerable ease, and the polysaccharide containing glucose -- with much difficulty. This supports the supposition of the existence in the plague microbe of two specific carbohydrate components: The capsular -- containing arabinose, and the somatic -- containing glucose.

Literature

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Table 1

Content of protein and polysaccharide in cells of the plague microbe and extracts from them

Preparation	Amount of protein				Amount of polysaccharide			
	at various incubation temperature							
	28°		37°		28°		37°	
	in mg.	in %	in mg.	in %	in mg.	in %	in mg.	in %
Cells	750	100	690	100	42	100	103	100
Extract 1	142	18.9	254	36.8	10.1	24	25.3	24.5
Extract 2	140	18.7	250	36.2	11.2	26.6	28.1	27.1
Extract 3	91	12.1	187	27.1	25.4	60.3	56.2	54.3

Table 2

Serological activity of cells of the plague microbe and extracts from them

Preparation	Time of appearance of precipitaion lines (in hours)		Titer of hemagglutination reaction	
	at various temperature			
	28°	37°	28°	37°
Cells	78	10	1:100 000	1:40 000
Extract 1	14	4	1:200	1:40
Extract 2	14	4	1:200	1:40
Extract 3	14	6	1:500	1:40

Remarks. When setting up the precipitation reaction we took 0.2 mg of dry cells or 0.1 ml of the corresponding extract. The titer of the hemagglutination reaction represents: For the cells -- the maximum dilution of hapten, prepared on the basis of 1 g of dry cells in 100 ml of solution; for the extracts -- the maximum dilution of hapten, prepared from the corresponding extract.

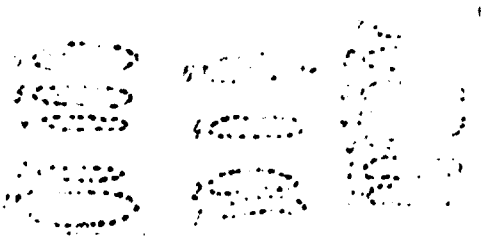

Figure 1. Carbohydrate composition of cells of the plague microbe, incubated at various temperatures.
Reference spots: 1 - galactose, 2 - glucose, 3 - mannose, 4 - arabinose, 5 - xylose, 6 - ribose, 7 - rhamnose.

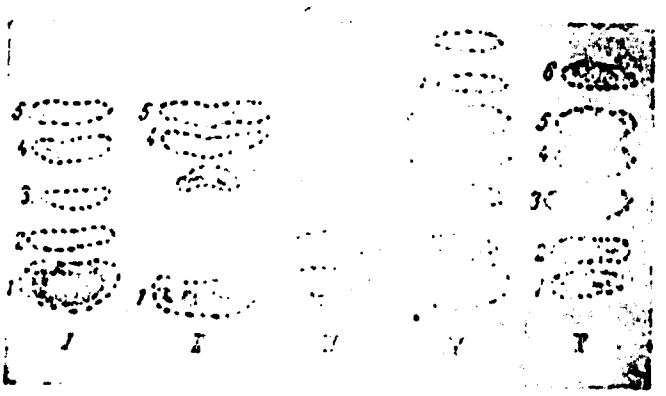

Figure 2. Carbohydrate composition of extracts and cells after extraction: I - cells after extraction in the cold; II - extract 1; III - cells after extraction with boiling; IV - extract 3; V - reference spots: 1 - galactose, 2 - glucose, 3 - arabinose, 4 - xylose, 5 - ribose, 6 - rhamnose.