PROPERTIES OF BACTERIAL PROTOPLASTS AND SPHEROPLASTS*

G. I. Fedorova

UDC 576.8.094

Lysozyme protoplasts of Bacillus megaterium, Micrococcus lysodeikticus, Salmonella typhi, and Salmonella typhimurium possess well-marked osmotic fertility, are Gram-negative, and their cell wall cannot be detected by the method. Penicillin and glycine spheroplasts of S. typhi and S. typhimurium have lower osmotic fragility than lysozyme spheroplasts, remnants of the cell wall can be detected in them by Knaysi's method, but they are Gram-negative. Lysozyme, penicillin, and glycine spheroplasts of S. typhi and S. typhimurium are agglutinated by antisera against whole cells, but in much lower titers than whole cells. Respiration of moderate intensity is found in the penicillin spheroplasts of Salmonella gallinarum and Escherichia coli, but it is weaker than respiration of the whole cells.

* * *

No systematic experimental studies of the properties of protoplasts and spheroplasts of different microorganisms could be found in the literature [1, 3, 4-13].

*The investigation was carried out in the Department of General Medical Microbiology, N. F. Gamaleya Institute of Microbiology and Epidemiology, Academy of Medical Sciences of the USSR, Moscow.

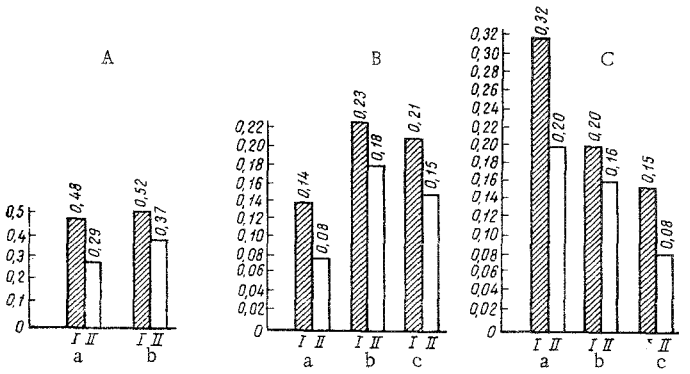

Fig. 1. Optical density of whole cells and their protoplasts and spheroplasts (determined in the FÉK-IM photoelectric colorimeter). I) Whole cells; II) protoplasts or spheroplasts. A: a) Lysozyme of B. megaterium No. 654; b) lysozyme of M. lysodeikticus No. 2665; B: a) lysozyme of S. typhi No. 5606; b) penicillin S. typhi No. 5606; c) glycine S. typhi No. 5606; C: a) lysozyme S. typhimurium; b) penicillin S. typhimurium No. 3048; c) glycine S. typhimurium No. 3048.

D. I. Ivanovskii Institute of Virology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. M. Zhdanov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 68, No. 10, pp. 66-69, October, 1969. Original article submitted February 6, 1968.

TABLE 1. Morphology and Osmotic Fragility of Bacterial Protoplasts and Spheroplasts

Types of bacteria	Protoplasts and spheroplasts	Morphology under phase-contrast microscope	Staining properties		Osmotic fragility			
					lysis		degree of destruction (in %) on centrifugation	
			by Gram's method	by Knaysi's method	distilled water	spontaneous	3000 rpm	6000 rpm
Gram-positive	Lysozyme protoplasts B. megaterium No. 654	Optically dense spheres 4-6μ in diameter	–	–	+	+	13.5	27
	Lysozyme protoplasts M. lysodeikticus No. 2665	Same, 5-7 μ in diameter	–	–	+	+	10.8	25.1
Gram-negative	Lysozyme spheroplasts S. typhi No. 5606	Optically dense spheres: diameter 3-5 μ	–	–	+	+	5.0	15.4
	Penicillin spheroplasts S. typhi No. 5606	"4-12"	–	+	+	–	3.6	8.9
	Glycine spheroplasts S. typhi No. 5606	"4-10"	–	+	±	–	1.6	2.1
	Lysozyme spheroplasts S. typhimurium No. 3048	"3-5"	–	–	+	+	4.8	8.2
	Penicillin spheroplasts S. typhimurium No. 3048	"4-11"	–	+	+	–	2.0	3.1
	Glycine spheroplasts S. typhimurium No. 3048	"5-11"	–	+	+	–	2.5	3.4

The object of the present investigation was to study the following properties of protoplasts and spheroplasts of Gram-positive and Gram-negative bacteria isolated by the writer: 1) optical density compared with that of whole cells; 2) morphology under the phase-contrast microscope; 3) behavior toward Gram's strain; 4) presence or absence of a cell wall (by staining by Knaysi's method); 5) osmotic fragility as characterized by lysis in distilled water, degree of destruction during centrifugation, and ability to undergo spontaneous lysis; 6) serologic properties; 7) respiration of spheroplasts of Gram-negative cultures.

EXPERIMENTAL METHOD AND RESULTS

The above-mentioned properties were studied in the following objects: 1) lysozyme protoplasts of Bacillus megaterium No. 654 and Micrococcus lysodeikticus No. 2665; 2) lysozyme, penicillin, and glycine spheroplasts of Salmonella typhi No. 5606 and Salmonella typhimurium No. 3048; 3) penicillin spheroplasts of Salmonella gallinarum No. 7979, S. gallinarum No. 398, Escherichia coli No. 344, and E. coli K-12.

The results obtained are given in Fig. 1 and Tables 1-3.

TABLE 2. Serologic Properties of Spheroplasts of Gram-Negative Bacteria

Strain of bacteria	Materials investigated	Titer in agglutination reaction with serum against whole cells
S. typhi № 5606	Whole cells	1:3 200(++++)
	Glycine spheroplasts	1:400(++++)
	Penicillin spheroplasts	1:400(+++)
	Lysozyme spheroplasts	1:100(+++)
S. typhimurium № 3048	Whole cells	1:3 200(++++)
	Glycine spheroplasts	1:800(+++)
	Penicillin spheroplasts	1:400(+++)
	Lysozyme spheroplasts	1:100(+++)

Note. Serologic properties of lysozyme, penicillin, and glycine spheroplasts of S. typhi No. 5606 were studied by the agglutination reaction with standard typhoid serum prepared by the L. A. Tarasevich State Control Institute for Medical Biological Preparations, and in the case of S. typhimurium No. 3048, with serum obtained in the laboratory by immunization of rabbits with S. typhimurium No. 3048 cells.

TABLE 3. Respiration of Penicillin Spheroplasts of Gram-Negative Bacteria

Strain	Respiration			No. of cells not converted into spheroplasts (in %)
	of spheroplasts	of whole cells	of cells not converted into spheroplasts (in %)	
S. gallinarum № 7979	50	278	—	1,4
» » № 398	37	248	9	0,5
E. col; № 844	56	169	10	2,2
» » K-12	94	182	23.	7,3

Note. Respiration measured in mm^3 oxygen absorbed/mm^3 suspension/30 min by Warburg's manometric method.

The investigations showed that the protoplasts and spheroplasts differed both morphologically and in their degree of osmotic fragility. Neither protoplasts nor spheroplasts stained by Gram's method. As a rule the penicillin and glycine spheroplasts were larger than the lysozyme. The cell wall of lysozyme protoplasts and spheroplasts did not stain by Knaysi's method. Remains of the cell wall were detected in penicillin and glycine spheroplasts of Gram-negative cultures. The protoplasts and spheroplasts differed in their optical density and osmotic fragility; lysozyme protoplasts and spheroplasts had a lower optical density and were more fragile than penicillin and glycine. This suggests that the penicillin and glycine spheroplasts were more stable than the lysozyme, probably because of partial preservation of their cell wall.

The low titers in the agglutination reaction with lysozyme spheroplasts were evidently associated with more complete destruction of the cell wall. Almost complete agreement between the titers in the agglutination reaction for types of spheroplasts obtained from both strains will be noted.

The moderately intensive respiration of the penicillin spheroplasts of Gram-negative cultures was observed, although it was always much lower than the respiration of whole cells: in this respect the present results differ from those obtained by other workers [1-3, 12] for respiration of lysozyme spheroplasts, according to whom their respiration was almost indistinguishable from that of whole cells.

LITERATURE CITED

1. V. N. Gershanovich, A. V. Avdeeva, and D. M. Gol'dfarb, Biokhimiya, No. 4, 700 (1963).
2. D. M. Gol'dfarb, A. V. Avdeeva, and N. B. Borisova, Mikrobiologiya, No. 5, 870 (1962).

3. A. I. Oparin, N. S. Gel'man, and I. G. Zhukova, Dokl. Akad. Nauk SSSR, 105, No. 5, 1036 (1955).
4. M. H. Jeynes, Exp. Cell Res., 24, 255 (1961).
5. J. Lederberg and J. St.-Claire, J. Bact., 75, 143 (1958).
6. R. McQuillen, Biochem. Biophys. Acta, 18, 458 (1955).
7. J. Z. Montgomerie, G. M. Kalmanson, and L. B. Guze, J. Lab. Clin. Med., 70, 539 (1967).
8. M. R. J. Salton and F. Shafa, Nature, 181, 1321 (1958).
9. J. Sud, Indian J. Path. Bact., 10, 226 (1967).
10. J. W. Vennes and P. Gerhardt, Science, 124, 535 (1956).
11. C. Weibull, J. Bact., 66, 688 (1953).
12. C. Weibull, Exp. Cell Res., 9, 139 (1955).
13. M. Welsh, J. Gen. Microbiol., 18, 491 (1958).