

# MICROBIOLOGY AND IMMUNOLOGY

## COMPARATIVE STUDY OF THE PHOSPHATASE ACTIVITY OF TYPHOID BACILLI OF VARIED VIRULENCE

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The study of the metabolic peculiarities of pathogenic microorganisms of different degrees of virulence is of both theoretical and practical importance. During investigation of phosphatases, enzymes widely distributed among bacteria, a definite relationship was observed between the pathogenic properties of staphylococci and micrococci and the presence of phosphatase activity in these organisms [2, 3, 5, 8, 10, 12]. It has been shown that virulent strains of mycobacteria possess much lower phosphatase activity than avirulent or saprophytic strains [7]. In the typhoid bacilli a relationship has also been established between phosphatase activity and virulence [11]. However, the investigation just cited was carried out on strains unconnected genetically with one another, so that inter-strain differences in the activity of the enzyme could not be ruled out [4, 6].

In the present investigation the phosphatase activity was studied in typhoid bacilli of different grades of virulence in groups of strains of identical origin.

### EXPERIMENTAL METHOD

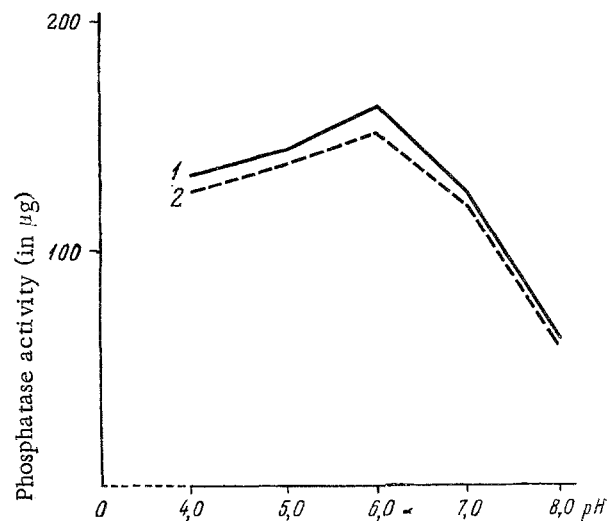
The following groups of strains were studied: 1) *Salmonella typhi* ty2 and an avirulent variant not containing Vi-antigen, obtained by the action of tryptaflavine, 2) *Salmonella typhi* 58 (Panama) and its rough variant 58R, having lost three antigens (H, O, and Vi); 3) *Salmonella typhi* B (laboratory strain) and two of its streptomycin-resistant variants: one little different as regards virulence (Bc4) and the second of low virulence (Bc4M), preserving its antigenic structure and immunogenicity.

Experiments were conducted on 18-h cultures of strains ty2, ty58, 58R, tyB, and Bc4, and 36-h cultures of variants with a slower rate of proliferation — Bc4M and ty2-tryptaflavine (at periods corresponding to the beginning of the stationary phase of growth of each of the test strains).

The bacterial mass was cultivated at 37° in 2% meat-peptone agar and washed with bidistilled water. The cells were separated by centrifugation and rinsed twice with water, and a 20% suspension was made of the washed bacteria. To determine the phosphatase activity 0.3 ml of suspension was placed in a test tube containing 1.4 ml of acetate (pH 4.0 and 5.0) or Tris-maleic (pH 6.0, 7.0, and 8.0) buffer solution, and 0.3 ml of a sodium  $\beta$ -glycerophosphate solution (in a final concentration of 0.01 M). Incubation was carried out at 37° for 1 h, after which 1 ml of 30% trichloroacetic acid solution was added to each tube and the inorganic phosphate was then estimated colorimetrically in the resulting protein-free centrifugate [1]. In the case of the control tube trichloroacetic acid was added previously, before addition of the bacteria.

### EXPERIMENTAL RESULTS

Preliminary experiments using a highly virulent strain ty2 and an avirulent variant 58R showed that of the five pH values tested (4.0, 5.0, 6.0, 7.0, 8.0), the optimal for decomposition of sodium  $\beta$ -glycerophosphate is pH 6.0 (see figure), which is in agreement with the data obtained for typhoid bacilli [9]. In the subsequent experiments the phosphatase activity was therefore studied at pH 6.0.



Effect of pH on phosphatase activity of typhoid bacilli.  
1) Strain ty2; 2) strain 58R.

Phosphatase Activity in Typhoid Bacilli of Different Grades of Virulence

Group	Note	LD <sub>50</sub>	Inorganic phosphorus (in micrograms per 10 mg dry weight of bacilli)	
1st	ty2	$3.1 \cdot 10^7$	Experiment dated June 7, 1962	Experiment dated June 14, 1962
			179	184
	ty2-trypaflavine	$1.0 \cdot 10^9$	165	160
2nd	ty58	$2.0 \cdot 10^8$	Experiment dated June 7, 1962	Experiment dated June 13, 1962
			196	168
	58R	$5.5 \cdot 10^8$	180	153
			Experiment dated April 24, 1962	Experiment dated April 26, 1962
3rd	tyB	$1.0 \cdot 10^8$	253	281
	Bc4	$2.0 \cdot 10^8$	274	301
	Bc4M	$4.6 \cdot 10^8$	278	274

Note. Two typical experiments with each group of strains are shown in the table.

It is clear from the table that all the strains of typhoid bacillus tested possessed comparatively high phosphatase activity. The results give some idea of the significance of strain differences in the enzymic activity of the cultures. Genetically related cultures are characterized by closely similar values of phosphatase activity. The indices of strains of ty2 and ty58 were close. The whole group of strain tyB possessed the highest acid phosphatase activity.

No relationship could be established between the phosphatase activity and the antigenic structure of the strains studied. Strains ty2 and ty58, possessing all three antigens Vi, O, and H, gave practically the same phosphatase activity as the corresponding variants without the Vi antigen alone (ty2-trypaflavine) or without all three antigens (58R). The difference of 8-9%, in our opinion, may be disregarded.

Nor did the enzymic activity of the bacilli reflect any differences in the virulence of strains which were different in origin (ty2, ty58, tyB) or which were genetically related (ty2 and ty2-trypaflavine; ty58 and 58R; tyB, Bc4, and Bc4M).

Our findings demonstrating that no relationship exists between the virulence of typhoid bacilli and their phosphatase activity do not agree with the results obtained by Shrivastava and co-workers [11]. The probable explanation of this difference is that the workers cited studied the activity of the enzyme at pH 8.0, whereas the optimum pH for decomposition of sodium  $\beta$ -glycerophosphate in our experiments was 6.0. This corresponds to the findings of other workers [9].

#### SUMMARY

The investigation covered three genetically associated cultural groups, which included the following strains: 1) ty2 and ty2-tryptaflavine, an avirulent mutant devoid of the Vi-antigen, derived from the first under the effect of tryptaflavine; 2) ty58-(Panama) and its rough avirulent variants 58R; 3) tyB and the mutants Bc4 and Bc4M derived from it and resistant to streptomycin, varying by their virulence.

It was demonstrated that all of the strains of the above bacteria break down sodium Betaglycerophosphate, at pH = 6, at a relatively high rate. The highest enzyme activity was found to be common to the strain tyB and to the variants derived from the latter. No differences in the phosphatase activity of virulent and avirulent bacterial cultures of the typhoid bacillus could be found after comparing strains of different origin, or genetically related strains.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.