

Aryl Sulphatase Activity in *Salmonella typhosa*

The presence of aryl-sulphatase in bacteria remained obscure till the studies of BARBER *et al.*¹ who tested 160 strains of *Micrococcus pyogenes* and seventy-five of coagulase negative staphylococci and found that two strains gave aryl-sulphatase positive reaction, whereas an aerobic spore forming bacillus showed a greater activity. Later WHITEHEAD *et al.*² in the course of a survey of a wide range of bacterial species, tested a few strains of *Salmonella* and *Mycobacteria* and found that most of the *Salmonella* strains with the exception of *S. paratyphosa* were aryl-sulphatase negative, whereas the *Mycobacteria* gave a positive reaction. ARORA *et al.*³ while surveying the aryl-sulphatase activity in various pathogenic bacteria reported faint aryl-sulphatase activity in *S. typhosa* on five days incubation. It was, therefore, considered of interest to see if there were any differences in the enzyme activity of the various antigenic strains of *S. typhosa*. The results obtained are described in the present communication.

The following strains of *S. typhosa* were used:

- (1) BHATNAGAR's ViI⁴ strain having predominantly the Vi antigen and no H antigen at all (O inagglutinable low virulence).
- (2) WATSON's V strain possessing all the three Vi, O and H antigens (O inagglutinable, relatively of high virulence).
- (3) Ty2 having all the three Vi, O and H antigens (O inagglutinable, higher virulence to mice than WATSON's V).
- (4) TyS₁ having all the three antigens (O agglutinable, virulent, locally isolated).
- (5) 0-901 possessing O antigen only (highly sensitive to O agglutinins and of low virulence).
- (6) H-901 having H and O antigen (O agglutinable, low virulence).

The substrate used was potassium-2-hydroxy-5-nitro-phenyl sulphate prepared by the method of SMITH⁵. 0.001 M of the substrate was incorporated in the papain meat broth and the pH was adjusted to 7.0. 5 ml amounts were dispensed in tubes, autoclaved and inoculated with a loopful of a 24 h growth of the cultures grown on the beef heart infusion agar of pH 7.4. After the specified period of growth as indicated in the table, 1 ml of 1 N NaOH was added to each tube and intensity of red colour was noted.

It will be observed that ViI culture showed the maximum activity giving a positive reaction at the end of 24 h whereas the 0-901 gave a faint reaction after five days and the H-901 and WATSON's V did not show any activity at all during the same period. Three cultures ViI, Ty2 and TyS₁ were further tested for aryl-sulphatase after mouse passage but were found to show no activity.

The results show marked differentiation between the three pure antigenic strains. ViI containing almost entirely the Vi antigen shows the best activity, while 0-901 shows a slight reaction on the 5th day and H-901

Aryl-Sulphatase Activity of the Different Strains of *Salmonella typhosa*

Days	Strains	1	2	3	5
	ViI	+	++	++++	++++
	0-901	—	—	—	±
	H-901	—	—	—	—
	WATSON's V	—	—	—	—
	Ty2	—	—	±	+
	TyS ₁	—	—	—	—

± faint orange; + pale orange; ++ orange; ++++ deep red; — no change.

no activity at all. On the other hand, WATSON's V and TyS₁ do not show any activity although Vi antigen is present in them in significant amounts. It is possible that as far as aryl-sulphatase activity is concerned, it is being interfered with by the presence of H and O antigens. This will find some support in the observations with Ty2 strain, which in spite of the presence of O and H antigens shows a slight activity probably because of a much larger content of Vi antigen than in case of WATSON's V and TyS₁. It may be pointed out that ViI has been reported earlier¹ to have the maximum metabolic activity towards L-glutamic acid and L-tyrosine.

The loss of aryl-sulphatase activity in strains ViI and Ty2 after the enhancement of their virulence by mouse passage is interesting. The significance of this in metabolism of *S. typhosa* as such or in relation to the host is not clear at present. This will have to await a more detailed investigation of the metabolism of the organism.

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Zusammenfassung

Es wurde die «Aryl Sulphatase»-Aktivität verschiedener Stämme von *Salmonella typhi* geprüft. Als Substrat wurde «K-2-hydroxy-5-nitro-phenyl-sulphate» verwendet.

ViI, Ty2 und 0-901 waren wirksam, wobei ViI die höchste Aktivität zeigte. Nach der Mäuse-Passage verloren die Stämme ViI und Ty2 ihre Aktivität.

¹ M. BARBER, B. W. L. BROOKSHANK, and S. W. A. KAUFER, J. Path. Bact. 63, 57 (1951).

² J. E. M. WHITEHEAD, A. R. MORRISON, and L. YOUNG, Biochem. J. 51, 585 (1952). — J. E. M. WHITEHEAD, P. WILDY, and H. C. ENGBAER, J. Path. Bact. 65, 451 (1953).

³ K. L. ARORA, A. T. DUDANI, and C. R. KRISHNAMURTI, J. Sc. Ind. Res. 12 [B], 502 (1953).

⁴ S. S. BHATNAGAR, C. G. J. SPEECHLY, and M. SINGH, J. Hyg. 38, 663 (1938).

⁵ J. N. SMITH, J. Chem. Soc. 1951, 2861.