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ISOLATION OF BACTERIOPHAGE RECEPTOR SUBSTANCES FROM SHIGELLAE

by J. Beumer and J. Dirkx.
(Pasteur Institute in Brussels)

It is well known nowadays that bacteriophages can be fixed and made inactive by the use of specific substances derived from sensitive bacteria. There is good reason to believe that these substances are simply the bacterial surface receptors to which the phages attach themselves at the moment of infection, and which hence govern the bacteria's sensitivity to a given bacteriophage. The phage particle attached to the isolated receptor is deactivated, either because the receptor prevents the phage from digging in with its tail, or because, as has been shown in several cases [1, 2] the phage's desoxyribonucleic acid has been expelled. In a small number of systems, it has been possible to discover the chemical nature of these receptors [3, 4].

This note describes the methods that enabled us to extract from a number of *Shigellae* strains the receptors for the phages of the lysogenic coliform *B. Lisbonne* (H-, H+ and V) described earlier [5].

Material — Recall that the H- phage, spread on the F6S, or H-F6S strain, yields from 200 to 1,000 times fewer stains when titred on the SHP B or F6R strain than it does on the F6S strain. After transfer to the SHP B strain, the phage obtained, known as H-SHP B, is about 1000 times as effective on the SHP B and F6R strains as is the H-F6S strain, but keeps the high titre of the latter on F6S [6].

The *Shigellae* strains used were described in earlier papers [7, 8]: an R strain of *Shigella dysenteriae* SHP B); an S strain of *Shigella flexneri* of serological type 5 (F6S) and its mutant R (F6R); a strain of *Shigella flexneri* of serological type 2a (F263). The sensitivity of these strains to the four phages studied is shown on Table 1.

TABLE I. — Spreading effectiveness of the phages of B. Lisbonne on the strains of Shigella studied (expressed in number of stains per millimeter).

Strains	Phage			
	H-F6S	H-SHP B	H/	V
SHP B	2.8×10^6	5×10^9	1.4×10^6	1.2×10^{10}
F6S	5.5×10^8	2.5×10^9	1.5×10^5	7.5×10^9
F6R	1.2×10^6	3.5×10^9	1×10^5	4×10^9
F 263	5×10^8	3.7×10^9	1.7×10^5	1.5×10^8

Methods. — During our preliminary tests, we experimented with a number of extraction methods, particularly the following: extraction by distilled water at 65º, or by urea 7M, or by pyridine at 50p.100 (Goebel, [9]), germs living or killed by heat, formol, phenol or chloroform; treatment of living germs with 0.4% soda (Weidel [4]); soda extraction at pH 10 of germs previously cold-treated with trichloracetic acid, either at pH 3.5 (Bordet & Beumer [10]) or in a 5% concentration (Beumer [11]). Details of the results obtained with all these methods will be published at a later date.

Two methods yielded us particularly active extracts: one was cold extraction with trichloracetic acid (Boivin & Mesrobeanu [12]), the other heat extraction with phenol (Westphal, Lüderitz & Bister [13]).

1º Cold extraction with trichloracetic acid. — The bacteria were cultured in a liquid medium, LB [14], with aeration, for 7 to 8 hours. They were then washed twice in physiological water, and suspended in distilled water; we added trichloracetic acid to an end concentration of 5%, then let it stand at +4ºC for 3 hours. After centrifuging, the remainder is neutralized and precipitated by 3 volumes of 94º ethyl alcohol to -10º, then kept overnight at that temperature. The precipitate obtained is dissolved in a small volume of distilled water and dialyzed for 5 days at +4ºC. The dialyzed solution is filtered over an L3 Chamberland lamp, and precipitated again with 3 volumes of 94º ethyl alcohol, under the same conditions as the first time. The precipitate is washed twice in alcohol and dried in a vacuum. This method, thanks to which Boivin and his co-workers, as well as many others since then, particularly A. M. Staub, obtained the somatic antigen for many of the Enterobacteria, is applicable only to the S bacteria.

2º Hot extraction with a water-phenol mixture. — The cultures, obtained and washed as described above, are suspended in distilled water

and brought to 68°; to this is added an equal volume of 90% phenol, and the mixture is kept at 68° for 20 minutes, shaking frequently. Centrifuging separates out the aqueous phase which contains the active substance. After dialysis in distilled water at 4° for 2 days, the extract is precipitated with 10 volumes of 94° ethyl alcohol at -10° overnight. The precipitate is washed twice in alcohol, then dried in a vacuum. Before use, the precipitate, dissolved again in distilled water, is filtered over an L3 Chamberland lamp. This method, which enabled us to get active extracts of R bacteria, is equally effective with S bacteria. Westphal and his co-workers used this method to isolate the specific lipopolysaccharides of several Enterobacteria, and Davies used it again more recently to prepare the lipopolysaccharide of an R strain of Shigella dysenteriae [15].

The demonstration of phage fixation by means of bacterial extracts obtained in this way is performed by mixing a dilute suspension of the phage containing about 5×10^3 particles per millimeter with decreasing doses of the extract, then letting the mixtures stand at 37° for 3 to 6 hours, according to the phages used. The composition of the medium in which the fixation occurs can be of extreme importance, as we point out elsewhere [16]. The non-fixed phage in each of the mixtures is then titred, and its percentage determined in relation to a control specimen incubated without extract under the same conditions. The activity of the extracts is generally expressed in receptor units per milligram (1 receptor unit - the smallest concentration of extract, leaving 50% of the phage unfixed under the conditions of the experiment).

RESULTS. — The trichloracetic extract (TCA) of the S strains has a very high fixing activity on the four phages studied. The aqueous phase of the extract obtained by treating these strains with hot phenol (Aq68) is generally less active than the TCA extract. On the other hand, the Aq68 extract of the R strains is very vigorous in fixing the H-SHPB and V phages; however, it shows very weak activity against the H-F6S and H/ phages. The TCA extract of the R strains fixes none of the phages studied (Table II).

TABLE II - Extract activity in receptor units per milligram

Extracts		Phages			
		H-F6S	II-SHP B	11/	V
SHP B	Aq 68	2 - 10	6.250	2 - 10	1.250
F6 S	TCA	6.250	6.250	>1.250	>250
F6 S	Aq 68	>250	250	250	>250
F6 R	Aq 68	10	1.250	<2	>1.250
F 263	TCA	31.250	>250	>250	>2 and <250
F 263	Aq 68	250 - 1.250	10 - 50	>2 and <250	>2 and <250

Extract activity is an accurate index to the sensitivity of bacterial strains to the H-F6S and H-SHPB phages. The Aq68 extract of the R strains is only mildly fixative against the former, but very active against the latter, to which they are far more sensitive. The TCA extract of the S strains, on the contrary, is highly fixative of both phages.

The slight activity of the Aq68 extract of the R strains on the H/ phage can be due to the fact that this extract contains only a very little of the receptor for this phage, or again to the fact that the media used hitherto for fixation demonstration are not suitable. Research is still continuing along this line.

The TCA extract of F6S is precipitated with anti-F6S serum, and the remaining liquid contains no trace of the fixative substance for the H-F6S or H-SHPB phages. We were unable to obtain any serum very active against the SHPB strain. However, although this serum is only a weak precipitant for the Aq68 extract of this strain, it does remove its capacity to fix the H-SHPB phage.

Physical and chemical study of the receptor substances, while still incomplete, has already made it possible to establish certain data, which we shall sum up briefly.

As we remarked earlier, filtration over a Chamberland L3 lamp does not diminish the activity of the TCA or Aq68 extracts of any of the several strains we studied.

1º TCA extract of F6S. — This extract apparently consists of a single substance; in any case, it has a homogeneous appearance under electrophoresis. The diffusion coefficient $D_{20º}$ is on the order of 6.10^{-8} $cm^2.sec^{-1}$, a very low value strongly indicative of marked molecular dissymmetry. Electrophoretic mobility is also very low, on the order of 6.10^{-6} $cm^2.volt^{-1}.sec^{-1}$ at 1ºC in a maleate buffer of Na 10^{-2}M, ionic power 0.03, pH 7.3. This low value, corresponding to a potential of about 2mV, is so weak that we can consider the molecule as having practically no charge.

Chemical analysis shows the presence of fatty acids, hexoses, methylpentoses, hexosamines, and small quantities of aldoheptoses. It would certainly seem therefore that this is a lipopolysaccharide similar to the one Jesaitis and Goebel extracted from Shigella sonnei, phase II /3/, which has the ability to combine with the phages of the T series, to which this bacterium is sensitive.

2º Aq68 extract of SHPB. — The Aq68 extract is heterogeneous, and, under electrophoresis, shows three components. One of these, which can be sedimented by ultracentrifuging, contains the active material. This

material is very unevenly distributed, with particle diameters ranging from 20 to 400 millimicrons. The fraction containing particles whose size lies between 50 and 100 millimicrons is itself heterogeneous under electrophoresis, revealing the presence of two components, the slower of which is slightly more active. The faster component, on the other hand, has an activity level identical with that of the particles of other sizes. With the exception of the slow component, all the particles, no matter what their size, have the same mobility rating of $1.335 \cdot 10^{-4}$ cm^2 $volt^{-1}$ sec^{-1} at $+1^{\circ}C$, or a potential of about 50mV, if we assume that von Smoluchowski's conditions are present. As for the slow component, its mobility at $+1^{\circ}C$ is $1.14 \cdot 10^{-4}$ $cm^2.volt^{-1}.sec^{-1}$, corresponding to a potential of about 42mV.

Unlike the TCA extract, the active substances in the Aq68 are highly charged, and markedly heterogeneous.

Chemical analysis of the active fraction of Aq68 is still fragmentary. It has shown the presence of fatty acids and sugars whose chemical nature has not yet been established. This work is currently under way.

SUMMARY

Isolation from Shigellae of receptor substances for bacteriophages.

Receptor substances for the phages of the lysogenic strain B. Lisbonne were prepared from Shigellae strains. Extraction in the cold with 5 p. 100 trichloracetic acid was found convenient for isolating the receptors from the S strains. Those from the R strains could be extracted by treating the bacteria suspended in water with an equal volume of 90 p. 100 phenol at 68° C. The receptor substances contain sugars and fatty acids and might be lipo-polysaccharids. Purification and chemical analysis of these receptors are in progress.

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