Aggregationsfähigkeit verloren, und zwar im Falle von Alanin stärker als von Valin.

Wichtig für die Frage der Temperatursensitivität, d. h. den Verlust der Fähigkeit zu geordneter Aggregation der Proteinuntereinheiten unter bestimmten Bedingungen, ist die Kombination einer bestimmten Aminosäure mit einer bestimmten Position innerhalb der Proteinkette. Es ist für diese Frage neben der Art des Aminosäure-Austausches (hydrophile, hydrophobe, saure, basische oder aromatische Seitenkette) die Lage des Austausches innerhalb der sekundären bzw. tertiären Struktur der Proteinuntereinheit wichtig; d. h., ob ein bestimmter Austausch in einem α-Helixbereich, in der Nähe der Oberfläche der Untereinheit oder mehr im Inneren liegt, ob die neue Aminosäure bestimmte, für die räumliche Struktur der Proteinuntereinheit wichtige Bindungen eingehen kann, und so weiter.

Sobald die räumliche Struktur der Proteinuntereinheiten bekannt sein wird, bilden die zahlreichen TMV-Mutanten, bei denen Aminosäure-Austausche lokalisiert worden sind, und besonders die Temperatur-sensitiven Mutanten ein ergiebiges Material für die Untersuchung strukturenchemischer Probleme, die mit der Aggregation und Stabilität der TMV-Proteinuntereinheit zusammenhängen.

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Phages-Adsorption - Test for Differentiation of Genus: Brucella*

Józef Parnas and Stefania Zalichta

Academy of Medicine, Department of Microbiology in Lublin (Poland)

Brucella-phages-adsorption test on killed (70°) cells of all types of Brucella in the pure "S" phase has orientating value for determination of the Genus: "Brucella".

Brucella abortus "S" strains are in 100% positive in this test. Brucella melitensis "S" strains are in 83% positive and in 17% weakly positive. Brucella suis "S" strains are in 100% positive. Brucella atypica "S" strains are mostly positive. All types of Brucella in "R" phase are negative in this test. This experiment is important from point of view of general systematics of bacteria and for the explanation of phage—specific receptors in the "S" and "R" phase.

Brucella colonies occur in great variety; colonies of "S" phase are identical in all the types of Brucella, but there are also similarities with colonies of other bacteria. The enzymatic, bacteriostatic and serological tests are different for the different types of Brucella.

The atypical and intermediate strains of Brucella also have different and varying features. A variety of tests had to be performed to get sufficient information on the Brucella Genus.

The Brucella phages (Russian, Polish and others) give no key to the definition of Genus Brucella in their typical spectrum. The Brucella abortus strains are mostly lysed by phages in a dose of 1 RTD and 10.000 RTD. The Brucella melitensis strains are mostly resistant to all Brucella phages.

Most strains of Brucella suis are resistant to 1 RTD and sensitive to 10.000 RTD. The atypical strains behave in different ways. The lytic spectrum is important for the taxonomy of Brucella types but not for the systematic differentiation of Genus Brucella.

Our efforts to find a polyvalent Brucella phage, analogous to Salmonella phage 0 — 1, that is one lysing all Brucella strains, remained unsuccessful.

* With the financial help of the Polish Academy of Sciences (Microb. Com.).
2 A. W. Koziński and M. Macierewicz, Med. důświadczalna Mikrobiol. 1591, 3.
The attempt to adapt Brucella phages to 3 types of Brucella was also fruitful. We have found an easy and simple adsorption test of Brucella phages on Brucella cells for orienting differentiation of Genus Brucella.

This test could be of value for medical and veterinary laboratories for differentiation and classification of freshly isolated Brucella strains.

### Material and Methods

We used the following Brucella strains: *Br. melitensis* 16 M ("S"); 21 strains of *Br. melitensis* "S" and 10 "R", (some of the strains were examined simultaneously in "S" and "R" phase) 16 *Br. abortus* strains "S" and 1 "R"; 10 strains *Br. suis* "S" and 1 "R"; 18 strains *Br. atypica* (9 "S" and 9 "R"). As control we used 11 *Pasteurella multocida* strains "S". The Brucella phage 212/XV in titer 4 x 10⁶ and indicator — strain *Br. ab. 12* "S" were also used. Each Brucella and Pasteurella — strain has been examined 2—5 times. From the results of examinations we made an mean average of phage negative colonies. All the examined strains of Brucella and Pasteurella were killed by heating at 70 °C for 3 hours. The "S" and "R" phase was defined by thermoagglutination.

Adsorption test² ³: 0.5 ml of bacteria suspension 3 x 10⁷ were mixed with 0.5 ml of Brucella phage (1,500 particles), and placed in a waterbath at 37 °C for 3 hours. As control there was the same quantity of phage suspension without bacteria. Then a few drops of a 24 hours culture of the indicator strain *Br. ab. 12* "S" was added, and 1.4% agar. This mixture was poured into Petri dishes with agar (the 2-layer-method, details of the method were described in a former communication)⁴. After 48 hours incubation the mean average of phage-negative colonies from 3 Petri dishes was calculated.

### Results

Fig. 3 shows the Brucella phages — adsorption on Brucella substrates. Fig. 1 and 2 show the results of adsorption tests: (+) on Fig. 1, (−) on Fig. 2.

Diagr. 1 presents the results of adsorption tests of Brucella and Pasteurella "S" strains.

Area I presents a positive result (+) from 0 to 100 negative colonies; area II a weak positive result (±) from 100 to 200 colonies; area III a negative result (−) from 200 to 300 colonies. In the control (K) we noted on average 330 colonies. The control Pasteurella strains showed negative results. All *Brucella suis* "S" strains showed positive results. The *Br. abortus* "S" strains were positive except for 2 strains that showed a little more than 100 colonies. 16 *Br. melitensis* "S" strains gave positive results, and 6 strains were weak positive. 6 *Br. atypica* "S" strains showed positive results, 2 strains showed weak positive and 1 strain was negative. On a


⁴ J. PARNAS and S. ZALICHTA, Zbl. Bakteriol. 98, 196 [1965].
Fig. 3. Brucella phages adsorption on Brucella.

Diagramm 1. Brucella phages adsorption test on killed cells “S” of Brucella.

Diagramm 2. Brucella phages adsorption test on killed cells “R” of Brucella.

In Diagr. 2 the results of this test with Brucella “R” strains are presented. Here the picture is quite different. In area I(+) there is only 1 Br. suis “R” strain. Analysis of this strain showed that it responds rather to the “Rs” phase: in spite of that this strain did not show more than 50 negative
colonies. *Br. melitensis* “R” strains are in area II (+ –) and III (–). One strain of *Br. abortus* “R” showed negative tests. The *Br. atypica* “R” strains are in area II (+ –) and III (–). From a general evaluation of Diagr. 2 we confirm that *Brucella* “R” strains behave in the same manner as controls; they do not adsorb *Brucella* phages, or adsorb them very slightly.5,6

**Conclusions**

In Table 1 we present the results of *Brucella* phage adsorption tests and the lytic spectrum of *Brucella* types.7 *Br. melitensis* “S” strains are usually (> ) not sensible to the lytic activity of phages, but they are positive in the adsorption test (83%), or weakly positive (17%). *Br. abortus* “S” strains are in the majority of cases (> ) sensible to the lytic activity of phages, and simultaneously almost 100% positive in the adsorption test. *Br. suis* “S” strains are generally (> ) not sensitive to the lytic activity of phages in 1 RTD, but they are sensitive in 10,000 RTD, and are 100% positive in adsorption tests. *Br. atypica* “S” strains behave in various ways in the lytic spectrum of phages; however in adsorption tests they are mostly positive. All *Pasteurella* “S” strains are negative in the adsorption test with *Brucella* phages. *Brucella* “R” strains are negative in the adsorption tests; strains in the “Rs” phase are weakly positive.5,6

Summarizing, we can say that the *Brucella* phages adsorption test on killed cells of *Brucella* in the “S” phase has orientating value for determination of the genus *Brucella*.

<table>
<thead>
<tr>
<th>Examined <em>Brucella</em>-strains</th>
<th>Control *</th>
<th>Adsorption test</th>
<th>Lytic spectrum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 RTD</td>
</tr>
<tr>
<td><em>Brucella melitensis</em></td>
<td></td>
<td></td>
<td>&gt; (–)</td>
</tr>
<tr>
<td>I, II, III</td>
<td>(–)</td>
<td>(++) (–)</td>
<td>&gt; (–)</td>
</tr>
<tr>
<td></td>
<td>&gt; 300 neg. colonies</td>
<td></td>
<td>&gt; (–)</td>
</tr>
<tr>
<td><em>Brucella abortus</em> I–IX</td>
<td></td>
<td></td>
<td>&gt; (–)</td>
</tr>
<tr>
<td></td>
<td>neg. colonies</td>
<td>(++) (–) (–)</td>
<td>&gt; (–)</td>
</tr>
<tr>
<td></td>
<td>&gt; 300</td>
<td></td>
<td>&gt; (–)</td>
</tr>
<tr>
<td><em>Brucella suis</em> I, II, III</td>
<td></td>
<td></td>
<td>&gt; (–)</td>
</tr>
<tr>
<td></td>
<td>neg. colonies</td>
<td>(++) (–)</td>
<td>&gt; (–)</td>
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<tr>
<td></td>
<td>&gt; 300</td>
<td></td>
<td>&gt; (–)</td>
</tr>
<tr>
<td><em>Brucella atypica</em></td>
<td>(–)</td>
<td>&gt; (+)</td>
<td>&gt; (+)</td>
</tr>
<tr>
<td></td>
<td>&gt; 300</td>
<td>negative</td>
<td>&gt; (+)</td>
</tr>
<tr>
<td>Other Bacteria</td>
<td>(–)</td>
<td>negative</td>
<td>&gt; (+)</td>
</tr>
</tbody>
</table>

Table 1. Behaviour of *Brucella* types in the phages adsorption test and the lytic spectrum. * *Brucellaphage and indicator strain.
