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AUTHORITY

SMUFD D/A ltr, 15 Feb 1972

TRANSLATION NO. 2023

DATE: 20 October 1967

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

TRANSMISSION OF INFECTIOUS RESISTANCE TO PASTEURELLA

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Path Microbiol
(Pathological Microbiology)
30: 1967, pp 103-121

The transferability of the Episoma F-Lac$^+$ from E. coli to P. pestis (Martin, 1962; Martin and Jakob, 1962) and of R-factors to P. pestis and P. pseudotuberculosis (Ginoza and Matney, 1963) as part of the investigations on the system involved in these two species led to the question as to whether P. pestis and P. pseudotuberculosis, because of their close relationship, differ from other Pasteurella species, such as for instance, P. multocida dn P. haemolytica, in terms of the absorption and transmission of infectious resistance.

As a result of the close relationship between P. pestis and P. pseudotuberculosis, which does not exist with respect to P. multocida and P. haemolytica and other Pasteurella species, it had been suggested on various occasions that both species be assigned to a new genus called "Yersinia" (van Loghem, 1945, 1946, et al) under the family of the Enterobacteriaceae (Thal, 1954, additional bibliography in Knapp, 1965). These suggestions are supported by the common cultural and serological relationships, the partial antigen community of P. pseudotuberculosis Type II and IV with Salmonella of the B-, D-, and E-subgroups, respectively, of P. pseudotuberculosis Type IV with respect to E. coli strains with O-antigen 77 (Knapp) and the lysability of various P. pseudotuberculosis, P. pestis, E. coli, and Sh. dysenteriae strains through the same phage strains (bibliography in Knapp, 1965). All of these factors have been established for both species. Furthermore, it was necessary to establish the presence of the identical phage- and also common pesticin-receptors for some of the pseudotuberculosis, pest, and coli strains (Brubaker and Surgalla, 1961; Smith and Burrows, 1962; Burrows, 1963; Knapp and Zwillenberg, 1964; Hartman, 1964; Brubaker and assoc, 1965). The results of the data evaluation which was performed with various methods finally made it possible to categorize P. pestis in the family of the Enterobacteriaceae between the genuses of Escherichia and Klebsiella (Talbot and Sneath, 1960; Sneath, 1962; Smith and Thal, 1965).

With a few exceptions (Kuwabara and assoc, 1963; Ginoza and Matney, 1963; Lebek, 1963) most of the information published so far concerned the transmission of the R-factors between bacteria strains of the various genuses

in the family of Enterobacteriaceae (bibliography in Watanabe, 1963; Lobek, 1965). Except for the observation by Ginoza and Matney (1963) for P. pestis and P. pseudotuberculosis, there are no reports on the behavior of the various pasteurella species with respect to R-infections.

In our article here today we want to report on investigations dealing with the following questions:

1. Are R-infections possible in P. pseudotuberculosis of the various serological types and in the only avirulent strains of P. pestis as well as Pasteurella "X" available to us?

(This type of bacteria is known under various species designations, such as Bact. enterocoliticum (Schleifstein and Coleman, 1939, 1943), P. pseudotuberculosis "Type B" (Dickinson and Mocquot, 1961), Pasteurella "X" (Daniels, 1963, Struwe, 1963, Knapp and Thal, 1963), or Yersinia enterocolitica (Frederiksen, 1963; Mollaret and Chevalier, 1964, 1965, et al); this type of bacteria has not yet been completely and definitively categorized in the system of bacteria. It seems to be closer to P. pestis and P. pseudotuberculosis than to the other Pasteurella species.)

2. Can we produce an R-infection also in strains of those Pasteurella species, such as P. multocida, P. haemolytica, P. pneumotropica and P. anatipestifer, which are not closely related to P. pseudotuberculosis and P. pestis?

3. Can we establish any differences in the transmission frequency between the various species?

4. Can R-infected Pasteurella strains act as R-donors?

5. Do the resistance qualities, which are transmitted through R-infection, remain stable when the strains are stored for a longer time?

6. Can we determine any special features in connection with the R-infection of Pasteurella strains?

Investigation Materials and Methods

A. Nutrient Media

The strains were kept and passaged in proteose solution (proteose peptone Difco No 5, 20.0, glucose 0.5, NaCl 5.0, sec disodium phosphate 5.0, aq dest 1,000.0 ml) respectively, on blood agar plates with the addition of 5% wether blood or in the case of P. multocida on YPC-medium with and without the addition of 5% sheep blood (Namioka and Murata, 1962). We used ammonium citrate agar (Simons Citratagar) to establish the R-infection of Klebsiella-Aerobacter by R-infected Pasteurella strains and we used endoagar plates to count the Coli colonies.

B. Bacteria Strains

I. R-Donors

For the R-donors we selected Coli strains whose transmission frequency onto strains of a wide variety of bacteria species within the family of the Enterobacteriaceae was about 10^{-3} to 10^{-4}. In preliminary experiments, the donor strains were not supposed to grow anymore on the blood agar plates which had been inoculated with 0.1 ml of an overnight culture and with the additions of antibiotics which were necessary for the selection of the R-infected acceptor strains.

We might note here that we did not perform R-infection experiments with each and every donor strain, using all of the acceptor strains listed below.

Bakterienart und Stammbezeichnung (a)		R-Faktor (b)	Herkunft der Stämme isoliert aus, bzw. übersandt von (c)
E. coli	4018/62	TCSKNSu	Säuglingsstuhl (d)
E. coli	3128/64	TCSSu	Patientenurin (e)
E. coli	6467/64	TCSSu	Patientenurin
E. coli	6649/64	TCSSu	Patientenurin
E. coli	11345/64	TSu	Patientenurin
E. coli	10265/64	TSu	Patientenurin
E. coli	14394/64	T	Vaginalabstrich (f)
E. coli	JE 51	TCSSu	Prof. Watanabe, Tokio, isoliert (g) von Sugino
E. coli	4242/64	TCSSu	Patientenurin
E. coli	2324/65	TCSSuA	Patientenurin

Key: a. bacteria species and strain designation
b. R-factor
c. Origin of strains isolated from or left by
d. Infant stool
e. Patient urine
f. Vaginal smear
g. Professor Watanabe, Tokyo, isolated from Sugino
T — Tetracycline; C — Chloramphenicol; S — Streptomycin; K — Kanamycin; N — Neomycin; A — Ampicillin; Su — Sulfonamide

II. R-Acceptors

In the Pasteurella strains used as acceptors we were working mostly with freshly isolated strains and partly with strains taken from strain collections; the latter were repeatedly inoculated into serum or ascites bouillon and on blood agar plates prior to the start of the experiment.

Key to following Table:
a. Pasteurella species, Number, Serological Type
b. Chromosome resistance
c. Isolation, man (M); animal (P), Fleas (F)
d. Origin of initial strains
e. against antibiotics listed below
f. primary (p), selected in vitro (s)
g. Our own strain collection in Bern
bzw — respectively

The acceptor strains marked with * were tested, after R-infection, against Klebsiella-Aerobacter (Strain No 8970/62) as acceptors, in their capacity as donors.

Pasteurellaart Nummer Serologischer Typ (a)	(b) Chromosomale Resistenz gegen u.g. Antibiotika (e)	primär (p) in vitro selektioniert (s) (f)	Isolierung Mensch (M) Tier (T) Floh (F) (c)	Herkunft der Ausgangsstämme (d)
1. a) *P. pseudotuberculosis*				
2I*	Polymyxin B bzw. Colistin	p	M	Eigene Stammsammlung Bern (g)
36/1728I	Streptomycin	s	M	Eigene Stammsammlung Bern (g)
18II	Polymyxin B bzw. Colistin	p	T	Prof. Thal, Stockholm
45II	Polymyxin B bzw. Colistin	p	M	Dr. Daniels, Rotterdam
43III	Polymyxin B bzw. Colistin	p	T	Prof. Thal, Stockholm
85III	Polymyxin B bzw. Colistin	p	M	Prof. K. F. Meyer, San Francisco
32IV	Streptomycin	s	T	Prof. Thal, Stockholm
32IV*	Kanamycin bzw. Neomycin	s	T	Prof. Thal, Stockholm
25V	Polymyxin B bzw. Colistin	p	T	Prof. Thal, Stockholm
9V	Polymyxin B bzw. Colistin	p	M	Eigene Stammsammlung Bern (g)
b) *P. pestis*				
TWJ*	Polymyxin B bzw. Colistin	p		Prof. K. F. Meyer, San Francisco
TWJ	Kanamycin bzw. Neomycin	s		Prof. K. F. Meyer, San Francisco
B1466	Kanamycin bzw. Neomycin	s	F	Prof. K. F. Meyer, San Francisco

Pasteurellaart Nummer Serologischer Typ (a)	(b) Chromosomale Resistenz gegen u.g. Antibiotika (a)	primär (p) in vitro sekundär (s) (a)	Isolierung Mensch (M) Tier (T) Fliege (F) (c)	Herkunft der Ausgangsstämme (d)
B868	Kanamycin bzw. Neomycin	s	F	Prof. K. F. Meyer, San Francisco
F7793	Polymyxin B bzw. Colistin	p	F	Prof. K. F. Meyer, San Francisco
B2764	Polymyxin B bzw. Colistin	p	F	Prof. K. F. Meyer, San Francisco
EV76	Polymyxin B bzw. Colistin	p	M	Prof. K. F. Meyer, San Francisco
c) *Pasteurella «X»*				
76	Streptomycin	s	T	Dr. Frederiksen, Kopenhagen
268	Streptomycin	s	T	Dr. Siegmann, Celle
1100	Streptomycin	s	T	Dr. Siegmann, Celle
18	Streptomycin	s	T	Dr. Becht, Zürich
978	Streptomycin	s		Dr. Daniels, Rotterdam
1055*	Kanamycin bzw. Neomycin	s	T	Dr. Daniels, Rotterdam
373*	Kanamycin bzw. Neomycin	s	T	Prof. Thal, Stockholm
71*	Kanamycin bzw. Neomycin	s	T	Dr. Frederiksen, Kopenhagen
271*	Kanamycin bzw. Neomycin	s	T	Dr. Siegmann, Celle
59	Kanamycin bzw. Neomycin	s	T	Dr. Becht, Zürich
2. a) *P. multocida*				
5117*	Kanamycin bzw. Neomycin	s	T	Prof. Fey, Bern
D417/64*	Kanamycin bzw. Neomycin	s	T	Prof. Fey, Bern
D299/60*	Kanamycin bzw. Neomycin	s	T	Prof. Fey, Bern

Pasteurellaart Nummer Serologischer Typ (a)	(b) Chromosomale Resistenz gegen u. g. Antibiotika (c)	prinzip (p) in vitro sensibilisiert (s) (f)	Isolierung Mensch (M) Tier (T) Pflanze (P) (g)	Herkunft der Ausgangsstämme (d)
595 *	Kanamycin bzw. Neomycin	s	T	Prof. Fey, Bern
W164/60 *	Kanamycin bzw. Neomycin	s	T	Prof. Fey, Bern
D2011/59	Kanamycin bzw. Neomycin	s	T	Prof. Fey, Bern
D199/60 *	Kanamycin bzw. Neomycin	s	T	Prof. Fey, Bern
D193/60 *	Kanamycin bzw. Neomycin	s	T	Prof. Fey, Bern
S5075/65	Kanamycin bzw. Neomycin	s	T	Prof. Fey, Bern
3503/65	Kanamycin bzw. Neomycin	s	M	Eigene Stammsammlung Bern (g)
13131/65	Kanamycin bzw. Neomycin	s	M	Eigene Stammsammlung Bern (g)
3316/65	Polymyxin B bzw. Colistin	p	M	Eigene Stammsammlung Bern (g)
b) *P. haemolytica*				
D125/65 *	Kanamycin bzw. Neomycin	s	T	Prof. Fey, Bern
D126/65 *	Kanamycin bzw. Neomycin	s	T	Prof. Fey, Bern
4434/61	Kanamycin bzw. Neomycin	s	T	Prof. Fey, Bern
c) *P. pneumotropica*				
D99 *	Kanamycin bzw. Neomycin	s	T	Prof. Fey, Bern
D103 *	Kanamycin bzw. Neomycin	s	T	Prof. Fey, Bern
d) *P. multocida*				
11948	Kanamycin bzw. Neomycin	s		Type culture collection, Washington

The approximate germ or virus content in every ml of the overnight cultures of donor and acceptor [recipient] strains was determined prior to the preparation of the mixed cultures by plating in each case 0.1 ml of the cultures which had been diluted down to 10^{-4} and 10^{-6} and by counting the colonies after the plates [dishes] had been incubated for 48 hours at 37° C.

We selected strains of the various Pasteurella species with a primary resistance against polymyxin B or with a selected resistance which would increase in vitro against doses of antibiotics (200 gamma/ml streptomycin, respectively, kanamycin/neomycin). In the transmission experiments we used only the resistant strains which would reveal a growth rate corresponding to the control cultures, without the addition of antibiotics, on blood agar plates with the addition of antibiotic required for selection against the donor strain, after we had spread 0.1 ml of an overnight culture in protease solution.

C. R-Infections and Selection of R-Infected Recipients

Overnight cultures of the donor (E. coli) and recipient strains (Pasteurella) were mixed in a ratio of 1:10 and the mixed cultures were incubated at 37° C. After 4 and 24 hours we plated 0.1 ml of the mixed cultures and as controls we plated the pure cultures of the particular donor and recipient strains on blood agar plates, adding antibiotics against which the recipient [acceptor] and the donor were sensitive. In addition, these blood agar plates contained one of the antibiotics against which the R-factor of the donor revealed resistance factors [determinants]. In other controls, we plated the pure cultures on blood agar plates, each time adding only one of these two antibiotics.

As a function of the resistance markers and the R-factors, we added to the nutrient media together 80 gamma/ml polymyxin B and 10 gamma/ml chloramphenicol or 50 gamma/ml streptomycin and 10 gamma/ml tetracyclin, respectively, 100 gamma/ml kanamycin and 10 gamma/ml chloramphenicol. The antibiotic which is mentioned first here, in each case, was used to inhibit the donor and the second-named antibiotic was used for the selection of the R-infected recipients.

The transmission experiments could be evaluated if there was no bacteria growth in the pure cultures of the partner strains on the blood agar plates, when both antibiotics were added and when the recipient, respectively, donor strain would grow only on the plates when we added that antibiotic against which the recipient was resistant and against which the donor was sensitive, or vice versa.

If we were able to observe colony growth on the selection plates containing both of the antibiotics, within 4 days, then we inoculated and investigated up to 10 colonies (clones). We tested the purity of the clones and their identity with the recipient strain by means of cultural-biochemical and, as much as possible, also by means of serological investigations and we also examined the resistance spectrum in the ring test.

We determined the resistance of the germs "viruse." quantitatively by inoculating solid nutrient media with the corresponding addition of antibiotics or we determined it qualitatively in the ring test with tooth-wheel-shaped test rings (according to Linzenmeier). The free ends of the teeth contained the following: 10 gamma streptomycin, 20 gamma tetracyclin, 12 gamma chloramphenicol, 20 gamma kanamycin, 1600 gamma sulfisoxazol, 50 gamma polymyxin B, and 40 gamma furazolidon.

The resistance was re-transmitted from the R-infected Pasteurella strains to the Klebsiella-aerobacter strain No 8970/62. We took our overnight cultures and we mixed 0.1 ml of the Klebsiella cultures, each, and 0.9 ml of Pasteurella cultures with these overnight cultures and these mixed cultures (0.1 ml) were then inoculated on Simons Citratagar plates after 4 and 24 hours of incubation at 37° C. By way of addition, these plates contained one of the antibiotics against which the R-factor contained resistance determinants.

The purity of the colonies which had grown within 2 days and their identity with the recipient strain were tested by seeding on endoagar plates (single-colony technique), by preparing a varied series, and by determining the resistance spectrum.

For a rough calculation of the transmission frequency, we started with the number of recipient germs per ml at the time of the mixture of the partner strains and from the number of the R-infected germs found in the mixed culture within 4 hours.

Our investigation results can be summarized as follows:

1. (a) R-Infections of P. pseudotuberculosis

We R-infected two strains, each, of the serological types I-V of P. pseudotuberculosis. The R-infection did not come out successful in each and every P. pseudotuberculosis strain with the same donor strain, respectively, in the same high frequency. We transferred R (T) from donor strain 1439/64 to P. pseudotuberculosis No $36/1720^{I}$, 85^{IV}, and 32^{IV} S^{r} in a frequency of 10^{-3} to 10^{-4} T (TSu) from the donor strains No 10285/64 and 11345/64 to P. pseudotuberculosis No $36/1720^{I}$ in a frequency of 10^{-6}, respectively, 10^{-5}; R (TCSSU) from the donor strains No 9667, 9849; JE51 and 4282/64 on P. pseudotuberculosis No 257^{II}, 43^{III}, 85^{III}, $32^{IV}K/K^{r}$, 9^{V}, and 25^{V}, and the R(TCSSuA), respectively, R(TCSK/MSu) from donor strains No 2234/65 and 4018/62 to P. pseudotuberculosis strains 32^{IV} KM^{r}, respectively 2^{I} and 25^{V} in a frequency of about 10^{-6}.

P. pseudotuberculosis strain 16^{II} could not be R-infected by any of the related donor strains. Because of the tremendous amount of work involved, it was impossible to perform transfer experiments with each of the donor strains listed under B I; these and the subsequent investigation results therefore do not enable us to make any numerical comparisons between

the donor strains, on the one hand, and the frequency, respectively, the differences in the receptivity of the various Pasteurella strains with respect to R-infections, on the other hand. On the basis of our observations we can certainly say that the Coli strains 14394/64 R(T), JE51 R (TCSSu), and 9849/64 R (TCSSu) were particularly suited as donors.

1. (b) R-Infections of P. pestis

Among the five, respectively, six donor and recipient strains, we were successful in transferring the R-factors R (TCSK/NSu and R (TCSSu) with donor strains No 4018, respectively, 9667, onto the P. pestis strain TWJ in a frequency of 10^{-6}, respectively, 10^{-5}, and using the donor strain JE51 we were able to transfer the R-factor R (TCSSu) onto the strains F7793, respectively, EV76 in a frequency of 10^{-6} and onto strain B2764 in the frequency of 10^{-5}. The other two pest strains could not be R-infected with these Coli strains and by means of the experimental technique selected here.

1. (c) R-Infections of Pasteurella "X"

The R-factors of the 5 donor strains selected, that is, 14394/64 R (T), 11345/64 R (TSu), JE51 R(TCSSu), 4242/64 R(TCSSu) and 2234/65 R(TCSSuA) could be transferred to 9 out of the 10 strains tested in frequencies between 10^{-3} and 10^{-6}. The best donors, with the highest transfer frequency, proved to be donor strains No 14393/64, JE51, and 11345/64. R-infection failed only in strain No 59.

2. (a) R-Infections in P. multocida and Other Pasteurella Species

The results of these experiments are shown in Table I, below. As donors we used the strains No JE 51, R(TCSSu), 4242/64 R(TCSSu and 2234/64 R(TCSSuA).

As we can see, the R-infections came out successful in most of the Pasteurella strains of the four different species shown in Table I. It is interesting to note here that the three strains of P. multocida which we had freshly isolated and which were of human origin could not be R-infected with the experimental technique employed here, although the transmission of the R-factors was accomplished with high frequency in most of the strains of animal origin which had been partly freshly isolated or which had been taken from the collection. Further investigations are being pursued with these strains.

The special features noted in the R-infections of the Pasteurella strains are listed under Point 6, below, and in Table IV.

Table I. Results of the R-Infections of Various Pasteurella Species (With the Exception of P. pseudotuberculosis, P. pestis, and Pasteurella "X")

Akzeptorstamme (a)	(b) Donatorstämme JK 81 R(TC35a)	4343/64 R(TC35a)	2234/65 R(TC35aA)	Akzeptorstamme (a)	(b) Donatorstämme JK 81 R(TC35a)	4343/64 R(TC35a)	2234/65 R(TC35aA)
P. multocida				*P. multocida*			
S11.71g	++	+	+	3316/65	–	–	–
D117/64	++	–	–	13131/65	▬	▬	▬
D200/60	++	+	+	3503/65	▬	▬	▬
D259/64	–	–	–				
S05	++	+	+	*P. haemolytica*			
W164/60	++	+	+	D125/65	++	+	+
D2011/59	++	–	–	D126/65	++	–	–
D193/60	++	–	–	4434/61	–	–	–
D199/60	+++	+	+				
S116/1g	▬	▬	▬	*P. pneumotropica*			
S575/65	–	–	–	D 99	++	+	–
				D 103	+++	+	+
				P. anatipestifer			
				11845	+++	+	+

Key: a. Recipient strains
b. Donor strains
Note: transfer frequency +++ = 10^{-3}–10^{-4}; ++ = 10^{-5}; + = 10^{-6};
– = negative experiment; ▬ = repeated negative experiments.

3. Differences in Transfer Frequency

Our experiments are not comprehensive enough to enable us to make reliable statements as to the differences in the frequency of acceptance of the various R-factors in the various Pasteurella species. But we think that we can say this: among all of the strains of the various Pasteurella types which we tested, the highest frequency in terms of R-infection was achieved in the strains of Pasteurella "X". Next came the strains of P. pseudotuberculosis, P. multocida, and P. pestis. Because of the small number of available strains there is really nothing we can say about the differences in the transfer frequency in the case of the other Pasteurella types. In this series of investigations we were concerned primarily with the question as to whether R-infections are possible only in the Pasteurella species P. pseudotuberculosis and P. pestis, including Pasteurella "X", which might possibly be categorized within the family of the Enterobacteriaceae, or whether these infections might not also be possible in other strains which are not related to P. pseudotuberculosis and P. pestis and which do not belong to the Pasteurella types that are under the Enterobacteriaceae. As our investigations revealed, R-factors could be transferred to the strains of all of the Pasteurella species tested.

4. Testing the R-Infected Pasteurella Strains as R-Donors

Out of the strains of each Pasteurella species, at least one R-infected clone was tested for its ability to transfer the absorbed R-factor further upon Klebsiella-Aerobacter (strain No 5970/62). As we can see in Table 2, below, this could be established in all strains. As donor, Pasteurella "X" revealed a transfer frequency that was by 1-2 powers of 10 greater than the strains of the other Pasteurella species. With respect to this property, Pasteurella "X" corresponded to most of the Coli strains.

5. Loss of R-Factors, Respectively, R-Determinants

Clones of Pasteurella strains of the various species, which had been tested for their purity and which had been R-infected, were kept in the refrigerator, in stab cultures sealed with rubber stoppers, with the exception of P. multocida. The cultures of P. multocida, which according to our experiences could withstand longer storage periods more safely and reliably at 37° C and -27° C than at ± 2° C, on the other hand, were kept in thermostats at 37° C. After 6 months we tested the inoculations — which at the same time had been transferred to blood and endoagar plates and in protease solution — for their purity, and their cultural-biochemical as well as, to the extent possible, their serological behavior. The resistance determination was made on the basis of the overnight cultures in protease solution. Table II, below, tells us about the loss of the R-factors, respectively, R-determinants.

These experimental results indicate that P. pseudotuberculosis and P. pestis strains, like Salmonella strains can lose most of their R-factors in case of longer storage of stab cultures, although these same factors did remain stable in most of the strains of the other Pasteurella species which we examined.

It was interesting to note here that, among the P. multocida strains, the strain 199/60 lost the resistance determinants only of [for] the R-factor of E. coli 2234/64 R(TCSSuA) but not of the R-factors of E. coli JE51 R(TCSSu) and E. coli 4242/64 R(TCSSu) (Table III). This seems to indicate that the ability to lose R-factors or R-determinants depends not only on the host cell but also on the R-factor.

The control of the cultural properties of R-infected Pasteurella strains -specially of P. multocida, was rendered very difficult because the resistance mutants, which had been selected in vitro, with and without R-factors, revealed poor growth properties. We will report on these observations elsewhere.

Table II. Experimentally R-Infected Pasteurella Strains as Transmitters of R-Factors Upon Klebsiella-Aerobacter (Strain No 8970/62)

Donator-stamm Nr. (a)	(b) Infiziert mit den R-Faktoren (f)	(b) Infiziert durch E. coli Nr. (g)	Übertragungs-frequenz (c)	Zahl der getesteten Kolonien (d)	Übertragene R-Determinanten (e)
P. pseudotuberculosis					
2[I]	TCSK/NSu	4015/62	10^{-4}	4	TCSK/NSu
32[IV]	TCSSu	JE 51	10^{-4}	10	TCSSu
25[V]	TCSK/NSu	4015/62	10^{-4}	4	TCSK/NSu
P. pestis					
TWJ	TCSK/NSu	4015/62	10^{-3}	4	TCSK/NSu
TWJ	TCSSu	8887/64	10^{-4}	2	TCSSu
Pasteurella X					
1055	TCSSu	JE 51	10^{-3}	10	TCSSu
373	TCSSu	4242/64	10^{-3}	10	TCSSu
71	TCSSu	JE 51	10^{-3}	10	TCSSu
271	TCSSu	JE 51	10^{-4}	10	TCSSu
P. multocida					
S1117	TCSSu	4242/64	10^{-5}	7	CSSu
D417/64	TCSSu	JE 51	10^{-4}	10	TCSSu
D299/60	TCSSu	JE 51	10^{-4}	10	TCSSu
S95	TCSSu	JE 51	10^{-5}	4	TCSSu
W164/60	TCSSu	JE 51	10^{-4}	10	TCSSu
D199/60	TCSSu	JE 51	10^{-4}	10	TCSSu
D193/60	TCSSu	JE 51	10^{-4}	10	TCSSu
P. haemolytica					
D125	TCSSu	JE 51	10^{-5}	10	TC
D126	TCSSu	JE 51	10^{-3}	10	TCSSu
P. pneumotropica					
D103	TCSSu	JE 51	10^{-4}	5	TCSSu
D99	TCSSu	4242/64	10^{-3}	10	1 Kol. = C, 5 Kol. = CSu, 1 Kol. = Su, 3 Kol. = TCSu

Key: a. Donor strain No.
b. Infected
c. Transduction frequency
d. No. of colonies tested
e. R-determinants transferred
f. with R-factors
g. By E. coli No
Kol = colony

Table III. Loss of R-Factors, Respectively, R-Determinants in Stab Cultures Stored 6 Months

Pasteurella-Art Stamm-Nr. Serolog. Typ (a)	(b) Infiziert durch E. coli Nr. (e)	mit dem R-Faktor (f)	Zahl der geprüften Klone (c)	Beurteilung der Ergebnisse (d)
P. pseudotuberculosis				
2[I]	4018/62	TCSK/NSu	1	<95 % Verlust des R-Faktors (RF) (g)
32[V]	JE 51	TCSSu	1	~80 % Verlust der T-Resistenz[a] (h)
55[V]	4018/62	TCSK/NSu	1	<95 % Verlust des RF (i)
P. pestis				
TWJ	4018/62	TCSK/NSu	1	<95 % Verlust des RF (i)
	9667/64	TCSSu	1	<95 % Verlust des RF (i)
Pasteurella «X»				
1055	JE 51	TCSSu	5	Kein Verlust des RF (j)
373	4242/64	TCSSu	2	1 Klon kein Verlust des RF (k)
				1 Klon Verlust der T-Resistenz (l)
71	JE 51	TCSSu	7	Kein Verlust des RF (j)
271	JE 51	TCSSu	5	Kein Verlust des RF (j)
P. multocida				
D199/60	2234/64	TCSSuA	4	2 Klone kein Verlust (m)
				1 Klon Verlust der T-, C- und A-Resistenz (n)
				1 Klon Verlust der T-, C-, S- und A-Resistenz (o)
7 Stämme	versch. Stämme	TCSSu oder TCSSuA	21	Kein Verlust des RF (j)
P. haemolytica				
D125/65	JE 51	TCSSu	1	Kein Verlust des RF
	4242/64	TCSSu	3	Kein Verlust des RF (j)
	2234/65	TCSSuA	4	Kein Verlust des RF
D126/65	JE 51	TCSSu	2	Kein Verlust des RF
P. pneumotropica				
D103	JE 51	TCSSu	1	Kein Verlust des RF
	2234/65	TCSSuA	1	Kein Verlust des RF (j)
D99	4545/64	TCSSu	2	Kein Verlust des RF
P. anatipestifer				
18845	JE 51	TCSSu	1	Kein Verlust des RF (j)
	2234/65	TCSSuA	4	Kein Verlust des RF

a. Pasteurella species, strain No, serological type
b. infected
c. No of clones tested
d. Evaluation of result
e. By E Coli No
f. With R-factor
g. Loss of R-factor(RF)(loss of all resistance properties obtained through R-infection in more than 95% of viruses of the clone tested
h. Loss of T-resistance (loss of some individual resistance properties)
i. Loss of RF
j. No loss of RF
k. One clone, no loss of RF
l. One clone, loss of T-resistance
m. 2 clones, no loss
n. 1 clone, loss of T, C, & A-resistance
o. 1 cone, loss of T, C, S, & A resistance

6. Special Features in Connection with the R-Infection of Pasteurella Strains

It is interesting to note that, in various Pasteurella strains, only some individual and not all resistance determinants turn up in the phaeno-type. A few examples are given in Table IV, below. In these experiments, the colonies, grown on selection media, were tested for their purity in the number indicated, on a blood plate not containing any antibiotic, and their resistance was then determined. The results show that only very few Pasteurella strains could be found to absorb the R-factor incompletely. It is interesting to note that 4 out of the 9 strains of Pasteurella "X" tested here expressed only a part of the resistance determinants of the R-factor in phaeno-typical terms. Investigations are now in progress in connection with the question as to whether the incomplete absorption here is only apparent and whether it might perhaps consist in the fact that the determinants of the missing resistance property are not expressed phaeno-typically (Lebek).

Table 4. Pasteurella Strains With Incomplete Absorption of R-Factor

Akzeptor Art und Stamm Nr. (a)	(b) Donator E. coli Nr.	R-Faktoren (e)	Anzahl der geprüften R-infizierten Klone (c)	Übertragene Resistenz-eigenschaften (d)	
P. pseudotuberculosis					
25V	9867/64	TCSSu	2	T	(1×)
				TCSSu	(1×)
25V	9849/64	TCSSu	4	TCSSu	(2×)
				TCSu	(1×)
				CSu	(1×)
P. pestis					
F 7793	JE 51	TCSSu	7	TCSSu	(1×)
				CSSu	(6×)
B 2764	JE 51	TCSSu	10	TCSSu	(9×)
				CS	(1×)
Pasteurella »X«					
71	JE 51	TCSSu	9	CSSu	(8×)
				TCSSu	(1×)
271	JE 51	TCSSu	2	CSSu	(2×)
373	JE 51	TCSSu	3	TCSSu	(2×)
				CSSu	(1×)
1065	JE 51	TCSSu	10	CSSu	(10×)
P. multocida					
184/60	4242/64	TCSSu	2	TCSSu	(1×)
				CSSu	(1×)
P. anatipestifer					
11845	2234/55	TCSSuA	4	TCSSuA	(3×)
				TCSSu	(1×)

Key: a. Recipient, species and strain number
b. Donor
c. Number of R-infected clones tested
d. Resistance properties transferred
e. R-factors

Inoculations of individual colonies of the primary selection plates were inoculated and tested.

Discussion of Findings

The observation that, among the experimental conditions selected by us, R-infections were possible in the strains of all species tested under what has so far been called the genus Pasteurella constitutes further proof that R-infections can occur also in strains from various genuses which do not belong to the family of the Enterobacteriaceae. This was supported by the observations on Vibrio cholerae (Kuwabara, and assoc, 1963) and Ps. aeruginosa (Lebek, 1963). It is interesting to note that our experiments involving P. pseudotuberculosis and P. pestis strains revealed the same inclination toward the spontaneous loss of the R-factors as had been reported for the Salmonella strains (Lebek, 1964). It was not to be observed in the strains of other Pasteurella species which we investigated. The strains of the various Pasteurella species thus differed in terms of their inclination to lose R-factors and not in terms of their capability of receiving them and transferring them.

Our investigation results bring up various questions which we cannot answer at this time. For instance, it was interesting to note that some Coli strains proved to be good donors with respect to the recipient Klebsiella-Aerobacter but not with respect to numerous Pasteurella strains which, for their part, easily accepted the R-factor from the other Coli strains. There was thus no inability to express the resistance determinants phaeno-typically. Here is another question which we cannot answer right now: we do not know why the Coli strains differ from each other in terms of the frequency with which they transfer their R-factors to the same recipient strains. The cause for these differences might perhaps be found in the type of the R-factor or in some of the as yet unknown properties of the acceptor [recipient] cells. It is entirely conceivable that further episomas or plasmides, in the donor or recipient cell, might inhibit the transfer or acceptance of the R-factors.

Similarly, we do not know why only P. pseudotuberculosis and P. pestis strains, respectively, strains of various Salmonella species, during longer storage time, again spontaneously lose their R-factors or some individual resistance determinants, in contrast to the strains of the other Pasteurella species and strains of other genuses from the family of Enterobacteriaceae. This might perhaps be due to the fact that the number of R-factors in the individual bacterium cell differs for the various species.

Summary

Investigations on the R-infection of strains of various Pasteurella species made it possible to establish the R-infection of all species tested. One difference between the Pasteurella species, such as P. pseudotuberculosis, P. pestis, and Pasteurella "X", respectively, the other Pasteurella species which are not related to them, such as P. multocida, P. haemolytica, P. pneumotropica, and P. anatipestifer — which might be categorised among the family of the Enterobacteriaceae, consisted in the fact that the two first-named species and Pasteurella "X" accepted the R-factors with a higher frequency than the other Pasteurella species. R-infected strains of all Pasteurella species transferred the R-factor to

an acceptor [recipient] strain. As we know for the case of the Salmonella the R-factors or individual R-determinants, the case of P. pseudotuberculosis and P. pestis, were not stable when the cultures were stored for 6 months, whereas, in the other Pasteurella species, a spontaneous loss of the R-factor could be established only in some individual cases.

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This study was prepared with the support of the Swiss National Fund. We want to thank our lady assistants B. Stemmer, Ch. Osterlag, and St. Dinter for their very careful technical support.

Received on 25 April 1966.