仮性結核菌に関する研究 II

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STUDIES ON *YERSINIA (PASTEURELLA) PSEUDOTUBERCULOSIS*

II. A NEW TYPE OF *Y. PSEUDOTUBERCULOSIS*, TYPE VI, AND SUBDIVISION OF TYPE V STRAINS

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In the previous investigation[12], serological typing was performed on 40 strains of *Yersinia pseudotuberculosis* isolated in Japan. Of these strains, three could not be classified into any of the known serotypes, I to V. At the same time, it was pointed out that the type V strains isolated in Japan were divided into two subtypes, but such a subdivision of type V strains had not been reported in any other country.

In the present study, biological and serological properties of the strains mentioned above were investigated intensively to complete the classification of *Y. pseudotuberculosis*.

MATERIALS AND METHODS

Strains used. (a) *Y. pseudotuberculosis*. Three untypable strains, #1, #3, and #14 strain, were supplied by Dr. Y. OCHI, of Azabu Veterinary College. They had been isolated from guinea pigs and classified into type I according to the typing scheme of OCHI et al.[8]. As type V strains for subtyping, 52, #12, #38, R2, R9, R22, R87, R94, R103, R108, and R112A strains were used. Of them, strain 52 had been supplied by Dr. H. H. MOLLARET of the Pasteur Institute, and strains #12 and #38, which had been derived from guinea pigs, by Dr. Y. OCHI. The remaining 8 strains had been isolated from rabbits by the authors. The origins of the other strains were described in detail in the previous paper[12]. (b) *Y. enterocolitica*. Two strains, MY79 and Albany5819, were received from Dr. R. SAKAZAKI, of the National Institute of Health, Tokyo.

Biochemical properties. Biochemical characteristics were examined by the conventional methods.

Phage susceptibility. Two phage strains, supplied by Dr. E. THAL (strain 97/II), of the National Institute of Veterinary Medicine in Sweden, and Dr. W. KNAPP (strain *PTS/Y. pseudotuberculosis* type IA), of Erlangen-Nürnberg University in Germany, respectively, were used. They were propagated on strain No. 2, which was of type IA of *Y. pseudotuberculosis*. The phage concentrations used for experiment were the routine test dilution (R.T.D.) and 10×R.T.D.

Agglutination. The techniques employed for the preparation of O-antigens and antisera and for the agglutination reactions were the same as described in the previous report[12]. H-antigens were prepared from 24-hour broth cultures at 22°C, to which formalin was added to a final concentration of 0.3 per cent. Before use, the organisms were subcultured five times in semi-fluid agar contained in a U-tube. The H-agglutination and absorption tests were performed according to the techniques for Salmonella organism.

RESULTS

1. Properties of strains #1, #3, and #14.

Table 1. Biological Characters of Strains #1, #3, and #14

Character	Strain #1	Strain #3	Strain #14	*Y. pseudotuberculosis*	*Y. enterocolitica* MY 79	*Y. enterocolitica* Albany 5819
Indol	−	−	−	−	+	+
V P	−	−	−	−	+	−
Gas production	−	−	−	−	+	+
Adonitol	+	+	+	+	−	−
Rhamnose	+	+	+	+	−	−
Xylose	+	+	+	+	+	+
Maltose	+	+	+	+	+	+
Salicin	+	+	+	+	−	−
Esculin	+	+	+	+	−	−
Melibiose	+	+	+	+	−	−
Sucrose	−	−	−	−	+	+
Sorbitol	−	−	−	−	+	+
Cellobiose	−	−	−	−	+	+
Arginine-decarboxylase	−	−	−	−	−	−
Ornithine-decarboxylase	−	−	−	−	+	+
ONPG	+	+	+	+	+	+
Phage susceptibility	+	+	+	+	−	−

Table 2. Pathogenicity of Strains for Experimental Animals (inoculated intraperitoneally)

Animal	Strain	Inoculum dose	Death / Tested
Mouse	# 1	1 mg	5/ 5
		0.5 mg	5/ 5
		0.1 mg	1/ 5
	# 3	1 mg	1/ 5
	#14	1 mg	3/ 5
	# 1	Broth culture 0.5 ml	5/10
		Broth culture 0.2 ml	2/10
	# 3	Broth culture 0.5 ml	0/10
	#14	Broth culture 0.5 ml	0/10
Guinea pig	# 1	1 mg	0/ 3
	# 3	1 mg	0/ 3
	#14	1 mg	0/ 3

a. Biochemical characteristics.

The biological characteristics of the 3 strains are shown in Table 1. All the three strains were classified into *Y. pseudotuberculosis* and differentiated from *Y. enterocolitica*. The other properties tested corresponded to those of *Y. pseudotuberculosis*.

b. Pathogenicity for experimental animals.

(1) Mice. Mice weighing 15 g were injected intraperitoneally with 0.1, 0.5, and 1.0 mg of nutrient agar culture and 0.2 and 0.5 ml of broth culture. The results obtained are indicated in Table 2. Macroscopic examination revealed that mice which died within a week after inoculation had been suffering from an enlarged spleen and congestion of visceral organs, and that mice which died after one week or more showed an enlargement of the spleen and the presence of white spots in the spleen and liver. Histologically, these white spots were necrotic lesions surrounded by epitheloid cells. All survivors were sacrificed on the 13th or 21st day following inoculation. They showed no macroscopic changes. Histologically, however, small necrotic foci were found in the liver, spleen, and kidney in a few of them. The organisms were recovered from the heart-blood, liver, and spleen in all mice which died, and from the liver and spleen in a few of the survivors sacrificed.

Table 3. Agglutination Reaction of Strains of Types I to V with Antiserum to Strain #1

Antiserum	Agglutination with		Agglutinated at titers
#1 (1:3,200)	Type I	5, 24, PSG-1, G-1, E-12, Nishigahara	50-200
	Type II	49, R16	≦25
	Type III	83, #35, #39, Miyazaki	≦25-100
	Type IV	51, E-2, E-9, R56, R80M, Ikegaki, Saisawa, Saru-2, Miyairi	<25
	Type V	52, R2, R9, R22, R87, R94, R103, R108, R111, R112A, #12, #13, #38	<25 or 200*

* Strain 52.

Table 4. Agglutination Reactions of Strains #1, #3, and #14 with Antisera to Types I to V Strains

Antiserum		Agglutination with #1	#3	#14
I	5 (1:3,200)	—	100	200
	E-12 (1:1,600)	—	—	—
II	49 (1:6,400)	—	50	200
	R16 (1:1,600)	—	25	50
III	83 (1:3,200)	50	100	200
	#39 (1:3,200)	—	—	—
IV	51 (1:3,200)	—	—	—
	Ikegaki (1:6,400)	—	—	—
V	52 (1:6,400)	—	50	100
	R2 (1:1,600)	—	—	—

—: No agglutination occurred at a dilution of 1:25.

Table 5. Absorption Test (1)

Antiserum	Absorbed by	Agglutination with* I	II	III	IV	V	#1	#3	#14
#1 (1:3,200)	—	50–200	≦25	≦25–100	—	<25,200	3,200	1,600	3,200
	5	—	—	—	—	—	1,600	800	400
	49	—	—	—	—	—	800	400	400
	#35	—	—	—	—	—	1,600	800	800
	52	—	—	—	—	—	1,600	800	400

* The antigens of types I to V are the same as listed in Table 3.

—: No agglutination occurred at a dilution of 1:25.

(2) Guinea pigs. Guinea pigs were inoculated intraperitoneally with a does of 1.0 mg of nutrient agar culture of the 3 strains, but they were not affected.

c. Serological properties.

(1) Antigenic relationship among the 3 strains. By the agglutinin-absorption test, the 3 strains were found to possess a common antigen.

(2) Cross-agglutination tests between the 3 strains and serotypes I to V strains. As shown in Table 3 and 4, reciprocal or unilateral weak reactions were found between the 3 strains and the strains of types I to V. Most of the strains of all the known serotypes, except type IV, agglutinated at a titer of 1:25 to 1:100.

Table 6. Absorption Test (2)

Antiserum	Absorbed by	Agglutination with #1	#3	#14
I 5 (1:3,200)	—	—	100	200
	#14	•	—	—
	5	•	—	—
	49	•	—	—
	#35	•	—	—
	52	•	—	—
II 49 (1:6,400)	—	—	50	200
	#14	•	—	—
	5	•	—	—
	49	•	—	—
	#35	•	—	—
	52	•	—	—
III 83 (1:1,600)	—	50	100	200
	#14	—	—	—
	5	—	—	—
	49	—	—	—
	#35	—	—	—
	52	—	—	—
V 52 (1:6,400)	—	—	50	100
	#14	•	—	—
	5	•	—	—
	49	•	—	—
	#35	•	—	—
	52	•	—	—

—: No agglutination occurred at a dilution of 1:25.

• : Not tested.

(3) Cross-absorption tests between the 3 strains and the strains of types I to V. As shown in Tables 5 and 6, it was found that the 3 strains possessed a unique antigen. The agglutinin, which had reacted to a heterogeneous combination, was absorbed by the strains of every serotype.

(4) H-antigen. The results of H-agglutination tests of the 3 strains revealed that all the strains possessed antigen "a".

2. Subdivision of strains of type V.

Two antisera against #38 and R103 which had been selected by the preliminary test were used. The results of agglutination and absorption tests are shown in Table 7. Eleven strains of type V were divided into two subtypes, one of which comprised 3 strains and the other 8 strains. This finding means that all strains of type V possess a common antigen and at least one other antigen specific to each subtype. Consequently, as shown in Table 8, the two subtypes were designated as A and B, respectively. The antigen common to both subtypes was designated as O–10, since it has been known hitherto as

Table 7. Absorption Tests of Antisera to Type V Strains

Antiserum	Absorbed by	Antigen	Titer	
			Before absorption	After absorption
#38 (1:2,560)	R103	52	2,560	320
		#12	1,280	320
		#38	2,560	320
		R2	320	—
		R9	320	—
		R22	640	—
		R87	320	—
		R94	640	—
		R103	320	—
		R108	640	—
		R112A	320	—
R103 (1:10,240)	#38	52	1,280	—
		#12	1,280	—
		#38	1,280	—
		R2	5,120	1,280
		R9	5,120	640
		R22	5,120	2,560
		R87	5,120	1,280
		R94	5,120	640
		R103	10,240	1,280
		R108	10,240	1,280
		R112A	10,240	1,280

—: No agglutination occurred at a dilution of 1:40.

Table 8. Somatic Antigens of Type V Strains

Type	Subtype	O-antigen	Strain
V	VA	10, 14	52, #12, #38
	VB	10, 15	R2, R9, R22, R87, R94, R103, R108, R112A

O-antigen of strains of type V. Specific antigens of subtypes A and B were named O–14 and O–15, respectively.

DISCUSSION

Many atypical strains of *Yersinia pseudotuberculosis* have been isolated from man and animals. They were classified as *Pasteurella pseudotuberculosis*[5] or reported as an unidentified microorganism resembling *Past. pseudotuberculosis*[1,9]. On the other hand, they had many synonyms, such as *Bacterium enterocoliticum*[10], *Past. pseudotuberculosis Type "b"*[3], *Pasteurella X*[2], and *Germ X*[6]. FREDERIKSEN[4] and MOLLARET[7] established a genus designated Yersinia, with *Yersinia enterocolitica* as the type species, for the above-mentioned organisms, and distinguished this species from *Y. pseudotuberculosis*.

At first, the authors doubted that the 3 untypable strains were *Y. enterocolitica*, because these strains did not belong to any hitherto known serotype, or because they showed little or no pathogenicity for mice and guinea pigs. The biochemical properties and phage susceptibility of these strains, however, were quite similar to those of *Y. pseudotuberculosis*, but distinctly different from *Y. enterocolitica*. Therefore, the authors are in the opinion that the 3 strains were of a new serological type of *Y. pseudotuberculosis*.

Y. pseudotuberculosis has been known to possess O–1 antigen, which is common to all strains. THAL[11] excluded this antigen, however, from the antigenic scheme of *Y. pseudotuberculosis*, because he thought this antigen to be an R-antigen. In the present experiment, the authors considered that the cross-agglutination reactions among the 3 strains of the new type and some strains of the other types were induce by the common antigen O–1. Consequently, the 3 strains were proved to possess a unique somatic antigen by the cross-absorption test. This antigen was designated as O–13, since it had not been shared by the strains of any other type. Therefore, the 3 strains were regarded to be of a new serotype, type VI, the antigenic structure of which was "13:a".

OCHI et al.[8] reported that serotype V according to their classification system was divided into two subtypes, V and V'. As reported previously[12], their type V was identical with type V of the established typing scheme. In other countries than Japan, however, no subdivision of type V strains has been reported. In the present experiment, strain 52, which had been supplied by Dr. H. H. MOLLARET, was classified into subtype

Table 9. Antigenic Formula of *Yersinia pseudotuberculosis* (by Modified THAL's scheme, 1967)

Type	Subtype	O-antigen	H-antigen
I	IA	2,3	a,c
	IB	2,4	a,c
II	IIA	5,6	a,d
	IIB	5,7	a,d
III		8	a
IV	IVA	9,11	b
		9,11	a,b
	IVB	9,12	a,b,d
V	VA	10,14	a,e, (b)
	VB	10,15	undetermined
VI		13	a

VA. From these results, it might be considered that strains of type V isolated in other countries than Japan fell into subtype VA. Strains of subtype VB were isolated only from rabbits by the authors in the Tottori district. They have not been detected from any other district in Japan. This fact suggests that the distribution of strains of subtype VB may be restricted within some limited areas.

THAL's antigenic scheme of *Y. pseudotuberculosis* is shown in Table 9, with supplements of a new additional type, type VI, and the subdivision of type V which was found in the present study. *Y. pseudotuberculosis* is classified into ten types and subtypes according to the combinations of 14 O-anigens; that is, types IA, IB, IIA, IIB, III, IVA, IVB, VA, VB, and VI.

SUMMARY

Biological and serological properties and pathogenicity for experimental animals were examined on 3 strains of *Yersinia pseudotuberculosis* which could not be classified into any of the hitherto known serotypes I to V. These strains were little or non-pathogenic for mice and guinea pigs, but their biological properties and phage susceptibility corresponded to those of *Y. pseudotuberculosis*. All the 3 strains possessed one common O-antigen, which was different from any of O–2 to O–12 antigen and thus designated as O–13. A new serotype was proposed for the 3 strains and designated as type VI. The antigenic structure of the type is O13:Ha.

Eleven strains of type V were divided into two subtypes, A and B. The compositions of O-antigens are O–10 and 14 in subtype VA and O–10 and 15 in subtype VB. One strain of type V received from France belonged to subtype VA.

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仮性結核菌に関する研究

II. 一新菌型の性状とV型菌の亜型

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第I報に記載したように，日本で分離された仮性結核菌は，血清学的に5型に分類されるほか，どの菌型にも属さない菌株が3株存在する．この菌株は，越智らによりI型に分類されたものである．また，越智らの分類によるV型は，VおよびV′型の2亜型に細分されているが，外国では，V型がさらに亜型に細分をされるという報告はない．

本試験は，型別不能の3株の性状と，V型に属する菌株の亜型について，検討するために行なわれた．

1. 型別不能株の性状

a. マウスに対し，1株 (#1) は弱い病原性を示したが，他の2株 (#3 および #14) は，ほとんど病原性を示さなかった．モルモットに対しては，3株とも，1 mg の腹腔内接種で病原性を示さなかった．

b. 生物学的性状およびファージ感受性については，3株とも仮性結核菌の性状を有し，*Yersinia enterocolitica* とは区別される．

c. 血清学的に，既知の菌型に知られていないO-抗原を保有した．この抗原をO-13とし，新しい菌型としてVI型に分類した．

2. V型菌の細分

a. V型菌は，AおよびBの亜型に細分された．両者はそれぞれ共通のO-抗原と，亜型特異抗原を有する．共通抗原を，従来V型に知られているO-10とし，VA型の特異抗原を，O-14，およびVB型の特異抗原をO-15とした．

b. この分類に従えば，フランスから送られたV型菌は，VA型に属する．

本試験の結果，仮性結核菌はIA, IB, IIA, IIB, III, IVA, IVB, VA, VBおよびVI型の10型に分けられ，O-抗原には，O-2からO-15までの14因子が明らかになった．

Note. Since this paper was submitted for publication, undetermined H-antigen of type VB was determined to "a" by THAL and KNAPP (*Symp. Series immunobiol. Standard.*, **15**, 219～222, 1971).