Serotypes and Antibiotic Resistance of *Erysipelothrix rhusiopathiae* Strains Isolated from Pigs Affected with Chronic Swine Erysipelas

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Introduction

Erysipelothrix rhusiopathiae is a causative agent of swine erysipelas, which causes great economic loss in pig production. Strains of *E. rhusiopathiae* are classified into 22 serotypes and N type, which does not produce any precipitating antibody against homologous and heterologous heat-stable antigens in rabbit.

In Japan, a few investigator[4,15,16] reported the results of serotyping of isolates from swine, fish, and birds by tube precipitation test with HCl- or CH_3COOH-extracted antigens. Likewise, there has been no report to attempt serotyping according to the system proposed by Kucsera.[11,12]

Although the drug susceptibility of *E. rhusiopathiae* had been dealt with some investigators,[1,2,7,8,9,10,14] the occurrence of antibiotic-resistant strains except for natural resistance to kanamycin and sulfonamide has not been reported. Antibiotics, especially penicillins, have been widely used for the treatment of this disease. However, in Japan, pigs are usually fed food containing various antibiotics, mainly tetracyclines and macrolides, for the purpose of growth stimulation. It seems, therefore, that long-term administration of antibiotics will give a selective advantage to antibiotic-resistant strains of *E. rhusiopathiae*.

The present report describes the serological classification and the antibiotic resistance in *E. rhusiopathiae* strains isolated from slaughter pigs affected with chronic swine erysipelas in Japan.

Materials and methods

1) *Sources of isolates*

The 258 isolates of *E. rhusiopathiae* submitted to determination of serotypes and drug susceptibility were listed in Table 1. They were isolated from cases of chronic swine erysipelas in 5 slaughter houses from October 1980 to December 1982. Infections associated with the isolates included arthritis (148 cases), lymphadenitis (65 cases), endocarditis (30 cases), and urticaria (15 cases).

2) *Preparation of antigenic extracts for serotyping*

Antigens were prepared by a modification of the methods described by Kucsera.[11,12] Typical smooth colonies from a 48-hr-old agar

Table 1. Sources of *Erysipelothrix rhusiopathiae* isolates from slaughter pigs affected with chronic swine erysipelas in Japan

Source	Date isolated (month/year)	No. of isolates				
		A*	L	E	U	Total
Shibaura Meat Inspectors Station, Bureau of Public Health, Tokyo Metropolitan Government	Jan./82–Oct./82	76	44	5	10	135
Tama Meat Inspectors Station, Bureau of Public Health, Tokyo Metropolitan Government	Oct./80–Nov./82	29	16	2	2	49
Tohbu Meat Inspection Office, Shizuoka Prefecture	Apr./82–Dec./82	14	3	2	2	21
Meat Inspection Office, Hamamatsu City	Apr./82–Dec./82	17	0	6	0	23
Meat Inspection Office, Saitama Prefecture	Sept./82–Oct./82	12	2	15	1	30
Total		148	65	30	15	258

* A: Arthritis, L: Lymphadenitis, E: Endocarditis, U: Urticaria.

plate culture of each isolate were inoculated into tryptic soy broth (pH 7.6, BBL) containing 0.1% Tween 80. Incubation was done at 37°C for 48 hr and the broth culture was centrifuged 3,500 r.p.m. for 20 min. The bacterial cells were washed 3 times with physiological saline and suspended in distilled water to 1/30 of the original volume. The cell suspension was heated in the autoclave at 121°C for 1 hr and clarified by centrifugation. The supernatant fluid was stored at 4°C as autoclave-extracted antigen.

3) Preparation of antisera for serotyping

Antisera were prepared in rabbits by hyperimmunization with killed whole cell antigens made from type reference strains of *E. rhusiopathiae* representing serotype 1 through 22 and N type. The bacterial suspension adjusted to an optical density of 1.8 (540 nm) in the saline was injected intravenously into rabbits at intervals of 3–4 days in dose successively increasing from 1 to 6 m*l*. Eight days after the final administration the rabbits were exsanguinated. Harvested sera were stored at −20°C after addition of 0.1% sodium azide. Antisera were also prepared against isolates whose serotypes could not determined by use of established typing sera.

4) Tests for determination of serotypes

Serotyping was carried out by the agar gel double-diffusion precipitation test. The diffusion medium consisted of 0.8% agar (Special-Agar-Noble, Difco) in the saline containing 0.1% sodium azide. About 12 m*l* of the melted medium was poured onto immunodiffusion plates (Seikagaku Kohgyo, 4.5×9.5 cm). The wells were arranged with a central well and 6 surrounding wells. Antigen was placed in the central well and antisera in the surrounding wells. Then, the plates were covered and placed at room temperature. Precipitin reactions were observed at 24 and 48 hr.

5) Tests for determination of drug susceptibility

Antimicrobial agents studied were penicillin G (PC-G), ampicillin (APC), erythromycin (EM), oleandomycin (OM), oxytetracycline (OTC), chloramphenicol (CP), dihydrostreptomycin (DSM), kanamycin (KM), and sulfadimethoxine (SDM). The minimal inhibitory concentration (MIC) was determined by an agar dilution method.[5] A 10^{-2} dilution of an overnight tryptic soy broth culture was inocu-

Table 2. Type strains of *E. rhusiopathiae* used in production of antisera and cross reactions in the agar gel double-diffusion precipitation test

Antigen		Antiserum																							
Type strain	Serotype	1a	1b	2a	2b	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	N
ME-7	1a	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
422/1E1	1b	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
R32E11	2a	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
NF4E1	2b	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Wittling	3	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Doggerscharbe	4	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Pécs 67	5	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Tuzok	6	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
P-43	7	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−
Goda	8	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Kaparek	9	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Lengyel-P	10	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−
IV 12/8	11	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−
Pécs 9	12	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−
Pécs 56	13	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−
Iszap-4	14	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−
Pécs 3597	15	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−
Tanzania	16	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−
545	17	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−
715	18	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−
2017	19	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−
2553	20	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−
Bãno 36	21	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−
Bãno 107	22	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−
MEW 22	N	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

lated with a microplanter (Ebara Works, Tokyo, Japan) onto Mueller-Hinton agar containing serial twofold dilution of the test antibiotic. The plates were incubated at 37°C for 48 hr. The MIC was defined as the lowest concentration of antimicrobial agent that prevented macroscopic growth.

6) Isolation method of plasmid DNA

Isolation of plasmid DNA was attempted by the method of Womble et al.[17] and Kado and Liu,[6] except for the use of sodium laurolyl sarcosinate (6%) in the procedure of bacteriolysis.

Results

1) Agar gel double-diffusion precipitation tests on type reference strains

Table 2 shows the results that all type antisera prepared from reference strains were tested with the antigens of the strains. Serotype 1a antigen gave reaction with antisera for 1a only, while 1b antigen gave reaction with 1a and 1b antisera. Subtypes 2a and 2b could not be distinguished by the precipitin lines. Cross reaction was observed between serotypes 7 and 14, while all other type strains and sera showed only homologous recations.

Table 3. Serotypes of 258 *E. rhusiopathiae* isolates from slaughter pigs affected with chronic erysipelas

Origin	Serotype										Total
	1a	1b	2	3	5	6	8	11	21	N	
Arthritis	21	6	107	1	1	4		5	1	2	148
Lymphadenitis	5	10	45			3	1			1	65
Endocarditis	3	2	25								30
Urticaria			14		1						15
Total	29	18	191	1	2	7	1	5	1	3	258

Table 4. Susceptibility of 258 isolates of *E. rhusiopathiae* to antimicrobial agents

Drug	No. of isolates with MIC (μg/ml) of:															MIC(μg/ml) breakpoint of resistance	No. of resistant strains(%)
	0.025	0.05	0.1	0.2	0.39	0.78	1.56	3.13	6.25	12.5	25	50	100	>100	>400		
PC-G[a]	23	213	22														
APC	23	162	73														
EM	2	54	177	10					3	6	4	2				0.78	15(5.8)
OM				6	10	174	55		5	8						3.13	13(5.0)
OTC				2	3	87	56		5	15	15	72	3			3.13	110(42.6)
CP							11	16	205	23	3						
DSM									173	24	7	4	5	45		100	45(17.4)
KM														258			
SDM															258		

a) Units per milliliter.

2) Serotypes of isolates

The distribution of serotypes of 258 *E. rhusiopathiae* isolates from slaughter pigs affected with chronic swine erysipelas is shown in Table 3. Of 148 isolates from the cases of arthritis, 107 (72.7%) belonged to serotype 2, 21 (14.2%) to serotype 1a, 6 (4.1%) to serotype 1b, 5 (3.4%) to serotype 11, 4 (2.7%) to serotype 6, 2 (1.3%) to type N, 1 (0.7%) to serotypes 3, 5, or 21, respectively. Of 65 isolates from the cases of lymphadenitis, 45 (69.2%) belonged to serotype 2, 10 (15.4%) to serotype 1b, 5 (7.7%) to serotype 1a, 3 (4.6%) to serotype 6, and 1 (1.5%) to serotype 8 or type N, respectively. Of 30 isolates from the cases of endocarditis, 25 (83.3%) belonged to serotypes 2, 3 (10.0%) to serotype 1a, and 2 (6.7%) to serotype 1b. Of 15 isolates from the cases of urticaria, 14 (93.3%) belonged to serotype 2 and 1 (6.7%) to serotype 5.

3) Drug susceptibility of isolates

The results of MIC determination are shown in Table 4. All of the strains were highly susceptible to PC-G and APC (MIC, 0.025 to 0.1 U or μg/ml) and moderately susceptible to CP (MIC, 1.56 to 25 μg/ml). KM and SDM showed no activity against the strains (MICs, >100 and >400 μg/ml, respectively). MICs of EM, OM, OTC, and DSM presented 2 distribution peaks. The MIC breakpoints of strains resistant to EM, OM, OTC, and DSM were assumed to be 0.78, 3.13, 3.13, and 100 μg/ml, respectively. The frequencies of isolation of *E. rhusiopathiae* strains resistant to each drug were as follows: OTC (42.6%), DSM (17.4%), EM (5.8%), and OM (5.0%).

Table 5. Relationship between resistance patterns, sources, and serotypes of 258 *E. rhusiopathiae* isolates

Resistance pattern[a]	No. of isolates								
	Source[b]				Serotype				Total (%)
	A	L	E	U	1a	1b	2	Other[c]	
EM OM OTC DSM	5	7	0	0	0	0	12	0	12 (4.6)
EM OTC DSM	2	1	0	0	0	0	3	0	3 (1.2)
OM OTC DSM	1	0	0	0	0	0	1	0	1 (0.4)
OTC DSM	20	3	1	4	0	0	28	0	28 (10.8)
OTC	44	11	4	7	0	3	59	4	66 (25.6)
DSM	0	1	0	0	0	0	1	0	1 (0.4)
Total Resistant	72	23	5	11	0	3	104	4	111 (43.0)
Susceptible	76	42	25	4	29	15	87	16	147 (57.0)

a) EM, resistant to erythromycin: OM, resistant to oleandomycin: OTC, resistant to oxytetracycline: DSM, resistant to dihydrostreptomycin.
b) A, arthritis: L, lymphadenitis: E, endocarditis: U, urticaria.
c) Includes serotypes 3, 5, 6, 8, 11, 21, and N.

Table 6. Stability of resistance of *E. rhusiopathiae* isolates after repeated serial passages in broth medium

Strain tested	No. of passage	MIC (μg/ml) of:				Resistance pattern
		EM	OM	OTC	DSM	
82–561	0	25	12.5	25	>100	EM OM OTC DSM
	10	25	12.5	25	>100	EM OM OTC DSM
82–583	0	0.2	1.56	50	>100	OTC DSM
	10	0.2	1.56	25	>100	OTC DSM
S–46	0	0.39	1.56	25	6.25	OTC
	10	0.2	1.56	50	3.13	OTC

4) Relationship between resistance patterns, sources, and serotypes of isolates

The relationship between antimicrobial resistance patterns, sources, and serotypes of 258 *E. rhusiopathiae* isolates are shown in Table 5. A total of 111 (43.0%) strains were resistant to EM, OM, OTC, or DSM. Six resistance patterns were found. Strains resistant only to OTC (25.6%) were most frequent, followed by those resistant to OTC and DSM (10.8%), EM, OM, OTC, and DSM (4.6%), EM, OTC, and DSM (1.2%), OM, OTC, and DSM (0.4%), and DSM (0.4%). In the *E. rhusiopathiae* strains isolated from cases of endocarditis, the frequency of resistance was significantly ($P<0.05$, Fisher's exact test) lower than in the isolates from other cases. Triple resistance and quadruple resistance were found only in isolates from cases of arthritis and lymphadenitis. Of the 111 resistant strains, 104 (93.7%) belonged to serotype 2. Only 7 (6.3%) strains belonging to serotypes 1b, 11, or type N were single resistant to OTC.

5) Stability of resistance and detection of plasmid DNA

The results of stability test on resistance of isolates after serial passages in broth

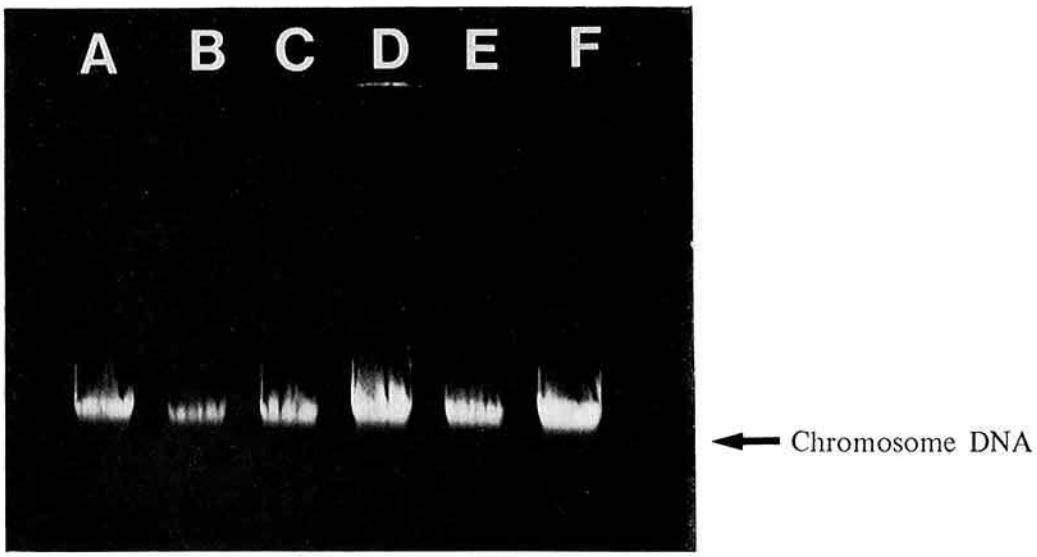

Plate 1. Agarose gel profile of purified DNA from drug resistant strains of *E. rhusiopathiae*
A: strain 582 resistant to EM, OTC, and DSM
B: strain 904 resistant to EM, OTC, and DSM
C: strain 900 resistant to EM, OM, OTC, and DSM
D: strain 878 resistant to OTC
E: strain 583 resistant to OM, OTC, and DSM
F: strain 860 resistant to OTC and DSM

medium are shown in Table 6. Resistance to EM, OM, OTC, or DSM was stable after 10 passages. Agarose gel profile of purified DNA from resistant strains of *E. rhusiopathiae* are shown in Plate 1. No plasmid DNA was detected in the strains showing the various resistance patterns, though the chromosomal DNA was found.

Discussion

The present study on serotyping demonstrated the presence of a wide variety of serotypes of *E. rhusiopathiae* strains isolated from the cases of chronic swine erysipelas, particularly arthritis or lymphadenitis. It has been generally known that most isolates of *E. rhusiopathiae* from pigs affected with clinical erysipelas fall into serotypes 1a, 1b, and 2, namely, most isolates from the cases of acute septicemia belonged to serotype 1a, from arthritis or urticaria belonged to serotype 2, and from endocarditis belonged to serotypes 1a, 1b, or 2.[18] Kucsera,[13] Cross and Claxton[3] reported 0.5 to 1.0% of isolates obtained from the cases of arthritis belonged to rare serotypes 4, 5, 7, or 10. The present results showed that about 90% of the isolates belonged to the major serotypes 1a, 1b, or 2. This is in general agreement with the results reported previously by others.[3,4,13,15,16] It should be noted, however, that 20 (7.8%) strains of serotypes 3, 5, 6, 8, 11, 21, and type N were isolated from the cases of arthritis, lymphadenitis, or urticaria, and that 26 (10.1%) strains of serotype 1a were isolated from the cases of arthritis or lymphadenitis. Serotypes 1a, 1b, and 2 of *E. rhusiopathiae* are still generally believed to be perhaps the only causes of clinical erysipelas in swine. The occurrence of strains of rare serotypes other than serotypes 1a, 1b, or 2 would indicate a possible role of these strains in the causes of clinical erysipelas. Assuming that the arthritis or lymphadenitis was consequent to temporary bacteriemia with strains of serotype 1a in

swine, the occurrence of serotype 1a in the cases of chronic erysipelas may be explainable.

The present results on susceptibility of *E. rhusiopathiae* isolates to PC-G, APC, CP, KM, and SDM are in general agreement with those reported previously by others,[1,2,7,8,9,10,14] indicating that penicillins remain the antibiotics of choice for the treatment of swine erysipelas. It should be noted, however, that 43.0% of *E. rhusiopathiae* isolates examined showed resistance to EM, OM, OTC, or DSM. This is the first report on resistant strains of *E. rhusiopathiae*. It seems probably that the acquirement of resistance in *E. rhusiopathiae* may be due to the chromosomal mutation, because no plasmid DNA in resistant strains was found and resistance was stable after serial passages in broth medium. In any case, frequent use of tetracyclines and macrolides for pig production will undoubtedly give a selective advantage to antibiotic-resistant strains of *E. rhusiopathiae*.

Our results also showed that most of the resistant *E. rhusiopathiae* strains belonged to serotype 2. It would be of great interest if there is any correlation between serotypes of *E. rhusiopathiae* and the occurrence of antibiotic resistance. Further epidemiological studies are necessary to clarify this correlation.

Summary

Serotypes and antibiotic resistance of 258 isolates of *E. rhusiopathiae* from slaughter pigs affected with chronic swine erysipelas during a period from 1980 to 1982 in Japan were determined. Predominant serotypes of 213 isolates from the cases of arthritis or lymphadenitis were serotypes 1a, 1b, 2, 6, and 11 (12.2, 7.5, 71.4, 3.3, and 2.3%, respectively). The other serotypes 3, 5, 8, 21, and type N composed 3.3% of isolates. Of 30 isolates from the cases of endocarditis, 3 belonged to serotype 1a, 2 belonged serotype 1b and 25 belonged to serotype 2. Of 15 isolates from the cases of urticaria, 14 belonged to serotype 2 and 1 to serotype 5.

A total of 111 (43.0%) strains were resistant to EM, OM, OTC, or DSM. Strains resistant only to OTC (25.6%) were most frequent, followed by those resistant to OTC and DSM (10.8%), EM, OM, OTC, and DSM (4.6%), EM, OTC, and DSM (1.2%), OM, OTC, and DSM (0.4%), and DSM (0.4%). Of the 111 resistant strains, 104 (93.7%) belonged to serotype 2. Plasmid DNAs were not detected in strains showing the various resistance patterns. This is the first report of resistance of *E. rhusiopathiae* to these antibiotics.

References

1) Azechi, H. et al.: Sensitivity of freshly isolated strains of *Erysipelothrix insidiosa* to antibiotics. *J. Jpn. Vet. Med. Assoc.*, 24, 92–97 (1971).
2) Cooper, M. S. & Allen, G. A.: The effect of chlortetracycline on immune response. II. Influence on development of protective antigen after vaccination with a live avirulent strain of *Erysipelothrix rhusiopathiae*. *J. Immunol.*, 83, 232–236 (1959).
3) Cross, G. M. R. & Claxton, P. D.: Serological classification of Australian strains of *Erysipelothrix rhusiopathiae* isolated from pigs, sheep, turkey, and man. *Aust. Vet. J.*, 55, 77–81 (1979).
4) Hashimoto, K., Yoshida, Y. & Sugawara, H.: Serotypes of *Erysipelothrix insidiosa* isolated from swine, fish, and birds in Japan. *Nat. Inst. Anim. Health Q. (Jpn.)*., 14, 113–120 (1974).
5) Ishiyama, S. et al.: On the standardization of method for determination of minimum inhibitory concentrations. *Chemotherapy (Tokyo)*, 16, 98–99 (1968).
6) Kado, C. I. & Liu, S. T.: Rapid procedure for detection and isolation of large and small plasmids. *J. Bacteriol.*, 145, 1365–1373 (1981).
7) Kalich, J. & Neubrand, D.: Tierexperimentelle Studien über die Rotlaufpathogese unter Berücksichtigung der Beifutterung von Antibiotica. *Mh. Tierheik.*, 9, 298–312 (1957). [In German]
8) Kalich, J. & Neubrand, D.: Zur Frageder Epidemiologie und Pathogenes des Schweinerotlaufs. *Mh. Tierheik.*, 11, 85–89 (1959). [In German]
9) Kielstein, P.: Zur bakteriellen Chemoresistenz des Rotlauferregers. *Arch. Exp. Vet. Med.*, 15, 1161–1171 (1961). [In German]

10) Kinjo, T. & Machida, S.: Sensitivity of *Erysipelothrix insidiosa* to antibiotics and trials of artificial induction of penicillin resistant strains in vitro. *Sci. Bull. Coll. Agr. Univ. Ryukyus Okinawa*, **20**, 335–341 (1973).
11) Kucsera, G.: Comparative study on special serotypes of *Erysipelothrix rhusiopathiae* strains isolated in Hungary and abroad. *Acta Vet. Acad. Sci. Hung.*, **22**, 251–261 (1972).
12) Kucsera, G.: Proposal for standardization of the designation used for serotypes of *Erysipelothrix rhusiopathiae* (Migula) Buchanan. *Int. J. Syst. Bacteriol.*, **23**, 184–188 (1973).
13) Kucsera, G.: Serological typing of *Erysipelothrix rhusiopathiae* and the epidemiological significance of the typing. *Acta Vet. Acad. Sci. Hung.*, **27**, 19–28 (1979).
14) Polityнskabans, E.: Susceptibility of various phage and serotypes of *Erysipelothrix insidiosa* to antibiotics. *Bull. Vet. Inst. Pulawy*, **9**, 78–83 (1965).
15) Sasaki, Y. et al.: Clinical, pathological and bacteriological observations on erysipelas arthritis in piglets. *Bull. Nat. Inst. Anim. Health*, **60**, 13–21 (1970) [In Japanese].
16) Seto, K., Muramatsu, M., & Yoshida, K.: Serotypes of isolates from pigs affected with 3 types of erysipelas and attenuated strains of *Erysipelothrix insidiosa*. *Ann. Rep. Nat. Vet. Assay Lab.*, **9**, 31–36 (1979) [In Japanese with English summary].
17) Womble, D. D., Taylor, D. P., & Rownd, R. H.: Method for obtaining more-accurate covalently closed circular plasmid-to-chromosome ratios from bacterial lysate by dye-buoyant density centrifugation. *J. Bacteriol.*, **130**, 148–153 (1977).
18) Wood, R. L.: Swine erysipelas—a review of prevalence and research. *J. Am. Vet. Med. Assoc.*, **184**, 944–949 (1984).

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