J. King Saud Univ., Vol. **2**, *Science* (1), pp. 5 – 12 (1410H/1990)

Effects of pH, Chemicals and Antibacterial Agents on Some Strains of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*

Ali Abdullah Salamah
Microbiology Unit, Botany Department, College of Science, King Saud University, P.O.Box 2455, Riyadh 11451, Saudi Arabia

(Received 4 October 1987; accepted for publication 28 June 1988)

Abstract. The effect of pH, some chemicals and antibiotics on the growth of three strains each of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* were studied. *Y. enterocolitica* was found to be more tolerant to the changes of pH and more resistant to the tested chemicals and antibacterial agents than *Y. pseudotuberculosis*. The concentrations of oxgall, sodium deoxycholate, cobaltous sulfate, glycine and chloramphenicol that are required for inhibition vary significantly between the two species under study. This variation makes these agents good candidates for any formulation of a selective medium for the selection of *Y. enterocolitica* from a sample which contains the two species. The concentrations of bile salts, novobiocin and methicillin that are required for inhibition were observed to be very similar for the different *Yersinia* strains. However, the concentrations required for inhibition are higher than the concentrations required for the inhibition of most of the Gram-negative bacteria and this may be useful in any formulation of a selective medium for the selection of the two *Yersinia* species from a sample with a mixed bacterial population.

Introduction

Yersinia enterocolitica and *Yersinia pseudotuberculosis* are Gram-negative rods of the family Enterobacteriaceae [1, pp. 448-506]. They share a variety of physiological, biochemical, serological, and virulence properties which include the following: Motility at 22–28°C but not at 37°C, possession of two plasmids with an identical size and roles, ability to produce a protein- lipoprotein complex (V and W antigen), and requirement of calcium for growth at 37°C but not at 26°C [2,3].

The two species have an animal reservoir such as domestic and wild mammals and birds. Their transmission to humans is through food contaminated by animal excreta or by direct contact with infected animals and consumption of infected meat

[4]. Further, *Y. enterocolitica* is also transmitted by water [5]. Once transmitted to humans, the virulent strains from the two species could lead to diarrhoeal diseases, both acute and chronic, and acute mesenteric lymphadenitis and terminal ileitis [6,7].

This study was under taken to compare the two species for sensitivity to some selected chemicals and antibiotic agents and for the effect of pH on their growth. This comparison should give some valuable results which might be used for the selection of either of the two species from a medium which contains both or selection of the two species from a sample with mixed bacterial population.

Materials and Method

Bacterial strains

Y. enterocolitica (strains NCTC 10460, ATCC 23715 and 8272) and *Y. pseudotuberculosis* (strains NCTC 10275, NCTC 824 and PB1) were obtained from the American Type of Culture Collection (ATCC), the National Collection of Type Cultures (NCTC) and from other sources (8272 and PB1) described by Salamah and Charnetzky [8]. They were grown in blood agar base slants (Difco) at 26°C and stored in the refrigerator with monthly transfers.

Effect of pH on growth

The effect of pH on growth was determined using Higuchi and Carlin [9] medium supplemented with 2.5 mM calcium. The pH value of the medium was adjusted at 5.5, 7.5 and 9.5. Media with different pH values was poured in 250 ml flasks (50 ml/flask), inoculated with an equivalent number of cells, and incubated with continuous shaking at 26°C. Samples were removed at 2 hr intervals, diluted by 0.85% saline, spread on plates of blood agar base supplemented with 2.5 mM calcium, and counted after 20 hr incubation at 26°C.

Effect of chemical agents

The effect of chemical agents (BDH) was studied by spreading an appropriate dilution of cells grown for 14 hr on blood agar base plates (10^2 colony-forming units/plate) containing different concentrations of the chemical agent under test. The blood agar base without any test chemical was used as a control. The colonies were counted after an incubation period of 48 hr at 26°C. The percent recovery was estimated by comparing the total count of cells plated on treated blood agar base to the count of cells on non-treated blood agar base.

Effect of the antibacterial agents

The effect of the antibacterial agents was determined by using a stock solution (2 mg/ml) of the dissolved antibiotics (WINLAB). The cells were spread on blood

agar base plates (10^4 colony-forming units/plate) containing different concentrations of the test antibiotic and on non-treated blood agar base plates as a control. The concentration of any antibiotic giving 3 colonies or less was recorded after an incubation period of 48 hr at 26°C.

Results

Effect of pH on growth

The effect of pH on the growth rates of four strains of *Y. enterocolitica* and *Y. pseudotuberculosis* is shown in Fig. 1. The strains of the two species grew at an equal rates at pH 7.5. The pH value higher or lower than 7.5 reduced the growth rate for all the tested strains, however, all the strains were more tolerant to alkaline pH than to acidic pH and the growth rates of *Y. pseudotuberculosis* strains were severely retarded at pH 5.5.

The results for the effects of pH on the growth rates of strains 8272 and PB1 were essentially the same as that shown for the other four strains.

Effect of the chemical agents

Table 1 shows the percent recovery of the strains of the two species in the presence of different selective chemical agents. The concentration of the chemical agent which was inhibiting the two species at the maximum variable and workable degree was chosen as the appropriate concentration for comparison which is presented here. It was observed that *Y. pseudotuberculosis* strains were inhibited more than *Y.*

Table 1. Effect of some selected chemicals on the growth of some selected strains of *Y. enterocolitica* and *Y. pseudotuberculosis*.

Chemical agent	Concentration %	Percent recovery					
		Y. enterocolitica strains			*Y. pseudotuberculosis* strains		
		NCTC 10460	ATCC 23715	8272	NCTC 10275	NCTC 824	PB1
Oxgall	5.5	88	79	82	44	56	51
Sodium azide	0.005	0	0	0	0	0	0
Sodium deoxycholate	1.5	84	81	82	52	39	46
Cobaltous sulfate	0.1	13	13	12	6	4	6
Glycine	0.1	83	83	80	46	53	52
Lithium chloride	0.2	45	47	45	36	31	35
Bile salts	5	87	81	82	80	77	77

enterocolitica strains by most of the tested chemicals. Bile salts, however, inhibited the strains of the two species to an approximately equal extent. Growth in the presence of 0.005% sodium azide was completely inhibited for both.

Effect of the antibacterial agents

The antibacterial agents used in this study are shown in Table 2. The *Y. pseudotuberculosis* strains were inhibited by a lower concentrations of chloramphenicol, ampicillin and kanamycin than *Y. enterocolitica* strains. The other antibiotics exhibited similar inhibitory concentrations for both species.

Table 2. Effect of some selected antibiotics on the growth of some selected strains of *Y. enterocolitica* and *Y. pseudotuberculosis*.

Antibiotic agent	Minimal inhibitory concentration (μg/ml)					
	Y. enterocolitica strains			*Y. pseudotuberculosis* strains		
	NCTC 10460	ATCC 23715	8272	NCTC 10275	NCTC 824	PBI
Chloramphenicol	10	12	9	2	1	2
Ampicillin	10	3	6	< 1	1	2
Novobiocin	200	200	205	195	180	185
Neomycin	3	5	4	2	2	1
Methicillin	120	140	120	115	110	105
Kanamycin	3	10	6	1	< 1	1
Gentamycin	1	2	1	1	< 1	< 1

Discussion

The increasing recovery of *Y. enterocolitica* and *Y. pseudotuberculosis* from foods and their cause of some human diseases necessitate the development of a good selection medium.

A number of studies were undertaken by several authors for the isolation of these two species from foods and water using a selective method based upon their stability at high sodium hydroxide concentrations [4,5,10,11]. This method, however, is not efficient for their isolation from a variety of sources. The development of a suitable selection medium which would be useful for their detection in samples from a variety of sources requires preliminary physiological and sensitivity studies. This paper deals with comparative studies between some selected strains of the two species. This approach will be useful not only for the development of a new selection medium which will allow the growth of the two species and inhibit the non *Yersinia*

species, but also for the development of a selection medium which will allow the growth of one species and inhibit the other.

It was observed during this study that *Y. enterocolitica* was more tolerant to changes in pH. This observation might be useful in the selection for *Y. enterocolitica* from a sample which contains both species, simply, by slowing the growth rate of *Y. pseudotuberculosis*.

The variations in the sensitivity of the two species to the antibiotics and the chemicals examined will also assist in the selective recovery of *Y. enterocolitica* strains, because the *Y. pseudotuberculosis* strains were inhibited at a lower concentration of most of the agents studied.

The tolerance of bile salts by the two species is very significant. Bile salts combine with the divalent cations inhibiting most of the Gram-negative bacteria [12]. The virulent species under study, however, requires calcium for growth at 37°C but not at 26°C [2,3]. Therefore, bile salts when used as a selective agent should be used at 26°C.

Gram-negative bacteria vary with respect of the sensitivity to the antibiotics studied [13]. Chloramphenicol inhibits the growth of a wide range of Gram-negative and Gram-positive bacteria. The minimum inhibitory concentrations obtained here vary significantly between the two species under study which makes this antibiotic useful as a selective agent for *Y. enterocolitica* strains, because they tolerated this antibiotic at a higher concentrations than *Y. pseudotuberculosis*.

Novobiocin and Methicillin were tolerated by the strains of the two species at a concentrations which are active against certain other Gram-negative bacteria, and therefore, they are good selective agents for the *Yersinia* species under investigation.

The studies reported here have shown clear differences between *Y. enterocolitica* and *Y. pseudotuberculosis* with respect to pH and sensitivity to some selected agents. These differences should be taken into consideration in any future studies concerning their selection from the samples with mixed bacterial populations.

Acknowledgement. This research (Bot/1404/22) was supported by the Research Center, College of Science, King Saud University, Riyadh, Saudi Arabia. I would like to thank Mr. Shamsher Khan for reading the manuscript.

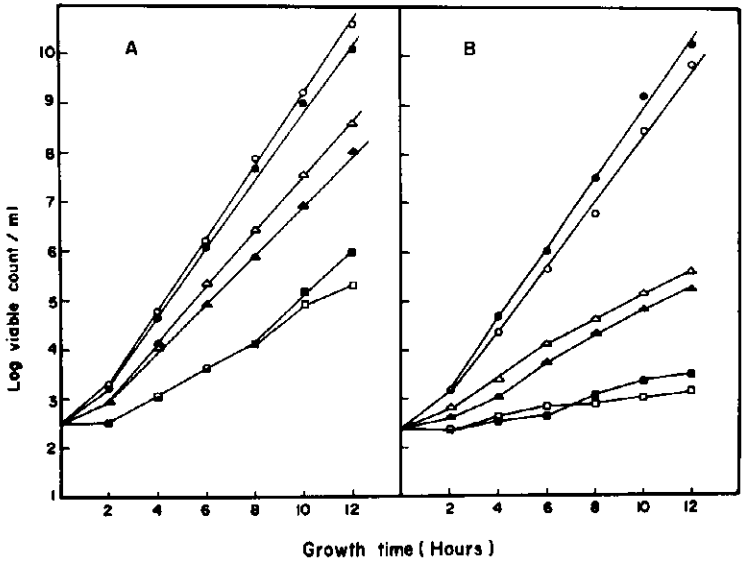

Fig. 1. Effect of pH (circles pH 7.5, triangles pH 9.5, squares pH 5.5) on the growth of *Y. enterocolitica* (panel A; closed symbols represent strain NCTC 10460, open symbols represent strain ATCC 23715) and *Y. pseudotuberculosis* (panel B; closed symbols represent strain NCTC 10275, open symbols represent strain NCTC 824).

References

[1] Bercovier, H. and Mollart, H.H. Yersinia. In: *Bergey's Manual of Systematic Bacteriology*, Vol. 1, (N.R. Krieg and J.G.Holt, Eds.) Baltimore: Williams and Wilkins Co., 1984.

[2] Brubaker, R.R. "The Genus *Yersinia*: Biochemistry and Genetics of Virulence." *Curr. Top. Microbiol.*, 57 (1972), 111–158.

[3] Portnoy, D.A., Wolf-Watz, H., Bolin, I., Beeder, A.B. and Falkow, S. "Characterization of Common Virulence Plasmids in *Yersinia* Species and Their Role in the Expression of Outer Membrane Proteins." *Infect. Immun.*, 43 (1984), 108–114.

[4] Aulisio, C.G., Mehlman, I.J. and Sanders, A.C. "Alkali Method for Rapid Recovery of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* from Foods." *Appl. Environ. Microbiol.*, 39 (1980), 135–140.

[5] Marinelli, G., Dinnocenzo, C., Fabiani, L. and Leoni, V. "Application of a Simplified Method for Recovery of *Yersinia enterocolitica* from Surface Waters." *Appl. Environ. Microbiol.*, 49 (1985), 1348–1349.

[6] Kohl, S., Jacobson, J. and Nahmias, A. "*Yersinia enterocolitica* Infections in Children." *J. Pediatr.*, 89 (1976), 77–79.

[7] Tertti, R., Granfors, K., Lehtonen, O.P., Mertsola, J., Makela, A.L., Valimaki, I., Hanninen, P. and Toivanen, A. "An Outbreak of *Yersinia pseudotuberculosis* Infection." *J. Infect. Dis.*, 149 (1984), 245–250.

[8] Salamah, A.A. and Charnetzky, W.T. "Effect of the Virulence Associated 47 Megadalton Plasmid of *Yersinia pestis* on Permeability to Gentian Violet and Sensitivity to Novobiocin." *Acta. Microbiol. Hung.*, 33 (1986), 193–202.

[9] Higuchi, K. and Karlin, C. "Studies on the Nutrition and Physiology of *Pasteurella pestis*. II. A Defined Medium for the Growth of *Pasteurella pestis*." *J. Bacteriol.*, 75 (1957), 409–413.

[10] Schiemann, D.A. "Development of a Two-step Enrichment Procedure for Recovery of *Yersinia enterocolitica* from Food." *Appl. Environ. Microbiol.*, 43 (1982), 14–27.

[11] Fukushima, H. "Direct Isolation of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* from Meat." *Appl. Environ. Microbiol.*, 50 (1985), 710–712.

[12] Kinner, J.A. and Moats, W.A. "Selective Action of Sodium Cholate-$MgCl_2$ Broth and Its Possible Use in Isolation of Salmonellae and Other Enteric Pathogens." *J. Food. Prot.*, 41 (1978), 638–642.

[13] Bryan, L.E. *Bacterial Resistance and Susceptibility to Chemotherapeutic Agents*. Cambridge: Cambridge University Press, 1982.

تأثير الرقم الهيدروجيني، المواد الكيميائية والمضادات البكتيرية على بعض سلالات البكتيريا يرسينيا بسيودوتيوبركيولوسس ويرسينيا انتيروكوليتيكا

علي عبدالله السلامة

وحدة الأحياء الدقيقة ـ قسم علم النبات ـ كلية العلوم ـ جامعة الملك سعود ـ ص.ب ٢٤٥٥ ـ الرياض ١١٤٥١ ـ المملكة العربية السعودية

(أستلم في ١١ صفر ١٤٠٨هـ، قُبل للنشر في ١٤ ذوالقعدة ١٤٠٨هـ)

ملخص البحث. تمت دراسة تأثير الرقم الهيدروجيني وبعض المضادات الحيوية والمواد الكيميائية على ثلاث سلالات من كل من البكتيريا يرسينيا بسيودتيوبركيولوسس ويرسينيا انتيروكوليتيكا ووجد أن سلالات البكتيريا يرسينيا انتيروكوليتيكا أكثر تحملا لتغيرات الرقم الهيدروجيني وأكثر مقاومة للمواد الكيميائية والمضادات الحيوية التي تم اختبارها. تركيزات الأوكسجال وكولات الصوديوم وكبريتات الكوبلت والجليسين والكلورامفينيكول اللازمة لتثبيط النمو تختلف بشكل مميز بين النوعين تحت الدراسة وهذا الاختلاف يجعل هذه المواد مناسبة جدا لعمل بيئة اختيارية لاختيار أحد الأنواع من عينة تحتوي على النوعين معا. لقد وجد أن تراكيز أملاح الصفراء والنوفوبيوسين والميثيسلين اللازمة للتثبيط متشابهة بالنسبة للنوعين ولكنها أكثر من التراكيز اللازمة لتثبيط غالبية البكتيريا السالبة لصبغة جرام مما جعل هذه المواد مفيدة في تركيب بيئة اختيارية يمكن استخدامها في اختيار هذين النوعين من عينة تحتوي على خليط من المجاميع البكتيرية.