The Journal of Medical Microbiology

Vol. 11, No. 3

DIFFERENTIATION OF *SERRATIA MARCESCENS* AND *SERRATIA LIQUEFACIENS* BY TESTS FOR LIPASE AND PHOSPHOLIPASE PRODUCTION

N. J. LEGAKIS, K. J. NICOLAS, M. XILINAS AND J. PAPAVALASSIOU

*Department of Microbiology, Faculty of Medicine, University of Athens,
Athens PO Box 1540, Greece*

SERRATIA spp. are almost exclusively encountered in nosocomial infections, especially in patients with compromised host defences or treated with broad-spectrum antibiotics (Bodey, Rodriguez and Smith, 1970; Dans *et al.*, 1970; Ball, 1976). Infection may produce conditions that endanger life, e.g., septicaemia (Dodson, 1968; Altemeier *et al.*, 1969) and endocarditis (Alexander, Reichenbach and Merendino, 1969). Furthermore *Serratia marcescens*, like certain other opportunistic pathogens such as *Pseudomonas aeruginosa*, is susceptible to the development of multiple antibiotic resistance.

The production of prodigiosin by *S. marcescens* has been used, together with biochemical reactions, to separate the organism from those of related genera (Wilson and Miles, 1964). However, the characteristic pigment is absent in most clinical isolates (Wilkowske *et al.*, 1970) and, even when present, it does not conclusively identify *S. marcescens*, as pigmented biotypes of *Serratia liquefaciens* (formerly *Enterobacter liquefaciens*) exist (Edwards and Ewing, 1972).

The genus *Serratia* presents special problems of identification because of biochemical and morphological similarity to other genera of the Enterobacteriaceae, notably *Klebsiella* and *Enterobacter* (Blazevic, 1969; Edwards and Ewing, 1972). The deoxyribonuclease (DNAase) test has been suggested for distinguishing *S. marcescens* from the other members of the Enterobacteriaceae (Rothberg and Swartz, 1965; Blazevic, 1969). However, *S. liquefaciens* also produces DNAase (Martin and Ewing, 1967) and may have biochemical reactions similar to those of *S. marcescens*. Nucleoside phosphotransferase production is also common to both *S. marcescens* and *S. liquefaciens* (Durand and Blazevic, 1970).

Received 19 July 1977; accepted 1 Oct. 1977

The purpose of this study was to investigate by means of thin-layer chromatography the production of lipase and phospholipase by *S. marcescens* and *S. liquefaciens*, as a method for the rapid differentiation of these organisms.

MATERIALS AND METHODS

Strains. Thirty-three strains of *S. marcescens* and 15 strains of *S. liquefaciens* were examined. Of the former, nine were obtained from the Department of Laboratory Medicine, University of Minnesota, and five from the National Collection of Type Cultures, London; the rest were clinical isolates. Of the *S. liquefaciens* strains, seven were obtained from the Department of Laboratory Medicine, University of Minnesota, and the others were clinical isolates. In addition, recent clinical isolates of other species were tested, including twenty strains of *Klebsiella pneumoniae*, nine of *Enterobacter aerogenes*, eight of *Enterobacter hafniae*, eight of *Enterobacter cloacae*, fifteen of *Escherichia coli*, seven of *Citrobacter freundii*, five of *Proteus mirabilis*, five of *Proteus vulgaris*, three of *Proteus rettgeri* and seven of *Proteus morgani*. The isolates were identified by conventional tests (Cowan and Steel, 1961; Ewing and Martin, 1974). DNAase was tested in DNAase Medium (Difco) supplemented with *o*-toluidine (Schreier, 1969).

Detection of lipase and phospholipase. Lipase (Legakis and Papavassiliou, 1974) and phospholipase (Legakis and Papavassiliou, 1975) activities were assayed by thin-layer chromatography after incubation of resting cells for 4 h at 37°C in 0.1M tris buffer, pH 8.0, containing triolein (Fluka, Switzerland) as substrate for lipase, and purified lecithin (Pangborn, 1951) as substrate for phospholipase. These procedures permitted the semi-quantitative determination of lipolytic activity by estimation of the unhydrolysed substrate.

RESULTS

Complete or almost complete (80–100%) hydrolysis of triolein was achieved by almost all the strains of *S. marcescens* and *S. liquefaciens* studied (table I). In contrast, under the same conditions there was no hydrolysis of triolein by any of the other bacterial strains tested, except three of *Ent. cloacae*, two of *P. mirabilis* and three of *P. vulgaris*; these hydrolysed 10–40% of the substrate.

The chromatograms of the lipase-positive strains showed that free fatty acids (FFA) were the only lipid-soluble products and that hydroxylation of oleic acid did not occur. With the procedures used there was no extraction of lipids from the bacteria themselves.

Table II shows that almost one-half of the *S. marcescens* strains possessed strong phospholipase activity that hydrolysed 70–100% of the lecithin in the test mixture. In contrast, most of the *S. liquefaciens* strains showed activity that was slight, and in only two of 15 strains was it strong. No phospholipase was detected in any of the other bacterial strains except one-half of the *Ent. aerogenes* and *Ent. hafniae* strains; these showed slight activity. With all phospholipase-positive strains, the hydrolysis of lecithin yielded FFA as the sole lipid product. There was no degradation of lecithin in tests with boiled cells or without bacteria. The study of unincubated reaction mixtures revealed that, although several compounds were extracted by the applied procedure, they moved in the chromatograms in front of FFA and were poorly stained by the indicator reagents. With pigmented strains of *S. marcescens*, the assay procedure revealed prodigiosin; however, this disappeared after treatment with the reagents used.

TABLE I
Hydrolysis of triolein by bacterial resting cells

Organism	Origin of strains	Number of strains tested	Triolein hydrolysis	
			Number of positive strains	Amount (%) hydrolysed
<i>Serratia marcescens</i>	NCTC (London)	5	2	10
			3	100
	University of Minnesota	9	1	30
			8	100
<i>Serratia liquefaciens</i>	Clinical isolates	19	5	20-40
			14	80-100
	University of Minnesota	7	1	10
			6	100
<i>Klebsiella pneumoniae</i>	Clinical isolates	8	2	10-20
			6	100
	Clinical isolates	20	0	...
<i>Enterobacter aerogenes</i>	Clinical isolates	9	0	...
<i>Enterobacter hafniae</i>	Clinical isolates	8	0	...
<i>Enterobacter cloacae</i>	Clinical isolates	8	3	20-30
<i>Escherichia coli</i>	Clinical isolates	15	0	...
<i>Citrobacter freundii</i>	Clinical isolates	7	0	...
<i>Proteus mirabilis</i>	Clinical isolates	5	2	10-40
<i>Proteus vulgaris</i>	Clinical isolates	5	3	20-40
<i>Proteus rettgeri</i>	Clinical isolates	3	0	...
<i>Proteus morganii</i>	Clinical isolates	7	0	...

TABLE II
Hydrolysis of lecithin by bacterial resting cells

Organism	Origin of strains	Number of strains tested	Lecithin hydrolysis	
			Number of positive strains	Amount (%) hydrolysed
<i>S. marcescens</i>	NCTC (London)	5	4	40-70
			1	100
	University of Minnesota	9	3	20-40
			3	60
<i>S. liquefaciens</i>	Clinical isolates	19	3	80-100
			5	30-40
			4	50-60
			10	70-100
<i>S. liquefaciens</i>	University of Minnesota	7	2	100
			3	40
<i>S. liquefaciens</i>	Clinical isolates	8	7	20-40
<i>K. pneumoniae</i>	Clinical isolates	20	0	...
<i>Ent. aerogenes</i>	Clinical isolates	9	4	20-30
<i>Ent. hafniae</i>	Clinical isolates	8	4	20-40
<i>Ent. cloacae</i>	Clinical isolates	8	0	...
<i>Esch. coli</i>	Clinical isolates	15	0	...
<i>C. freundii</i>	Clinical isolates	7	0	...
<i>P. mirabilis</i>	Clinical isolates	5	0	...
<i>P. vulgaris</i>	Clinical isolates	5	0	...
<i>P. rettgeri</i>	Clinical isolates	3	0	...
<i>P. morganii</i>	Clinical isolates	7	0	...

The lipase and phospholipase activity of the individual strains of *S. marcescens* and *S. liquefaciens* together with their DNAase production and ability to ferment rhamnose, arabinose and raffinose, is shown in table III. It will be noted that lipase activity paralleled phospholipase activity in most *S. marcescens* strains, but in *S. liquefaciens* lipase activity sometimes occurred alone. Table III shows that only three strains of *S. marcescens* fermented any of the three sugars, while most strains of *S. liquefaciens* fermented arabinose, but not rhamnose or raffinose. DNAase was produced by all except two strains of *S. marcescens* and one strain of *S. liquefaciens*.

DISCUSSION

The results obtained with *S. marcescens* indicate that lipolytic activity, produced by lipase and phospholipase, is a rather general characteristic of the species. *S. liquefaciens* on the other hand usually showed only weak phospholipase production, although most strains possessed strong lipase activity. With other members of the Enterobacteriaceae examined, the ability to hydrolyse triolein and lecithin was slight or absent. All strains of *Esch. coli*, *Ent. aerogenes* and *Ent. hafniae* failed to hydrolyse triolein while three of eight strains of *Ent. cloacae* were weakly positive. No phospholipase was found in these species except in one-half of the *Ent. aerogenes* and *Ent. hafniae* strains; these possessed slight activity. The findings agree with those in previous studies (Esselmann and Liu, 1961; Edwards and Ewing, 1972) in which lipase and phospholipase were detected by other methods.

The action of *S. marcescens* on purified lecithin produces FFA as the sole lipid-soluble product, but this does not indicate which kind of lecithinase is involved. Lipase activity is characteristic of strains of *S. marcescens* (Hugo and Beveridge, 1962; Edwards and Ewing, 1972; Owens, 1974). The absence of mono- or di-glycerides as hydrolysis products indicated that the lipase released all the fatty acids of triolein with equal facility, independently of their position.

The differences between various *S. marcescens* strains in terms of their enzymatic activity may be due either to the quantities of lipase and lecithinase produced or to the special characteristics of the enzymes.

This study showed that most strains of *S. liquefaciens* fermented arabinose and possessed slight lecithinase and strong lipase activity.

DNAase production has been considered helpful in identifying *S. marcescens* in the diagnostic laboratory (Blazevic, 1969), and it is correlated with nucleoside phosphotransferase production. However, these two enzymes are produced by both *S. marcescens* and *S. liquefaciens* (Durand and Blazevic, 1970).

Although *S. marcescens*, alone among the Enterobacteriaceae, produces lipase and phospholipase, neither enzyme forms the basis of any test routinely used as an aid in identification. Triolein has proved a suitable substrate for assaying lipase (Legakis and Papavassiliou, 1974) while the use of purified lecithin instead of egg-yolk emulsion is a prerequisite for the detection of true lecithinase activity (Legakis and Papavassiliou, 1975). The determination of lipolytic activity by thin-layer chromatography is a simple and rapid procedure.

TABLE III

Lipase activity, phospholipase activity, DNAase production and sugar fermentations of S. marcescens and S. liquefaciens

Organism	Origin of strains	Strain no.	Percentage hydrolysis of		DNAase activity	Fermentation of		
			triolein	lecithin		rhamnose	arabinose	raffinose
<i>S. marcescens</i>	NCTC (London)	1337	100	100	+++	—	—	—
		10211	10	40	+	—	—	—
		10861	10	60	+++	—	—	—
		2847	100	70	+++	—	—	—
		2446	100	40	+	—	—	—
	University of Minnesota	S-114	100	20	+++	—	—	—
		S-113	100	40	+++	—	+	—
		S-194	100	60	+++	—	—	—
		S-140	100	60	+++	—	—	—
		S-145	30	60	+	—	—	—
		S-146	100	80	+++	—	—	—
		S-148	100	80	—	—	—	—
		S-M6	100	100	+++	—	—	—
		S-168	100	20	+++	—	—	—
	Clinical isolates	24/76	100	70	+++	—	—	—
		25/76	100	40	+++	—	—	—
		26/76	100	60	+++	—	+	+
		27/76	100	70	+++	—	—	—
		28/76	20	40	+	—	—	—
		33/76	100	60	+++	—	—	—
		34/76	40	80	—	—	—	—
		36/76	30	50	+++	—	—	—
		37/76	100	40	+++	—	—	—
		38/76	80	30	+++	—	—	—
		39/76	100	70	+++	—	—	—
		41/76	20	30	+++	—	—	—
		43/76	100	100	+++	—	+	+
		44/76	80	60	+++	—	—	—
		48/76	100	70	+++	—	—	—
		49/76	100	100	+++	—	—	—
		50/76	100	80	+++	—	—	—
		52/76	80	50	+++	—	—	—
		54/76	100	80	+++	—	—	—
<i>S. liquefaciens</i>	University of Minnesota	E-39	100	40	+++	—	—	—
		E-21	10	100	+++	+	—	+
		E-14	100	100	+++	—	—	—
		E-11	100	40	+++	—	+	—
		E-9	100	0	+++	—	+	—
		E-8	100	0	+++	—	+	—
		E-38	100	40	+++	—	+	—
	Clinical isolates	21/76	10	0	+++	—	+	—
		22/76	100	40	+++	—	—	—
		23/76	20	30	+++	—	+	—
		42/76	100	30	+++	—	+	—
		45/76	100	20	+++	—	+	—
		46/76	100	40	—	—	+	—
		47/76	100	30	+++	—	+	+
		56/76	100	20	+++	—	+	—

In conclusion, the lipase and phospholipase assays confirm the close relationship between *S. marcescens* and *S. liquefaciens* and provide another tool for investigating their classification. We believe that the assay of these enzymes and of DNAase or nucleoside phosphotransferase, together with sugar fermentation tests, offers a better basis for differentiating *S. marcescens* and *S. liquefaciens* than does a study of sugar fermentation alone.

SUMMARY

The production of lipase and phospholipase by certain members of the Enterobacteriaceae was examined by thin-layer chromatography of resting-cell suspensions incubated with triolein or lecithin. Most strains of *Serratia marcescens* produced both enzymes while most strains of *Serratia liquefaciens* exhibited strong lipase but only a minor phospholipase activity. *Enterobacter* spp. (25 strains), *Klebsiella pneumoniae* (20 strains), *Escherichia coli* (15 strains), *Citrobacter freundii* (7 strains) and *Proteus* spp. (20 strains) lacked both types of enzymic activity except for the following: three strains of *Enterobacter cloacae*, two of *Proteus mirabilis* and three of *Proteus vulgaris* possessed slight lipase activity; about one-half of the *Enterobacter aerogenes* and *Enterobacter hafniae* strains examined produced slight phospholipase activity. It is suggested that tests for lipase and phospholipase should be used in conjunction with those for DNAase production and sugar fermentation for the differentiation of *S. marcescens* and *S. liquefaciens*.

We are indebted to Professor Donna J. Blazevic (Department of Laboratory Medicine and Pathology, University of Minnesota) for kindly supplying some of the *serratia* organisms used in this study.

REFERENCES

- ALEXANDER, R. H., REICHENBACH, D. D. AND MERENDINO, K. A. 1969. *Serratia marcescens* endocarditis: a review of the literature and report of a case involving a homograft replacement of the aortic valve. *Archs Surg., Chicago*, **98**, 287.
- ALTEMEIER, W. A., CULBERTSON, W. R., FULLEN, W. D. AND McDONOUGH J. J. 1969. *Serratia marcescens* septicaemia: a new threat in surgery. *Archs Surg., Chicago*, **99**, 232.
- BALL, A. P. 1976. *Serratia marcescens* infections. Selection of an antibiotic. *J. antimicrob. Chemother.*, **2**, 317.
- BLAZEVIC, D. J. 1969. Identification of *Serratia* in the diagnostic microbiology laboratory. *Am. J. clin. Path.*, **51**, 277.
- BODEY, G. P., RODRIGUEZ, V. AND SMITH, J. P. 1970. *Serratia* spp. infections in cancer patients. *Cancer*, **25**, 199.
- COWAN, S. T. AND STEEL, K. J. 1961. Diagnostic tables for the common medical bacteria. *J. Hyg., Camb.*, **59**, 357.
- DANS, P. E., BARRETT, F. F., CASEY, J. I. AND FINLAND, M. 1970. *Klebsiella-Enterobacter* at Boston city hospital, 1967. *Archs intern. Med.*, **125**, 94.
- DODSON, W. H. 1968. *Serratia marcescens* septicaemia. *Archs intern. Med.*, **121**, 145.
- DURAND, A. M. AND BLAZEVIC, D. J. 1970. Differentiation of *Serratia* from *Enterobacter* on the basis of nucleoside phosphotransferase production. *Appl. Microbiol.*, **19**, 134.
- EDWARDS, P. R. AND EWING, W. H. 1972. Identification of Enterobacteriaceae, 3rd ed., *Minneapolis*, p. 309.
- ESSELMANN, M. T. AND LIU, P. V. 1961. Lecithinase production by gram-negative bacteria. *J. Bact.*, **81**, 939.

- EWING, W. H. AND MARTIN, W. J. 1974. Enterobacteriaceae. In Manual of clinical microbiology, 2nd ed., edited by E. H. Lennette, E. H. Spaulding and J. P. Truant, *Washington*, p. 109.
- HUGO, W. B. AND BEVERIDGE, E. G. 1962. A quantitative and qualitative study of the lipolytic activity of single strains of seven bacterial species. *J. appl. Bact.*, **25**, 72.
- LEGAKIS, N. J. AND PAPAVALASSIOU, J. 1974. A thin-layer chromatographic technique for rapid estimation of bacterial lipases. *J. appl. Bact.*, **37**, 341.
- LEGAKIS, N. J. AND PAPAVALASSIOU, J. 1975. Thin-layer chromatographic technique for rapid detection of bacterial phospholipase. *J. clin. Microbiol.*, **2**, 373.
- MARTIN, W. J. AND EWING, W. H. 1967. The deoxyribonuclease test as applied to certain gram-negative bacteria. *Can. J. Microbiol.*, **13**, 616.
- OWENS, J. J. 1974. The egg-yolk reaction produced by several species of bacteria. *J. appl. Bact.*, **37**, 137.
- PANGBORN, M. C. 1951. A simplified purification of lecithin. *J. biol. Chem.*, **188**, 471.
- ROTHBERG, N. W. AND SWARTZ, M. N. 1965. Extracellular deoxyribonucleases in members of the family Enterobacteriaceae. *J. Bact.*, **90**, 294.
- SCHREIER, J. B. 1969. Modification of deoxyribonuclease test medium for rapid identification of *Serratia marcescens*. *Am. J. clin. Path.*, **51**, 711.
- WILKOWSKE, C. J., WASHINGTON, J. A., MARTIN, W. J. AND RITTS, R. E. JR 1970. *Serratia marcescens*: biochemical characteristics, antibiotic susceptibility patterns and clinical significance. *J. Am. med. Assoc.*, **214**, 2157.
- WILSON, G. S. AND MILES, A. A. 1964. Topley and Wilson's Principles of bacteriology and immunity, 5th ed., *London*, p. 839.