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LIPID COMPOSITION OF POLYMYXIN B SENSITIVE AND RESISTANT STRAINS OF *SERRATIA MARCENCENS* GROWN IN TWO TYPES OF MEDIA

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ABSTRACT

The lipid composition of polymyxin B sensitive and resistant strains of *Serratia marcescens*, grown on either an enriched or an inorganic medium, was analyzed. No differences were observed for the contents of total extractable lipid, but the amount of phospholipid was higher when the cells were grown on an enriched medium. However, there were significant differences in the fatty acid composition of the lipid fractions from the sensitive and resistant strains. The proportion of unsaturated fatty acids, particularly in the phospholipid fraction, was higher in the sensitive strains grown on both enriched and inorganic media. Overall the unsaturated fatty acid content was higher on the strains grown on the enriched medium. Our results suggest that the correlation observed between the proportion of unsaturated fatty acid and the sensitivity to polymyxin B is independent of the type of growth medium used. The decrease in the total amounts of phospholipid and unsaturated fatty acid seen in the cells grown on inorganic medium may be due to a deficiency of growth factors which might be important in the synthesis of these components.

It is being recognized with increasing frequency that *Serratia marcencens*, once considered to be nonpathogenic, can cause various nosocomial diseases (Clayton and von Graevenitz, 1966; Wilfert et al., 1968; Wilfert et al., 1970; Davis et al., 1970; Maki et al., 1973). Most of the clinical isolates of *S. marcencens* have been found to be resistant to multiple drugs, especially the polymyxins (Clayton and von Graevenitz, 1966; Thornton and Cramer, 1970; Wilfert et al., 1970; Maki et al., 1973). It has been suggested that differences in the total lipid or fatty acid composition, or both, are associated with increased antibiotic resistance in various bacteria, and that the lipid composition may be of importance in preventing the entrance or binding of the antibiotic

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to the cell (Hugo and Stretton, 1966; Dunnick and O'Leary, 1970; Norrington and James, 1970; Chang et al., 1972; Miller et al., 1973; Pechey et al., 1974; Button et al., 1974; Wade and Tsang, 1975).

In an earlier study it was shown that, in Serratia marcescens, an increase in the proportion of unsaturated fatty acid in the extractable phospholipid may be associated with an increase in the sensitivity to polymyxin B (Wade and Tsang, 1975). It was explained that the possible decrease in hydrophobic interactions between these membrane fatty acids and the 6-methyloctanoic acid of the polymyxin B enables the antibiotic to penetrate with greater ease to the site of lethal action. However, it has been established that various growth factors and conditions can have an effect on fatty acid composition (Montgomerie et al., 1973; Cronan, 1974; Naik and Kaneda, 1974). In order to ascertain that the correlation observed between the degree of unsaturation of the fatty acids of S. marcescens and the sensitivity to polymyxin B is independent of the types of media used, the lipid composition of polymyxin B sensitive and resistant strains of Serratia marcescens grown on two types of media was analyzed.

METHODS AND MATERIALS

The strains used in this study were 08 (resistant) and Bizio (sensitive). Both strains are nonclinical, but they are representative in their response to polymyxin B. The minimal inhibitory concentrations of polymyxin B for these strains have been reported (Tsang et al., 1974). The cells were grown on both an enriched medium (2.0% Casamino acids, 0.1% glycerine, 0.5% NaCl and 0.3% meat extract) (Tsang et al., 1974) and an inorganic salts medium (0.1% NH_4Cl, 0.02% $MgSO_4 \cdot 7\ H_2O$, 0.5% KH_2PO_4 and 0.8% glucose) (Gladstone, 1937) with aeration (500 ml/min) at room temperature (20-25 deg C). The cells were harvested during the late log phase. Lyophilized whole cells were extracted with chloroform: methanol (2:1, v/v) (Huston and Albro, 1974), washed according to Folch (Folch et al., 1957), dried and then weighed. The extracted lipids were separated preparatively into neutral lipid, phospholipid and phospholipid component fractions by thin-layer chromatograph (TLC) on Silica Gel G as previously described (Wade and Tsang, 1975). The individual phospholipids were identified by comparison of the R_f values with those of chromatographically pure phospholipids. The phospholipid content of the extracted lipids and the percent phospholipid component distribution were determined by the method of microphosphorus analysis (Bartlett, 1958). The methods followed for preparation and analysis of the fatty acid methyl esters were reported previously (Wade and Tsang, 1975).

RESULTS AND DISCUSSION

Differences in the total lipid or fatty acid composition, or both, have been associated with increased antibiotic resistance in

various bacteria (Hugo and Stretton, 1966; Dunnick and O'Leary, 1970; Norrington and James, 1970; Chang et al., 1972; Miller et al., 1973). In Serratia marcescens an increase in the proportion of unsaturated fatty acid in the extractable phospholipid was suggested to correlate with an increased sensitivity to polymyxin B (Wade and Tsang, 1975). However, the fatty acid composition can be influenced by various growth factors and conditions, including the composition of the growth medium (Montgomerie et al., 1973; Cronan, 1974; Naik and Kaneda, 1974). To determine if the correlation observed between the degree of unsaturation in S. marcescens and sensitivity to polymyxin B is due to the type of medium used, the lipid composition of sensitive and resistant strains grown on two types of media was analyzed.

It was found that the percentages of total extractable lipid were similar for the sensitive (10-11%) and resistant strains (9-10%) on the two media used (Table 1). There were differences, however, in the amounts of total phospholipid in the strains grown on different media (Table 1). Although the phospholipid percentage is similar for the sensitive and resistant strains grown on the same medium, it is higher in the strains grown in enriched medium (80-82%) than in inorganic medium (65-71%). This result is similar to that of Ballesta and Schaechter (1971) who observed that when cells of E. coli are shifted down from a rich to a limited growth medium, the synthesis of the major phospholipid component, phosphatidylethanolamine, is inhibited. However, since there was no significant difference observed in the phospholipid distribution of S. marcescens grown on rich and limited media (Table 1), it may be that in this organism a reduction in phospholipid synthesis may affect all components to a similar degree.

The analysis of the fatty acid composition of the total extractable lipid (TEL), total phospholipid (TPL) and phospholipid components showed that, regardless of the type of growth medium, S. marcescens sensitive to polymyxin B has a higher proportion of unsaturated fatty acid, or conversely, a lower proportion of saturated fatty acid, than the resistant strain (Tables 2 and 3). This difference is particularly evident in the total phospholipid fraction (Table 2) where the ratio of saturated to unsaturated fatty acid is higher in the resistant strain 08 (1.2 in enriched, 9.5 in inorganic) than in the sensitive strain Bizio (0.6 in enriched, 3.4 in inorganic). This relationship is also reflected in the fatty acid composition of the phosphatidylethanolamine in which the ratio of saturated to unsaturated fatty acid in the resistant strain is higher than that in the sensitive strain (Table 3). These results confirm the relationship observed previously between the degree of unsaturation in S. marcescens and sensitivity to polymyxin B (Wade and Tsang, 1975). It is also observed that the total amount of unsaturated fatty acid was significantly lower in the strains grown in inorganic medium

(10-23% in TPL) than those grown in enriched medium (45-61% in TPL). This result may be due to the deficiency of certain important growth factors in the inorganic medium, such as the vitamins nicotinamide in NADP, riboflavin in FAD and panthothenic acid in coenzyme A which are involved in the desaturation reactions, and certain amino acids which can serve as the sources of acetyl CoA and, thus, enhance the synthesis of fatty acids (Sakawa et al., 1968; White et al., 1968).

In conclusion, the correlation observed between the degree of unsaturation of fatty acids in Serratia marcescens and the sensitivity to polymyxin B is independent of the type of growth medium used. The increased ratio of unsaturated fatty acid/saturated fatty acid observed previously in sensitive strains grown on enriched medium was seen also in the strains grown on inorganic medium. The decrease in the total amounts of phospholipid and unsaturated fatty acid seen in the strains grown in inorganic medium may be the result of a deficiency of vital growth factors which may be important in the synthesis of these components.

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Table 1. Lipid contents of polymyxin B sensitive and resistant strains of *Serratia marcescens* grown on two types of media

Strains and growth media	Antibiotic Susceptibility	MIC[a] (ug/ml)	Total Extractable Lipid[b]	Composition of Total Extractable Lipid		Phospholipid Distribution		
				Neutral Lipid[c]	Phospho-Lipid	PE[d]	PG	CL
			%	%	%	%	%	%
Enriched								
08	Resistant	1,000	8.5[e]	18.4	81.6	66.4	15.8	17.8
Bizio	Sensitive	7.8	11.3	19.7	80.3	63.2	16.4	20.5
Inorganic								
08	Resistant	1,000	9.6	35.1	64.9	63.2	19.0	17.8
Bizio	Sensitive	7.8	10.1	29.2	70.8	67.7	14.5	17.9

[a]minimal inhibitory concentration

[b]expressed as percent of dry cells

[c]calculated from the difference between total extractable lipid and phospholipid

[d]PE = Phosphatidylethanolamine; PG = Phosphatidylglycerol; CL = Cardiolipin (Polyglycerol phosphatide)

[e]values reported are an average of three runs

Table 2. Fatty acid composition of the total extractable lipid and total phospholipid of polymyxin B sensitive and resistant strains of Serratia marcescens grown on two types of media

Strains and growth media	Fatty Acid %[a]											
	Total Extractable Lipid						Total Phospholipid					
	C_{14}	C_{15}	C_{16}	$C_{16:1}$	$C_{18:1}$	saturated / unsaturated	C_{14}	C_{15}	C_{16}	$C_{16:1}$	$C_{18:1}$	saturated / unsaturated
Enriched												
08	3.8	T[b]	40.8	25.3	29.9	0.8	3.4	T	51.4	28.1	17.2	1.2
Bizio	3.1	T	35.8	18.3	42.8	0.6	2.3	T	36.3	17.4	44.0	0.6
Inorganic												
08	3.2	3.9	82.3	6.3	4.3	8.4	2.8	3.8	83.9	5.1	4.4	9.5
Bizio	6.8	1.7	65.6	1.9	23.9	2.9	6.1	1.9	69.3	1.5	21.2	3.4

[a]average of two runs

[b]trace

Table 3. Fatty acid composition of the phospholipid components of polymyxin B sensitive and resistant strains of Serratia marcescens grown on two types of media

Strains and growth media	Fatty Acid %[a]																
	Phosphatidylethanolamine							Phosphatidylglycerol					Cardiolipin				
	C_{14}	C_{15}	C_{16}	$C_{16:1}$	C_{18}	$C_{18:1}$	sat. unsat.	C_{14}	C_{15}	C_{16}	C_{18}	$C_{18:1}$	C_{14}	C_{15}	C_{16}	C_{18}	$C_{18:1}$
Enriched																	
08	3.1	T[b]	71.4	6.6	4.4	14.5	3.8	T	T	88.4	8.1	2.9	3.4	T	88.1	8.6	T
Bizio	2.6	T	50.6	4.9	14.2	27.6	2.1	8.5	T	91.5	T	T	T	T	75.3	13.3	11.3
Inorganic																	
08	2.8	4.5	89.1	T	T	3.6	26.8	1.5	2.1	88.0	8.4	T	2.2	3.4	94.3	T	T
Bizio	6.9	2.1	71.3	T	T	19.7	4.1	2.2	T	91.0	6.2	T	7.1	1.4	91.5	T	T

[a]average of two runs

[b]trace