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Effects of Carbon Sources on Antibiotic Resistance in *Pseudomonas aeruginosa*

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The metabolism of branched-chain amino acids, branched-chain acyl derivatives, D-glucose, L-glutamate, and Mueller-Hinton medium was investigated to determine their effects on the growth, lipid composition, and antibiotic susceptibility of *Pseudomonas aeruginosa*. The unsaturated fatty acid content of the readily extractable lipids was altered by growth on selected branched-chain amino acids and their acyl derivatives. Bacteria grown on branched-chain acyl derivatives became more susceptible to polymyxin B and colistin. The effect acyl derivatives had on increasing susceptibility was also manifest in mixed media which contained both an acyl derivative and a carbon source which did not increase susceptibility. Growth on branched-chain amino acids gave mixed results which were dependent on a number of factors, including unique manifestations of individual amino acids, growth conditions, and availability of other carbon sources. The cultural conditions which altered susceptibility to polymyxin antibiotics did not correlate with similar effects on susceptibility to carbenicillin and gentamicin. An adaptive resistance to polymyxin B was observed when the sole carbon source was D-glucose or L-glutamate.

The recognition of *Pseudomonas aeruginosa* as a major opportunistic pathogen in compromised patients has given increased relevance to studying its renowned lack of susceptibility to antibiotics. The pseudomonads differ from most bacteria in that antibiotic-inactivating enzymes are not the primary mechanism of resistance. There is substantial evidence that pseudomonads are refractory to most chemotherapeutic regimens by excluding the antimicrobial agents from the target site (4). This intrinsic lack of susceptibility presents a formidable therapeutic problem because the antibiotic must first circumvent a sophisticated exclusion mechanism before reaching its site of action. Although pseudomonads may acquire antibiotic resistance from other species at a low frequency (15), the occurrence of resistant strains which lack antibiotic-inactivating enzymes indicates that exclusion is the primary mechanism of resistance (4). The polymyxins are one of the few groups of antibiotics that can be used successfully against pseudomonal infections. This success is attributed to its detergent-like mechanism of action which attacks the protective envelope directly (16). It is then logical that changes in envelope components such as phospholipids which interact with polymyxins would elicit changes in susceptibility to polymyxin antibiotics.

A number of workers have demonstrated that microbial lipids may be altered by the carbon sources provided to the microbe (7, 16). These differences in cellular components no doubt contribute to differences in antibiotic susceptibility among different species and strains. Pseudomonads possess extraordinary nutritional capabilities which enable them to catabolize a diverse array of organic compounds, such as the branched-chain amino acids and their resultant catabolites (14). This metabolic prowess facilitates studying the effects of many carbon sources not metabolized by species lacking this physiological versatility. If these metabolic conditions can cause alterations in the lipid composition of the protective envelope of *P. aeruginosa*, we thought it reasonable that changes in antibiotic susceptibility may result.

The purpose of this study was to determine what effects growth on selected carbon source(s) would have on the susceptibility of *P. aeruginosa* to antibiotics commonly used in the treatment of pseudomonal infections. This report describes a series of experiments in which branched-chain amino acids and their acyl derivatives were used as carbon sources to determine their effects on growth, lipid composition, and antibiotic susceptibility in *P. aeruginosa*.

MATERIALS AND METHODS

Organisms. *P. aeruginosa* 39229, a strain resistant to tobramycin, gentamicin, and carbenicillin and susceptible to polymyxin B and colistin, was obtained

from the Center for Disease Control, Atlanta, Ga. *P. aeruginosa* 015, a clinical isolate furnished by University Hospital, Oklahoma City, Okla., was susceptible to these same antibiotics. Stock cultures were maintained on Mueller-Hinton slants and transferred every 3 weeks.

Growth conditions. Mueller-Hinton agar medium (Difco Laboratories; lot 645524) and Mueller-Hinton broth (Difco; lot 6483831) were used as controls throughout this study. All references to resistance and susceptibility were made relative to values obtained with Mueller-Hinton medium. Jacobson medium was used as the base medium when individual carbon sources were used to determine their effects on susceptibility (10). This was a defined medium in which the final concentrations of trace elements and nitrogen source (NH_4Cl) were not growth limiting. Final pH was 6.8 to 7.0. Carbon source concentration in defined medium was 25 mM with the exception of isoleucine-leucine-valine (ILV) and DL-2-methylbutyrate-isovalerate-isobutyrate (MII) media, in which each respective component was 10 mM. Solid medium was prepared from the defined medium by the addition of 0.15% agar (Difco).

Doubling times for individual carbon sources were calculated from 50-ml samples grown in 250-ml side arm flasks. Doubling times for mixtures of carbon sources were determined from 40-ml samples grown in 250-ml side arm flasks. Controls for the mixed medium contained 40 ml of either D-glucose or Mueller-Hinton medium. Test flasks contained 20 ml of the control medium plus 20 ml of supplemental carbon medium. Inoculum was 1 ml of an overnight culture grown in control medium. Optical density for all experiments was read at 660 nm with a Bausch and Lomb Spectronic 20 spectrophotometer. Aeration was provided by vigorous shaking in a Lab-Line Environ-Shaker 3597-2 shaker. All growth was accomplished at 37°C.

Lipid analyses of cells grown on different carbon sources. Approximately 3 g (wet weight) of *P. aeruginosa* 015 cells grown on either L-valine, L-isoleucine, DL-2-methylbutyrate, or Mueller-Hinton medium were used in each extraction. Growth was monitored spectrophotometrically and allowed to reach early stationary phase. Cells were harvested by centrifugation and washed twice in 50 mM phosphate buffer, pH 7.5.

Readily extractable lipids were extracted and washed by the method of Folch et al. (9). The extracted lipids were transmethylated with boron trifluoride methanol reagent (Applied Science Laboratories) by the procedure of Morrison and Smith (13). The fatty acid methyl esters were extracted by hexane and separated from the nonesterified fatty acids by using thin-layer chromatography. The methylated fatty acids were then dissolved and stored in cyclohexane. Identification of fatty acids was accomplished with a Hewlett-Packard 5840A gas chromatograph equipped with a flame ionization detector. The glass column (6 feet [ca. 183 cm] by 4 mm) was packed with 10% SP-2330 on 100/120 Chromosorb. The temperatures of the injection port and detector were 200 and 225°C, respectively. The column temperature was programmed to run for 4 min at 100°C, followed by a 10°C increase per min to a final temperature of 200°C. The flow rate of the nitrogen carrier gas was 40 ml/min. Bacterial fatty acids were identified by comparing peak retention times with fatty acid standards.

Disk diffusion susceptibilities. Disk diffusion susceptibility tests were performed by a modification of the method of Bauer et al. (2). Overnight cultures in Mueller-Hinton broth were transferred to fresh medium (1 ml/100 ml). Growth was followed spectrophotometrically to mid-exponential growth phase at which time the cultures were diluted in physiological saline to an optical density of 0.10. Bacterial lawns were prepared by spreading 0.1-ml samples with a glass rod. Analysis with a Coulter Channelyzer indicated that the inoculum contained 7×10^6 cells. Zone sizes were read after 24- and 48-h incubations at 37°C. This growth period was longer than what normally would be expected because growth on many of the carbon sources used in this study was not sufficient for susceptibility determinations after 24 h of growth. Zone diameters on medium supporting faster growth did not significantly change between 24 and 48 h. Difco Dispens-O/Discs of the following concentrations were used: carbenicillin, 100 μg; colistin, 10 μg; gentamicin, 10 μg; and polymyxin B, 200 U. Data regarding zone diameters in this study were the mean of 6 to 10 separate experiments.

Determination of MIC. Minimal inhibitory concentration (MIC) was measured by a modification of the method of Washington and Barry (17). Antibiotics were dissolved in double-distilled water at a concentration of 1 mg/ml. All solutions were filter-sterilized through membrane filters (0.45 M; Millipore Corp.) and frozen until used. Each antibiotic was serially diluted in growth medium for a final volume of 0.5 ml/tube. Overnight cultures of the test strain were transferred to fresh Mueller-Hinton broth (1 ml/100 ml of fresh medium). Growth was monitored to mid-exponential growth phase (optical density, 0.70 to 0.90). Inoculum for the MIC determinations was prepared by diluting these fresh cultures to an optical density of 0.05 in the same medium in which the antibiotics had been diluted. The diluted antibiotic solutions were brought to a volume of 1.0 ml by the addition of 0.5 ml of this inoculum. The inoculum contained 1.6×10^7 cells as indicated by a Coulter Channelyzer.

This protocol was somewhat modified for those experiments which measured susceptibility to antibiotics with multiple carbon sources. The antibiotic was diluted in the growth medium as previously described. However, in these experiments the inoculum was prepared by diluting the freshly grown cultures in medium containing another carbon source (supplemental medium). Equal volumes of growth medium and supplemental medium (0.5 ml each) resulted in a final concentration of approximately 12.5 mM of each carbon source. The MIC obtained on each carbon source was determined at 24 and 48 h. All MIC values were the mean of four to six separate determinations.

RESULTS

The utilization of either L-valine, L-isoleucine, or DL-2-methylbutyrate as the sole carbon source resulted in substantial changes in the

extractable lipids of *P. aeruginosa* 015 (Table 1). The comparative fatty acid profile of cells grown in Mueller-Hinton medium indicated that these differences were both quantitative and qualitative. Cells grown on L-isoleucine, L-valine, and DL-2-methylbutyrate had peaks corresponding to pentadecanate (C_{15}) with a relative retention time (RRT) of 0.95. All three of these carbon sources are catabolized to propionyl-coenzyme A, which might enhance the possibility of increased odd-chain fatty acid synthesis. These possibilities in substrate ambiguity would be accentuated by growth conditions in which all lipids are derived from odd-chain precursors, such as propionyl-coenzyme A, which are normally found only in minute quantities. Palmitate (C_{16}) appeared to be synthesized at a constant rate regardless of carbon source (RRT, 1.000). Mueller-Hinton medium-grown cells had higher concentrations of palmitoleate ($C_{16:1}$; RRT, 1.04), and oleate ($C_{18:1}$; RRT, 1.17) than did cells grown on the other carbon sources. Cells grown on L-valine, L-isoleucine, and DL-2-methylbutyrate had higher levels of linoleate ($C_{18:2}$; RRT, 1.25) than did Mueller-Hinton medium-grown cells.

Effects of carbon source on susceptibility to antibiotics. The phenotypic expression of susceptibility and resistance to antibiotics in *P. aeruginosa* was influenced significantly by the carbon source. The effects that growth on single or multiple carbon source(s) had on susceptibility to antibiotics in strain 39229 are illustrated in Table 2. The MICs of polymyxin B and colistin indicated that growth in D-glucose, L-glutamate, and ILV medium enabled cells to resist higher concentrations of these antibiotics than did growth in a rich medium, such as Mueller-Hinton medium. Conversely, growth in L-isoleucine, L-valine, isovalerate, isobutyrate, and MII medium rendered the cells more vulnerable to the action of polymyxin B than did growth in Mueller-Hinton medium. The MICs for gentamicin remained greater than 500 μg/ml in this resistant strain regardless of growth medium.

The apparent development of resistance in strain 015 was related to exposure time between bacteria and antibiotic (Table 3). A comparison of MICs at 24 h indicated that cells grown with D-glucose, L-glutamate, and ILV medium were more susceptible to polymyxin B than were cells grown in Mueller-Hinton medium. After 48 h of growth, the MICs of polymyxin B in these same cultures were 3 to 11 times higher than their Mueller-Hinton medium counterparts. This apparent increase in resistance noted in these media was not due to slower growth per se, as shown by the sensitizing effects of acyl derivatives which support an even slower growth rate (Table 4). Subsequent investigations found this resistance to be adaptive and transitory in nature. Bacteria subcultured in various other media from these apparently resistant cultures exhibited the same susceptibility to antibiotics as

TABLE 1. *Effects of carbon source on readily extractable lipids of P. aeruginosa 015*

RTT[a]	Area % with the following as carbon source[b]			
	Mueller-Hinton medium	L-Valine	L-Isoleucine	DL-2-Methylbutyrate
0.76	0.09		0.05	0.21
0.86	0.27	1.46	0.30	0.59
0.89	1.22	0.95	0.54	1.00
0.95	0.62	2.28	3.97	2.65
0.97	0.35	2.91	0.64	0.26
1.00	28.94	25.85	27.56	26.26
1.04	19.14	14.69	9.73	8.02
1.06			2.65	1.59
1.09	2.00	4.36	4.86	9.25
1.12	1.20	1.19	1.16	1.99
1.17	40.58	32.64	36.72	21.49
1.25	4.64	8.17	11.80	23.70

[a] RTT was calculated by dividing peak retention time by retention time of a methyl palmitate standard. Standard methyl esters were chromatographed under the same conditions which yielded RTTs as follows: methyl pentadecanate, 0.95; methyl palmitate, 1.00; methyl palmitoleate, 1.04; methyl oleate, 1.17; and methyl linoleate, 1.25.

[b] Average of three separate determinations. The carbon source was the sole source of carbon and energy. Cells were harvested at an optical density of 0.90 to 1.10.

TABLE 2. *Relationship between carbon source and susceptibility to antibiotics in P. aeruginosa 39229*

Growth medium[a]	MIC at 48 h[b] (μg/ml)		
	Polymyxin B	Colistin	Gentamicin
Mueller-Hinton	13	27	<500
D-Glucose	167↑	291↑	<500
L-Glutamate	375↑	250↑	<500
L-Isoleucine	2↓	10	<500
DL-2-Methylbutyrate	0.5↓	0.5↓	<500
L-Leucine	20	4↓	<500
Isovalerate	1↓	2↓	<500
L-Valine	3↓	13	<500
Isobutyrate	0.5↓	0.5↓	<500
ILV mixture	156↑	217↑	<500
MII mixture	0.5↓	0.5↓	<500

[a] The growth medium was the sole carbon source.

[b] All MICs were compared with those obtained in Mueller-Hinton broth, which served as the controls. Differences in susceptibility to each antibiotic which were greater than twofold for each respective antibiotic when another carbon source was used are indicated by the following: ↑, increase in resistance; ↓, increase in susceptibility.

TABLE 3. *Correlation among susceptibility to antibiotics, carbon source, and exposure time in P. aeruginosa 015*

Growth medium[a]	MIC[b] (μg/ml)					
	Polymyxin B		Colistin		Gentamicin	
	24 h	48 h	24 h	48 h	24 h	48 h
Mueller Hinton	5.2	11	19	37	4.5	11
D-Glucose	1.6	83↑	61	125	14	37
L-Glutamate	2.6	125↑	25	250↑	19	19
L-Isoleucine	2	6	8	7	4	32↑
DL-2-Methylbutyrate	—[c]	0.5	—	2	—	13
L-Leucine	—	22	—	7	—	16
Isovalerate	—	2	—	8	—	43
L-Valine	—	2	—	6	—	16
Isobutyrate	—	0.3	—	3	—	11
ILV mixture	1.3	33↑	4.6	25↑	7	28↑
MII mixture	0.5	0.5	0.9	2	6.2	8

[a] The growth medium was the sole carbon source.

[b] MICs were determined at 24 and 48 h for each combination of growth medium and antibiotic. An increase of greater than twofold between the 24-h MIC and the 48-h MIC is indicated by ↑.

[c] —, Insufficient growth at 24 h.

TABLE 4. *Doubling time of P. aeruginosa strains grown on different carbon sources*

Growth medium[a]	Doubling time (h) of strain:	
	39229	015
Mueller-Hinton	0.67	0.78
D-Glucose	0.91	0.89
L-Glutamate	0.67	0.77
L-Isoleucine	2.63	1.58
DL-2-Methylbutyrate	2.78[b]	1.43[b]
L-Leucine	6.02	11.49
Isovalerate	2.38	1.47
L-Valine	4.34	6.33
Isobutyrate	3.70[b]	3.93[b]
ILV mixture	1.51	1.96
MII mixture	1.82	2.44

[a] The growth medium was the sole carbon source.

[b] There was a lag period of 24 h or greater before significant growth began.

did the parent strain. This was true for both strains utilized in this study, which were tested by disk diffusion and broth dilution. This pattern of temporally dependent alterations in susceptibilities to antibiotics was also noted in strain 39229 with essentially the same observations. Growth in L-leucine medium enhanced susceptibility to colistin in both strains but had a diminished effect on susceptibility to polymyxin B.

The MICs of polymyxin B for strain 015 were determined at concentrations of 10, 20, 30, and 40 mM DL-2-methylbutyrate and L-glutamate. The MIC with each carbon source was unchanged at the various concentrations. This consistency implied that medium constituents at these concentrations neither inactivated polymyxin B nor competed for cellular binding sites.

There was no apparent nutritionally dependent correlation between susceptibility to polymyxin B and colistin and susceptibility to carbenicillin. *P. aeruginosa* 39229 (carbenicillin resistant) remained resistant to carbenicillin at concentrations of greater than 500 μg/ml regardless of carbon source. Carbenicillin MICs for *P. aeruginosa* 015 (carbenicillin sensitive) varied depending upon the carbon source but did not correlate with susceptibility to polymyxin B and colistin.

Effects of combined carbon sources on susceptibility to antibiotics. Pseudomonads grown on single carbon sources either increased or decreased their resistance to polymyxin B, depending on the carbon source. The ability of individual carbon sources to influence resistance was observed even when they were combined with carbon sources which exerted dissimilar effects (Table 5). The addition of D-glucose, L-glutamate, and ILV medium to Mueller-Hinton medium increased the MIC of polymyxin B four- to ninefold compared with the MIC with Mueller-Hinton medium alone. These experiments also demonstrated that resistance could be modulated toward susceptibility with the proper combinations of growth media. L-Glutamate- and D-glucose-grown cells were relatively resistant to polymyxin B when each was used as the sole carbon source (Tables 2 and 3). The addition of either isobutyrate, isovalerate, or DL-2-methylbutyrate to either glutamate or glucose medium drastically reduced the MIC of polymyxin B.

Similar observations were made with the mod-

TABLE 5. *Effects of carbon source combinations on P. aeruginosa 015 susceptibility to polymyxin B and colistin*

Antibiotic	Base medium[a]	Supplemental medium[a]	MIC at 48 h[b] (μg/ml)
Polymyxin B	Mueller-Hinton	Mueller-Hinton	6
		D-Glucose	37
		L-Glutamate	58
		L-Leucine	20
		Isovalerate	5
		L-Isoleucine	24↑
		DL-2-Methylbutyrate	3
		L-Valine	63↑
		Isobutyrate	3
		ILV mixture	39↑
		MII mixture	3
	L-Glutamate	L-Glutamate	125
		L-Valine	35
		Isobutyrate	4↓
		L-Leucine	94
		Isovalerate	4↓
		L-Isoleucine	125
		DL-2-Methylbutyrate	4↓
	D-Glucose	D-Glucose	32
		L-Valine	42
		Isobutyrate	7↓
		L-Leucine	47
		Isovalerate	4↓
		L-Isoleucine	47
		DL-2-Methylbutyrate	2↓
Colistin	Mueller-Hinton	Muller-Hinton	47
		D-Glucose	375↑
		L-Glutamate	500↑
		L-Valine	125
		Isobutyrate	24
	D-Glucose	D-Glucose	250
		L-Valine	375
		Isobutyrate	16↓

[a] Each tube contained 0.5 ml each of base medium and supplemental medium prepared as described in the text.

[b] All MICs were compared with susceptibility demonstrated when base and supplemental media were identical (Mueller-Hinton, L-glutamate, D-glucose). Differences in MICs of greater than twofold are compared with their controls as indicated by the following: ↑, increase in resistance; ↓, increase in susceptibility.

ulation of susceptibility to colistin. The addition of D-glucose, L-glutamate, and L-valine to Mueller-Hinton medium increased resistance to colistin (Table 5). The addition of isobutyrate to Mueller-Hinton medium resulted in an increase of susceptibility to colistin. Colistin resistance in D-glucose medium was increased by L-valine and decreased by isobutyrate.

The role of branched-chain amino acids in influencing susceptibility to polymyxin B was somewhat erratic, compared with the effects of their acyl derivatives. The addition of either L-isoleucine, L-leucine, or L-valine to either Mueller-Hinton or D-glucose medium increased resistance (Table 5). The addition of L-leucine and L-isoleucine had little effect in L-glutamate medium, although L-valine decreased the MIC of polymyxin B. This was in sharp contrast to the utilization of L-isoleucine and L-valine as the sole carbon source, which increased the susceptibility of the cell to polymyxin B.

It was noted that the individual branched-chain amino acids had dissimilar effects on susceptibility to antibiotics depending on whether they were (i) the sole source of carbon, (ii) a supplemental carbon source, or (iii) a combination carbon source (e.g., ILV medium). This was not the case for the branched-chain acyl acids which increased cellular susceptibility to polymyxin B and colistin regardless of the experimental protocol.

Effects of carbon source as measured by disk diffusion. Determination of susceptibility to antibiotics by the disk diffusion technique indicated that susceptibility and resistance to antibiotics on solid medium was influenced by the carbon source in a manner analogous to that observed in liquid cultures. Cells grown with

branched-chain amino acids, branched-chain acyl acids, or combinations thereof were more susceptible to polymyxin B and colistin than were cells grown on Mueller-Hinton agar. The zones of inhibition for polymyxin B ranged from 13 mm for Mueller-Hinton agar to 36 mm for isobutyrate medium. Other representative zones included: D-glucose, 16 mm; L-glutamate, 15 mm; L-isoleucine, 20 mm; DL-2-methylbutyrate, 23 mm; L-leucine, 28 mm; isovalerate, 24 mm; L-valine, 28 mm; ILV mixture, 18 mm; and MII mixture, 23 mm. The response to colistin resembled that of polymyxin B on all media. There were no significant differences between the responses of *P. aeruginosa* 015 and 39229 to either polymyxin B or colistin. The adaptive resistance to the polymyxins noted in liquid media between 24 and 48 h was not observed on solid media.

Gentamicin resistance in strain 39229 (carbenicillin and gentamicin resistant) was not altered by the carbon source in solid medium, although the branched-chain acyl derivatives appeared to enhance resistance to some degree. Susceptibility testing for carbenicillin in strain 39229 resulted in confluent growth on all media. Susceptibility to gentamicin in strain 015 was not influenced by the carbon source and did not correlate with susceptibility or resistance to polymyxin B and colistin. Carbenicillin susceptibility testing indicated that *P. aeruginosa* 015 was slightly more susceptible when the carbon source was D-glucose, L-isoleucine, DL-2-methylbutyrate, L-leucine, isovalerate, L-valine, and isobutyrate, compared with susceptibility on Mueller-Hinton medium. Susceptibilities to carbenicillin were unchanged when the growth medium was L-glutamate, ILV, and MII.

Influence of carbon source on growth rates. The doubling times of both strains were less than 1 h when the carbon source was Mueller-Hinton medium, D-glucose, or L-glutamate (Table 4). Doubling times were much slower when either L-leucine or L-valine was the sole carbon source. The cells grew on all three of the branched-chain acyl derivatives, but a lag period of 24 h or longer before exponential growth commenced was observed when the carbon source was DL-2-methylbutyrate or isobutyrate. L-Valine was growth inhibitory to strain 015 grown in D-glucose medium but had no effect when added to Mueller-Hinton medium (Fig. 1). L-Isoleucine and L-leucine did not affect growth of strain 015 in either Mueller-Hinton or D-glucose medium. DL-2-Methylbutyrate inhibited growth in the same media. Isovalerate and isobutyrate inhibited growth in D-glucose medium but had little effect when added to Mueller-Hinton medium. All inhibitions were more pronounced in D-glucose medium than in Mueller-Hinton medium. Combinations of either

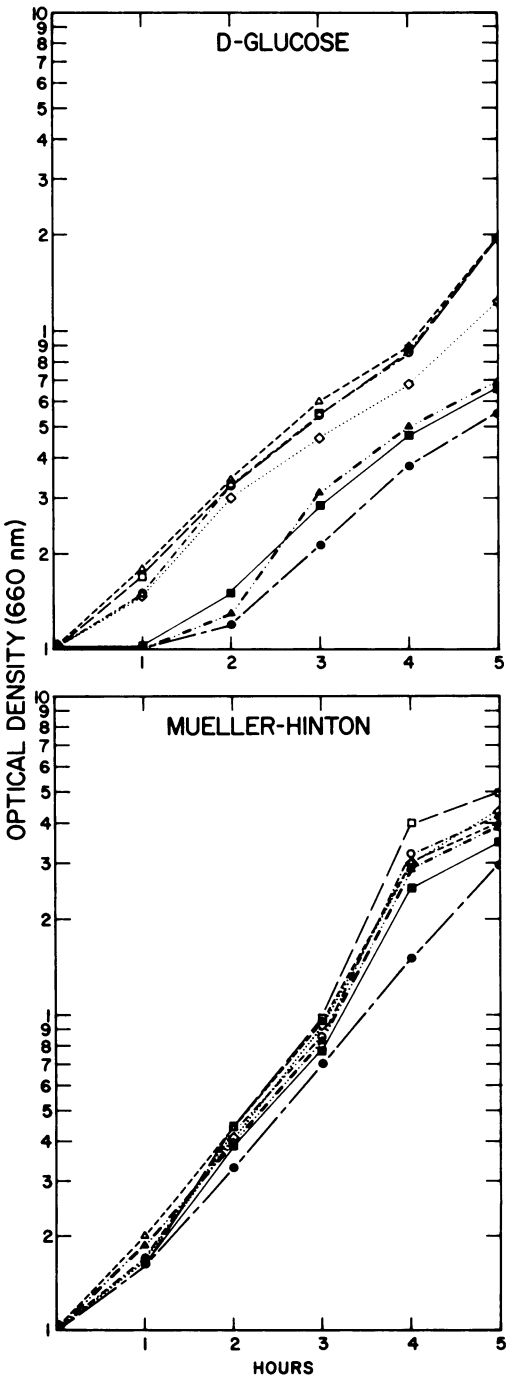

FIG. 1. *Effects of branched-chain amino acid and acyl derivative media on growth of P. aeruginosa 015 in D-glucose and Mueller-Hinton media. Control and test media were present in equal volumes at zero time. Symbols: △- - -△, control; ◇·····◇, L-valine; ○-·-·-○, L-isoleucine; □— —□, L-leucine; ●- —— -●, DL-methylbutyrate; ▲···-···-▲, isovalerate; and ■——■, isobutyrate.*

branched-chain amino (ILV medium) or acyl (MII medium) acids supported growth better than did any of these compounds used individually.

DISCUSSION

The metabolism of branched-chain amino acids and their acyl derivatives had profound effects upon *P. aeruginosa* susceptibility to polymyxin B and colistin. The metabolism of selected carbon sources also had marked effects on the quantitative and qualitative fatty acid composition of the readily extractable lipids found in *P. aeruginosa* (Table 1). Cells grown on branched-chain amino acids as the sole carbon source did not have the same susceptibility to antibiotics as did cells grown on the acyl analogs. Growth on L-isoleucine and L-valine as the sole carbon source resulted in increased susceptibility to antibiotics. The addition of either L-isoleucine, L-leucine, or L-valine medium to Mueller-Hinton and D-glucose media increased resistance to polymyxin B (Table 5). Cells grown on combinations of branched-chain amino acids (ILV medium) were also more resistant to polymyxin B.

The metabolism of branched-chain acyl derivatives resulted in increased susceptibility to polymyxin B and colistin regardless of experimental protocol. The sensitizing effects of acyl derivatives was expressed even when they were incorporated into either D-glucose or L-glutamate medium. Either of these media increased resistance when used as the sole carbon source. The dissimilarities in the effects of branched-chain amino acids and their acyl derivatives indicate that the metabolism of the amino acid and/or its keto acid analog plays a pivotal role in determining susceptibility to polymyxin B and colistin. The catabolism of branched-chain amino acids proceeds via a common catabolic pathway in which the amino acids are deaminated to keto acids, followed by decarboxylation to the acyl derivatives (12). The growth of bacteria on branched-chain amino acids as the sole carbon source would undoubtedly result in abnormally high intracellular concentrations of the respective amino and keto acids. These accumulations would not be found in cells grown on acyl derivatives because the oxidative decarboxylation to the acyl derivative is an irreversible metabolic reaction.

The mechanism of action of polymyxin B involves interaction between antibiotic and cellular phospholipids (15). Therefore, alterations in cellular phospholipids should result in changes in susceptibility if the target site has been sufficiently modified. The growth conditions used in this study resulted in significant changes in chain lengths and degree of saturation. The addition or elimination of double bonds to long-chain fatty acids results in significant stereochemical modifications to biological membranes. It is most likely not coincidental that alterations in cellular lipids are accompanied by changes in susceptibility to antibiotics. It should be noted that the carbon source exerting the greatest sensitizing effect (DL-2-methylbutyrate) also resulted in the greatest changes in fatty acid composition. Additional proof of the relationship between changes in lipids and susceptibility to polymyxin B was indicated by the fact that the carbon source did not significantly alter susceptibility to either gentamicin or carbenicillin. This incongruity would be consistent with the diverse mechanisms of action for the three classes of antibiotics represented here. This distinctiveness in susceptibility suggests that alteration in susceptibility to polymyxin is a separate phenomenon not necessarily related to metabolic vulnerability in peptidoglycan biosynthesis (carbenicillin) or protein synthesis (gentamicin).

It is not a novel idea that susceptibility to antibiotics is related to cellular lipid content. Anderess et al. noted differences in extractable lipids of pseudomonal strains both susceptible and resistant to quatarnary ammonium compounds and chloramphenicol (1). Similar observations have been made in the enterics with a variety of antibiotics (5). It has been established that the lipid composition of gram-negative bacteria varies as a function of other parameters, such as temperature (8), carbon source (6), presence of plasmids (11), and cation concentrations (3).

Our results indicate that growth on the carbon sources used in this study resulted in quantitative and qualitative changes in readily extractable lipids of *P. aeruginosa*. We are presently in the process of further analyzing the effects of the nutritional environment on *P. aeruginosa*, with particular emphasis on the phospholipids of the readily extractable lipid fraction. If these lipid alterations can be correlated to changes in susceptibility to antibiotics, it may be possible to demonstrate a causal relationship between resistance or susceptibility to polymyxin B and the metabolism of specific lipids or classes of lipids.

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