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Pseudomonas

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Lipids of *Pseudomonas* 4

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1. INTRODUCTION

Lipids are generally defined as fatty acids, alcohols, hydrocarbons, and compounds containing these substances which are soluble in organic solvents. The lipids most commonly found in bacteria are phospholipids, glycolipids, ornithine amide lipids, fatty acids, and lipopolysaccharides. Phospholipids generally constitute ~40% of the cytoplasmic membrane of bacteria and up to 25% of the outer membrane (mainly localized in the inner leaflet). A generalized structure for a *Pseudomonas* membrane is shown in Figure 1. It has been found that the predominant phospholipid in both the inner and outer membranes in most *Pseudomonas* species is phosphatidylethanolamine (Wilkinson, 1988). Ornithine amide lipids are localized in the outer membrane. Lipopolysaccharides are located in the outer leaflet of the outer membrane of gram-negative bacteria. Glycolipids are generally found as storage lipids located in intracellular inclusions but can also be found in the membranes of *P. diminuta* and *P. vesicularis* and gram-positive bacteria (Wilkinson, 1988). Carotenoids and hydrocarbons may be found in the cytoplasmic membrane.

2. LIPIDS OF THE GENUS PSEUDOMONAS

2.1. Membrane Lipids

2.1.1. Phospholipids

Phospholipids usually constitute 90% of the cellular lipids of fluorescent pseudomonads. The primary phospholipids in *Pseudomonas* are

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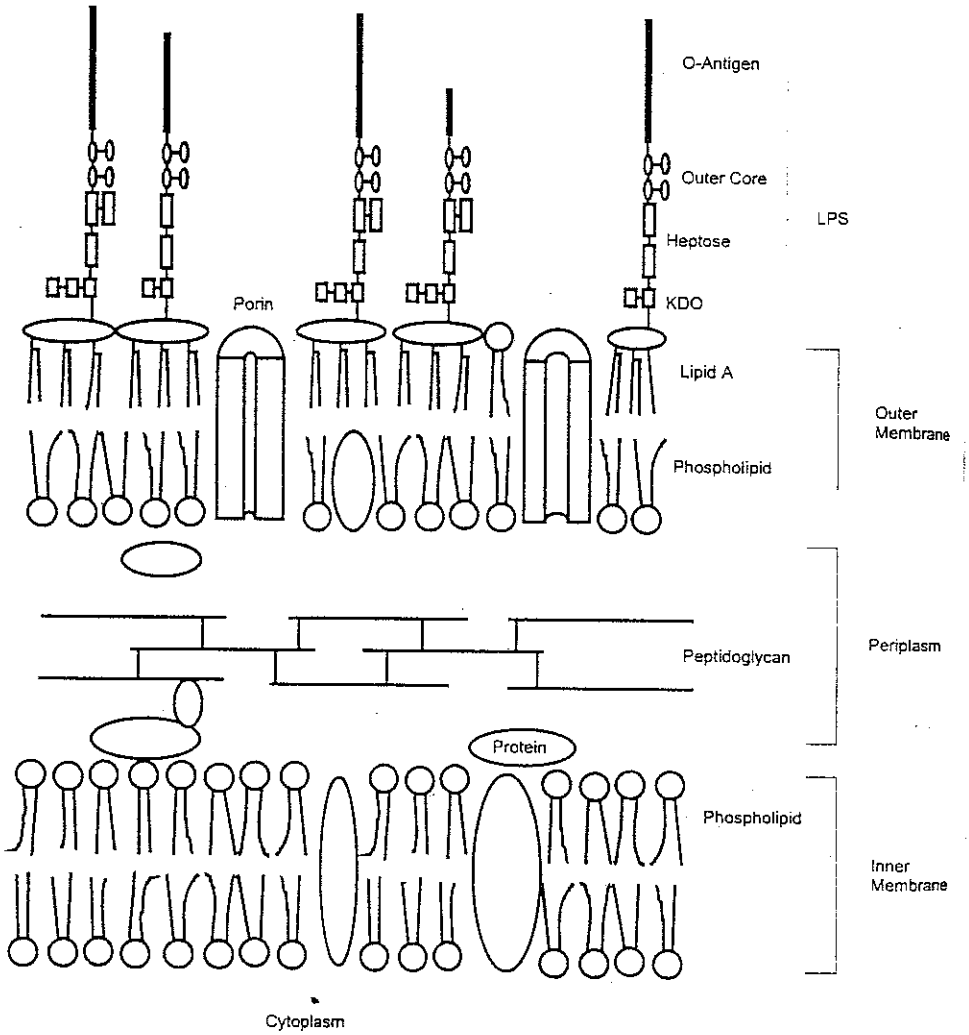
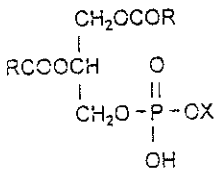


Figure 1. Schematic representation of a cell envelope typical of *Pseudomonas*. KDO, 2-keto-3-deoxyoctulosonate; LPS, lipopolysaccharide.

phosphatidylethanolamine, phosphatidylglycerol, and diphosphatidylglycerol (cardiolipin). Phosphatidylcholine, lysophosphatidylethanolamine, methyl-substituted and dimethyl-substituted phosphatidylethanolamine, and glucosyl-substituted phosphatidylglycerol have been identified in *Pseudomonas* (Table I). Phosphatidylserine is detected in

Table I. Phospholipids in *Pseudomonas* and Their Structures[a]



Lipid	—X
Phosphatidic acid	—H
Phosphatidylglycerol	$—CH_2CH(OH)CH_2OH$
Diphosphatidylglycerol (cardiolipin)	$—CH_2CH(OH)CH_2OOPO_2H$-sn-1,2-diacylglycerol
Phosphatidylserine	$—CH_2CH(NH_2)CO_2H$
Phosphatidylcholine (lecithin)	$—CH_2CH_2N^+(CH_3)_3$
Phosphatidyl-*N*-monomethylethanolamine	$—CH_2CH_2NHCH_3$
Phosphatidyl-*N*,*N*-dimethylethanolamine	$—CH_2CH_2N(CH_3)_2$

[a]R = fatty acyl groups.

most *Pseudomonas* strains in trace amounts but has not been shown to be a major phospholipid (Table II). The inner cytoplasmic membrane and the inner leaflet of the outer membrane are comprised of these phospholipids. Table II lists the phospholipids currently known in *Pseudomonas* species. *P. diminuta* and *P. vesicularis* (recently reclassified as *Brevundimonas*) are unusual in their lack of phosphatidylethanolamine and diphosphatidylglycerol, and both contain 6-*O*-phosphatidylglucosyldiacylglycerol (Fig. 2). The halophile *P. halosaccharolytica* contains glucosyl-substituted phospholipids, as shown in Figure 3 (Wilkinson, 1988).

2.1.2. Ornithine Amide Lipids

Acylornithines (ornithine amide lipids) are quite common in some members of the *Pseudomonas* genus. They have been isolated most commonly from the fluorescent pseudomonads and from *P. diminuta* and *P. vesicularis* (Segers *et al.*, 1994). For the strains studied, these lipids serve functions similar to those of phospholipids. In phosphate-limited cultures, *P. fluorescens* produces ornithine lipids as the sole polar lipid (Minnikin and Abdolrahimzadeh, 1974). A generalized structure for

Table II. Phospholipids in the Genus *Pseudomonas*

Strain	PE	PG	DPG	PC	PS	LysoPE	glucosaminyl PG	6-O-phosphatidylglucosyldiacylglycerol	Phosphatidyl-N-methyl ethanolamine	Phosphatidyl-N,N-dimethyl-ethanolamine
Pseudomonas aeruginosa[a]	+	+	+							
Pseudomonas alcaligenes[a]	+	+	+	+		+				
Pseudomonas aureofaciens[a]	+	+	+							
Pseudomonas carboxydovorans[a]	+	+	+	+						
Pseudomonas carophylli[b,h]	+	+	+							
Pseudomonas cepacia[a,h]	+	+								
Pseudomonas coronafaciens[c]	+	+	+							
Pseudomonas diazotrophicus[d]	+			+					+	+
Pseudomonas diminuta[c,h]		+						+		
Pseudomonas fluorescens[a]	+	+	+							
Pseudomonas gladioli[b,h]	+	+	+							
Pseudomonas gardneri[e]	+	+	+							
Pseudomonas halophila[f]	+	+								
Pseudomonas halosaccharolytica[a]	+	+	+				+			
Pseudomonas mildenbergii[a]	+	+	+							
Pseudomonas pickettii[b,h]	+	+	+							
Pseudomonas pseudomallei[g,h]	+	+								
Pseudomonas putida[a]	+	+	+							
Pseudomonas rubescens[c]	+	+								
Pseudomonas savastanoi[c]	+	+	+							
Pseudomonas solanacearum[a,h]	+	+	+							
Pseudomonas stutzeri[c]	+	+	+			+				
Pseudomonas syncyanea[c]	+	+	+							
Pseudomonas syringae[c]	+	+	+							
Pseudomonas vesicularis[a,i]		+						+		

[a]Wilkinson, 1988; [b]Galbraith and Wilkinson, 1991; [c]Wilkinson *et al.*, 1973; [d]Taylor *et al.*, 1993; [e]Bouzar *et al.*, 1994; [f]Franzmann *et al.*, 1990; [g]Yabucchi *et al.*, 1992; [h]Now classified as *Burkholderia* (Yabuuchi *et al.*, 1994); [i]Now classified as *Brevundimonas* (Segers *et al.*, 1994).

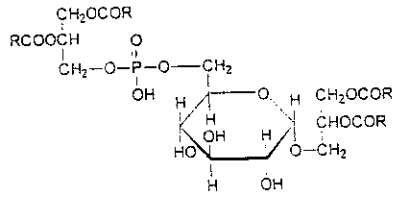


Figure 2. Structure of 6-*O*-phosphatidylglucosyldiacylglycerol found commonly in *P. diminuta and P. vesicularis*. R = fatty acyl groups.

acylornithine lipids is shown in Figure 4. The definitive mechanism for tproduction of amino lipids is still under investigation, and their true function is still unknown.

2.2. Fatty Acids

2.2.1. Non-Hydroxy Fatty Acids

The fatty acids found in *Pseudomonas* are shown in Tables III and IV. High concentrations of free fatty acids are not found in bacteria because they would lyse the membrane. Detection of free fatty acids in an extract usually means that some hydrolysis has occurred. However, reports of fatty acid methyl esters and ethyl esters produced by *P. fluorescensw* and *P. fragi* have been documented. The production of these compounds as associated with decomposition of refrigerated beef (Edwards *et al.*, 1987). Non-hydroxy fatty acids are generally found in the inner and outer membranes, covalently linked to phospholipids via ester and amide bonds. It has been shown that phospholipid inner leaflet of the outer membrane contains mainly saturated fatty acids, whereas the cytoplasmic membrane contains more of a distribution of saturated and unsaturated fatty acids. Almost all *Pseudomonas* species contain the saturated fatty acids hexadecanoic acid and octadecanoic acid. Branced-chain fatty acids are not common in *Pseudomonas* but can be found in some species (Table III). These fatty acids may occur as saturated or unsaturated forms. Unsaturated fatty acids are common in *Pseudomonas* and commonly constitute 25–40% of the total fatty acids (although these

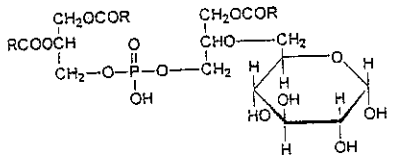


Figure 3. Structure of 1-*O*-glucosylphosphatidylglycerol found in *P. halosaccharolytica*. R = fatty acyl groups.

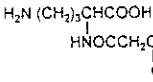


Figure 4. Generalized structure of an ornithine amide lipid. R = fatty acyl groups.

percentages may vary depending on growth conditions). Many species can make monounsaturated forms of hexadecanoic acid and octadecanoic acid. Monounsaturated fatty acids in *Pseudomonas* have the unsaturation predominantly in the cis configuration. Localization seven carbons from the alkyl (ω) end of the molecule suggests biosynthesis via the "anaerobic bacterial pathway" (Wilkinson, 1988). These bacteria also can make the fatty acids cyclopropyl 17:0 and cyclopropyl 19:0 (Table IV). Because of this variety of fatty acid structures, fatty acid profiles can be used in both clinical and agricultural applications to classify strains (Denny *et al.*, 1988; Franzmann and Tindall, 1990; Galbraith and Wilkinson, 1991; Janse, 1991; Rosello-Mora *et al.*, 1994; Stead, 1992; Yabuuchi *et al.*, 1992). These fatty acids are most useful for differentiating species rather than major groups of *Pseudomonas* if the isolates are grown under carefully specified conditions.

2.2.2. Hydroxy Fatty Acids

Almost all gram-negative bacteria contain Lipid A, which constitutes the inner portion of LPS (Fig. 1). Many hydroxy fatty acids are localized to Lipid A. Lipid A usually also contains amide and ester-linked non-hydroxy fatty acids in small amounts (primarily hexadecanoic acid). Hydroxy fatty acids are commonly used in combination with non-hydroxy fatty acid profiles to classify *Pseudomonas*. The hydroxy fatty acids are most useful for differentiating *Pseudomonas* into groups, whereas the non-hydroxy fatty acids are most useful for further differentiation into species (Stead, 1992). Table V shows the distribution of these types of fatty acids among *Pseudomonas*. Hydroxy fatty acids range in chain length from ten to eighteen carbons and may be saturated or unsaturated. The saturated forms are more common than the unsaturated forms, and branching is uncommon in *Pseudomonas* species, although it has been detected in *P. rubescens*. While the hydroxy fatty acids and non-hydroxy fatty acids demonstrate great utility in classifying *Pseudomonas* groups and species, it must be kept in mind that lipid profiles can vary greatly with environmental conditions (see section, "Lipid Alteration in Response to Environmental Conditions"). Great care must be taken with respect to growth conditions when classifying organisms by using lipid profiles.

Recently a distinct group of gram-negative motile rods, previously

Table III. Hydroxy Fatty Acids of *Pseudomonas* Species

Strain	Hydroxy fatty acids												
	3OH10	3OH11	2OH12	3OH12	3OH12:1	3OH13	2OH14	3OH14	2OH16	2OH16:1	3OH16	2OH18:1	3OH18
Pseudomonas aeruginosa[a]	+		+	+									
Pseudomonas alcaligenes[a]	+	+		+									
Pseudomonas andropogonis[a]									+	+	+	+	
Pseudomonas aureofaciens[a]	+		+	+	+								
Pseudomonas carboxydovorans[b]				+									+
Pseudomonas carophyllic[a]								+	+	+	+	+	
Pseudomonas cepacia[a,e]								+	+	+	+	+	
Pseudomonas cichorii[a]	+		+	+									
Pseudomonas coronavenenans[d]				–					+	+	+	+	
Pseudomonas coronafaciens[d]	+		+	+									
Pseudomonas corrugata[a]	+		+	+	+	+					+		
Pseudomonas diazotrophicus[e]								+					+
Pseudomonas diminuta[f]				+				+					
Pseudomonas fluorescens[a,g]	+		+	+									

(continued)

Table III. (*Continued*)

Strain	Hydroxy fatty acids												
	3OH10	3OH11	2OH12	3OH12	3OH12:1	3OH13	2OH14	3OH14	2OH16	2OH16:1	3OH16	2OH18:1	3OH18
Pseudomonas gladioli[c,p]	+												
Pseudomonas gardneri[g]		+		+		+		+	+	+	+	+	
Pseudomonas halophila[h]													
Pseudomonas halosaccharolytica[i]													
Pseudomonas indigofera[d]	+												
Pseudomonas marginalis[a]	+		+	+									
Pseudomonas marginata[i]	–			+			+	+	+		+	+	
Pseudomonas mendocina[d]	+			+									
Pseudomonas mildenbergii[k]	+			+									
Pseudomonas oleovorans[l]								+					
Pseudomonas pickettii[c,p]									+	+		+	
Pseudomonas pseudoalcaligenes[a]	+			+									
Pseudomonas pseudomallei[m,p]								+	+	+	+	+	+
Pseudomonas putida[a]	+		+	+									
Pseudomonas rubescens[f,r]		+		+		+		+					
Pseudomonas rubrisubalbicans[a]	+		+	+			+			+			

Pseudomonas saccharophila[d]				+					
Pseudomonas savastanoi[d]	+	+	+						
Pseudomonas solanacearum[n,p]					+	+	+		+
Pseudomonas stutzeri[o]	+		+						
Pseudomonas syncyanea[f]	+	+	+						
Pseudomonas syringae[n]	+	+	+						
Pseudomonas vesicularis[i,q]			+						
Pseudomonas viridiflava[d]	+	+	+						
Pseudomonas woodsii[d]						+	+	+	+

[a]Stead, 1992.
[b]Mayer *et al.*, 1989.
[c]Galbraith and Wilkinson, 1991.
[d]MIDI.
[e]Taylor *et al.*, 1993.
[f]Wilkinson *et al.*, 1973.
[g]Bouzar *et al.*, 1994.
[h]Franzmann and Tindall, 1990.
[i]Wilkinson, 1988.
[j]Dees *et al.*, 1983.
[k]Roussel and Asselineau, 1980.
[l]de Smet *et al.*, 1983.
[m]Yabuuchi *et al.*, 1992.
[n]Janse *et al.*, 1991.
[o]Rosello–Mora *et al.*, 1994.
[p]Now classified as *Burkholderia* (Yabuuchi *et al.*, 1994).
[q]Now classified as *Brevundimonas* (Segers *et al.*, 1994).
[r]Contains small amounts of br3OH13 and br3OH 15.

Table IV. Saturated and Branched Fatty Acids of *Pseudomonas* Species

Strain	Saturated fatty acids							Terminally branched saturated fatty acids								
	10:0	12:0	14:0	15:0	16:0	17:0	18:0	i11:0	i13:0	i14:0	i15:0	i15:1	a15:0	i16:0	i17:0	i17:1	a17:0
Pseudomonas aeruginosa[a]		+	+		+		+										
Pseudomonas alcaligenes[a]	+	+	+	+	+	+	+								+		
Pseudomonas andropogonis[a]			+		+		+										
Pseudomonas aureofaciens[a]		+	+		+		+										
Pseudomonas carboxydovorans[b]			+				+										
Pseudomonas carophylli[c,p]			+	+	+	+	+										
Pseudomonas cepacia[a,b]			+	+	+	+	+										
Pseudomonas cichorii[a]		+			+		+										
Pseudomonas cocovenenans[d]			+		+		+										
Pseudomonas coronafaciens[d]		+	+		+		+										
Pseudomonas corrugata[a]	+	+			+		+										
Pseudomonas diazotrophicus[e]					+		+										
Pseudomonas diminuta[f,q]			+	+	+	+	+										
Pseudomonas fluorescens[a]		+	+		+		+										
Pseudomonas gladioli[c,p]			+	+	+	+	+										
Pseudomonas gardneris			+					+			+		+	+	+		
Pseudomonas halophila[h]		+	+		+		+				+		+		+		+
Pseudomonas halosaccharolytica[i]			+		+												
Pseudomonas indigofera[d]	+	+	+	+	+		+										
Pseudomonas marginalis[a]		+	+		+		+										
Pseudomonas marginata[j]			+		+	+	+										
Pseudomonas mendocina[d]		+	+	+	+	+	+										
Pseudomonas mildenbergii[k]	+	+			+		+					+					
Pseudomonas oleovorans[l]		+	+		+												

Pseudomonas pickettii[c,p]		+	+	+	+	+					
Pseudomonas pseudoalcaligenes[a]	+		+	+	+	+					+
Pseudomonas pseudomallei[m,p]	+	+	+	+	+						
Pseudomonas putida[a]	+			+		+					
Pseudomonas rubescens[f,q]	+	+	+	+	+	+	+	+	+	+	+
Pseudomonas rubrisubalbicans[a]	+	+		+		+					
Pseudomonas saccharophila[d]		+	+	+							
Pseudomonas savastanoi[d]	+			+		+					
Pseudomonas solanacearum[n,p]		+	+	+	+	+					
Pseudomonas stutzeri[o]	+	+		+		+					+
Pseudomonas syncyanea[f]	+	+	+	+	+	+					
Pseudomonas syringae[n]	+			+		+					+
Pseudomonas vesicularis[i,q]		+	+	+							
Pseudomonas viridiflava[d]	+			+		+					
Pseudomonas woodsii[d]		+		+							

[a]Stead, 1992.
[b]Mayer *et al.*, 1989.
[c]Galbraith and Wilkinson, 1991.
[d]MIDI.
[e]Taylor *et al.*, 1993.
[f]Wilkinson *et al.*, 1973.
[g]Bouzar *et al.*, 1994.
[h]Franzmann and Tindall, 1990.
[i]Wilkinson, 1988.
[j]Dees *et al.*, 1983.
[k]Roussel and Asselineau, 1980.
[l]de Smet *et al.*, 1983.
[m]Yabuuchi *et al.*, 1992.
[n]Janse *et al.*, 1991.
[o]Rosello–Mora *et al.*, 1994.
[p]Now classified as *Burkholderia* (Yabuuchi *et al.*, 1994).
[q]Now classified as *Brevundimonas* (Segers *et al.*, 1994).

Table V. Unsaturated and Cyclopropyl Fatty Acids of *Pseudomonas* Species

Strain	Unsaturated fatty acids									Cyclopropyl FA	
	15:1	16:1ω7cis	16:1ω7trans	16:1ω9cis	17:1ω8cis	17:1ω8trans	18:1ω7cis	18:1ω7trans	18:1ω9cis	cyclo 17:0	cyclo 19:0
Pseudomonas aeruginosa[a]		+	+				+			+	+
Pseudomonas alcaligenes[a]		+			+					+	
Pseudomonas andropogonis[a]		+					+			+	+
Pseudomonas aureofaciens[a]		+					+			+	+
Pseudomonas carboxydovorans[b]							+				
Pseudomonas carophylli[c,p]		+					+			+	+
Pseudomonas cepacia[a,p]		+					+			+	+
Pseudomonas cichorii[a]		+					+			+	+
Pseudomonas cocovenenans[d]		+								+	+
Pseudomonas coronafaciens[d]		+									

Pseudomonas corrugata[a]	+						+				+	+
Pseudomonas diazotrophicus[e]							+					+
Pseudomonas diminuta[f,g]	+				+	+						+
Pseudomonas fluorescens[a]	+	+					+				+	+
Pseudomonas gladioli[c,p]	+						+				+	+
Pseudomonas gardneri[o]	+		+	+								
Pseudomonas halophilas[s,r]	+		+				+	+				
Pseudomonas halosaccharolytica[h]	+						+				+	+
Pseudomonas indigofera[d]	+											
Pseudomonas marginalis[a]	+										+	
Pseudomonas marginata[i]	+						+				+	+
Pseudomonas mendocina[d]	+				+							
Pseudomonas mildenbergii[i]	+						+				+	+

(*continued*)

Table V. (*Continued*)

Strain	Unsaturated fatty acids									Cyclopropyl FA	
	15:1	16:1ω7cis	16:1ω7trans	16:1ω9cis	17:1ω8cis	17:1ω8trans	18:1ω7cis	18:1ω7trans	18:1ω9cis	cyclo 17:0	cyclo 19:0
Pseudomonas oleovorans[k]		+					+			+	+
Pseudomonas pickettii[c,p]		+					+			+	+
Pseudomonas pseudoalcaligenes[a]		+	+		+		+			+	+
Pseudomonas pseudomallei[l,p]		+					+			+	+
Pseudomonas putida[a]		+	+				+	+		+	+
Pseudomonas rubescens[f,r]	+	+			+		+				
Pseudomonas rubrisubalbicans[a]		+					+				
Pseudomonas saccharophila[d]		+									
Pseudomonas savastanoi[d]		+					+			+	+
Pseudomonas solanacearum[m,p]	+	+					+			+	+

Pseudomonas stutzeri[n]		+			+	+	+
Pseudomonas syncyanea[f]	+	+		+	+		
Pseudomonas syringae[m]		+			+	+	+
Pseudomonas vesicularis[h,q]		+					
Pseudomonas viridiflava[d]		+			+	+	+
Pseudomonas woodsii[d]		+	+				+

[a]Stead, 1992.
[b]Mayer *et al.*, 1989.
[c]Galbraith and Wilkinson, 1991.
[d]MIDI.
[e]Taylor *et al.*, 1993.
[f]Wilkinson *et al.*, 1973.
[g]Franzmann and Tindall, 1990.
[h]Wilkinson, 1988.
[i]Dees *et al.*, 1983.
[j]Roussel and Asselineau, 1980.
[k]de Smet *et al.*, 1983.
[l]Yabuuchi *et al.*, 1992.
[m]Janse *et al.*, 1991.
[n]Rosello–Mora *et al.*, 1994.
[o]Bouzar *et al.*, 1994.
[p]Now classified as *Burkholderia* (Yabuuchi *et al.*, 1994).
[q]Now classified as *Brevundimonas* (Segers *et al.*, 1994).
[r]Contains 10ME18.

called *Pseudomonas*, has been reclassified as *Sphingomonas*. The genus *Sphingomonas* forms a phylogenetically tight group in the α-4 subclass of the *Proteobacteria* based on 16S rRNA sequence homology (Takeuchi *et al.*, 1994). Some *Sphingomonas* are nonmotile and nonfermentative, but all contain a class of unusual 'signature' components: 18–21-carbon straight-chain, saturated, monounsaturated, and cyclopropane-containing dihydrosphingosines in a ceramide glycolipid containing uronic acid and amide-linked 2-hydroxy straight-chain saturated fatty acids. In addition, they conatin a long-chain respiratory benzoquinone with a side chain of 10 isoprenoid units (ubiquinone Q-10) (Yabuuchi *et al.*, 1990). *Sphingomonas spp.* do not contain detectable ester or amid-linked, 3-OH fatty acids and lack the lipopolysaccharide components or structures characteristic of gram-negative bacteria. These former pseudomonads have important roles in biotechnology including the ability to degrade a diverse range of environmental pollutants (White *et al.*, 1996).

2.3. Storage Lipids

2.3.1. Polyhydroxyalkoanates (PHA)

PHA is a storage lipid common to many *Pseudomonas* species. *Burkholderia* (*Pseudomonas*) *cepacia*, *B. pickettii*, *B. pseudomallei*, *B. carophyllii*, *B. gladioli*, *B. solanacearum*, and *P. saccharophila* all produce and accumulate poly-β-hydroxybutyrate (PHB) as a carbon storage polymer, a characteristic which as been used to distinguish these organisms from other pseudomonads (Figure 5). Although some fluorescent pseudomonads cannot make PHB, they can accumulate other forms of PHA, most notably poly-β-hydroxydecanoate, which has been studied in *P. putida and poly-β-hydroxyoctanoate in P. oleovorans. In all cases, these polyhydroxyalkoanates accumulate in intracellular inclusions. Biosynthesis of these polymer depends on a combination of nutrient limitation (especially nitrogen limitation) and carbon source excess (Anderson and Dawes, 1990). PHA accumulation is greatest in fluorescent pseudomonads when they grow on decanoate, but accumulation also accurs when they frow on other fatty acids (hydroxy and non-hydroxy), glucose, fructose, and glycerol. P. oleovorans* accumulates poly-β-hydroxyoctanoate when gown on C_8–C_{12} alkanes, 1-octene, and C_8–C_{10} alcohols (de Smet *et al.*, 1983). Several investigators have shown that PHA monomer length depends on the growth source. The monomer is generally the carbon length of the growth substrate, or one or two C_2 units shorter following beta oxidation (Huijberts *et al.*, 1992). Interestingly, the concentration of unsaturated monomers in PHA depends on growth temperature, simi-

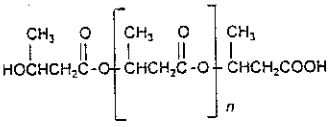


Figure 5. Structure of poly(hydroxybutyrate), n = up to 10,000.

lar to the cell envelope concentration of unsaturated fatty acids (Huijberts *et al.*, 1992).

Production of PHA requires the enzymes 3-ketothiolase, acetoacyl-CoA reductase, and PHA synthase. In *P. putida*, PHA is synthesized from monomers generated from one of three sources. It has been shown that PHA can be produced from de novo fatty acids synthesis, β-oxidation of fatty acids, and fatty acid elongation. The PHA synthase of *P. putida* preferentially incorporates C_8 and C_{10} CoA thioesters, C_6 and CoA thoesters larger tha C_{10} are invorporated less efficiently into PHA (Juijberts *et al.*, 1994). The PHA synthase of *P. oleovorans* accepts CoA thoesters in the range of C_6–C_{14}.

The organization of the genes involved in the biosynthesis of PHA has been most extensively studied in *Alcaligenes eutrophus*. However, investigators have cloned genes of the PHA biosynthetic pathway in *Pseudomonas*, most notably *P. oleovorans* and *P. aeruginosa*. In both of these strains, PHA synthases 1 and 2 have been identified as well as a PHA depolymerase and a protein of unknown function (Steinbuchel *et al.*, *1992). These proteins have similar molecular weights and amino acid sequence similarities ranging from 53.7–79.6%. In P. aeruginosa* two transcriptional start sites have been identified, one of which is preceded by a σ^{54}-dependent promoter, and the other by a σ^{70}-dependent promoter. An intact RpoN σ factor is required for PHA accumulation from gluconate in *P. aeruginosa*. Theis factor is not required in *P. oleovorans*. It is also notable that *P. oleovorans* can produce only PHA from β-oxidation-derived monomers butnot from luconate, even when complemented with PHA synthases from *P. aeruginosa*. An intact RpoN GS FACTOR IS NOT REQUIRED FOR PHA PRODUCTION FROM GLUCONATE IN *P. putida*, indicating yet another regulatory pathway for this species (Timm and Steinbuchel, 1992).

One final note of interest in the production of PHA by *Pseudomonas* species is the packaging of PHA material. As noted previously, the production of PHB versus longer carbon chain length PHA is usually mutually exclusive. Organisms capable of PHB production do not make longer chain length PHA molecules (and vice versa). However, those that have been engineered to do so through the addition of a plasmid accu-

mulate PHB exclusively in some intracelular grnules, while accumulating the longer carbon chain PHA molecules in other, separate granules (Preusting *et al.*, 1992).

2.4. Exolipids

2.4.1. Rhamnolipid

This glycolipid is produced by *Pseudomonas aeruginosa*. It is of special interest for two reasons. It is one of the few extracellular lipids produced by *Pseudomonas*, and it also possesses surfactant qualities. The surfactant qualities of rhamnolipid cause serious problems in the respiratory tracts of cystic fibrosis patients. Rhamnolipid releases glycoconjugates from tracheal cells (Somerville *et al.*, 1992) and damages tracheal cilia (Hastie *et al.*, 1986), thus increasing airway mucus output and helping to maintain infection. It has also been implicated in aggravating the oxidative burst response of lung macrophages which further damage lung tissue (Kharami *et al.*, 1989). The surfactant qualities of rhamnolipid are helpful in removing hydrocarbons from soil (Van Dyke *et al.*, 1992), and some investigators have shown that biodegradation rates of hydrocarbons increase when rhamnolipid is added to the growth medium (Zhang and Miller, 1992).

P. aeruginosa can synthesize both the mono-and di-rhamnolipids (Rendell *et al.*, 1990) shown in Figure 6. The most common rhamnolipid isolated from *P. aeruginosa* is rhamnosyl-3-hydroxydecanoyl-3-hydroxydecanoate. Rhamnolipids containing the fatty acids 3-hydroxyoctananoate, 3-hydroxydodecanoate, and 3-hydroxydodecenoate substituted

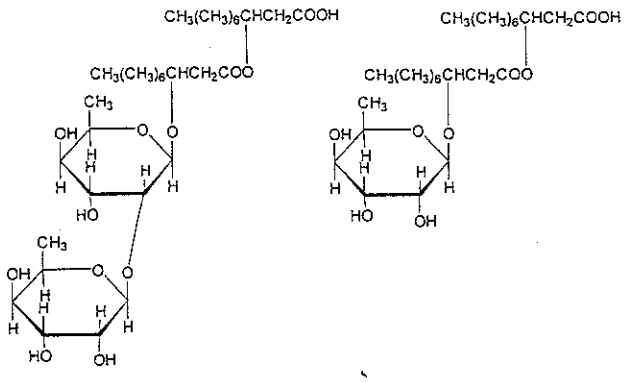


Figure 6. The two forms of rhamnolipid commonly found in *P. aeruginosa*.

at one of the 3-hydroxydecanoate positions have also been identified (Rendell *et al.*, 1990). Rhamnolipid production is greatest during the stationary phase of growth when grown on glycerol, n-alkanes, or glucose, and under some circumstances constitute up to 38% of cell dry weight (de Andres *et al.*, 1991). Production of rhamnolipid also increases under nitrogen limitation and limitation of divalent cations, such as magnesium and iron (Syldak *et al.*, 1985).

A biosynthetic pathway for rhamnolipid has been proposed (Burger *et al.*, 1963) based on studies with the radiolabelled substrates acetate and glycerol. The initial substrate for rhamnolipid biosynthesis is thymidine-diphospho-rhamnose (TDP-rhamnose), a precursor of the O-antigen region of LPS. TDP-rhamnose is the donor molecule for the rhamnose moiety of rhamnolipid. The rhamnose is donated to the β-hydroxydecanoyl-β-hydroxydecanoate molecule via a specific rhamnosyltransferase enzyme. The β-hydroxydecanoyl-β-hydroxydecanoate molecule is the product of a condensation reaction between two β-hydroxydecanoate molecules, although the exact mechanism for this reaction is unknown. The source of the β-hydroxydecanoate can be from one of two sources (Boulton and Ratledge, 1987). The first source is the fatty acid β-oxidation pathway. This is the most likely source if the organism is grown on fatty acids or alkanes. The second source of β-hydroxydecanoate is de novo fatty acid synthesis.

There are four genes in the *rhl* gene cluster (Ochsner and Reiser, 1995). The first two genes, *rhlA* and *rhlB*, code for the two subunits of the rhamnosyltransferase enzyme. *rhlR* codes for a transcriptional activator, and *rhlI* codes for an autoinducer synthetase. The *rhlR* gene is expressed at a low constitutive level from a σ^{70} promoter. The *rhlR* gene product becomes a fully functional transcriptional activator of rhlAB following binding to the autoinducer, *N*-acyl homoserine lactone. The *rhlAB* operon follows a σ^{54} promoter. Autoinducer molecules are produced at a low constitutive level. A high cell density is required to accumulate enough autoinducer locally to activate transcription of the *rhlAB* genes. Autoinducer-mediated activation of transcription in *P. aeruginosa* is a well-studied phenomenon and is linked to expression of elastase (Passador *et al.*, 1993; Pearson *et al.*, 1995), chitinase (Winson *et al.*, 1995), and pyocyanin (Brint and Ohman, 1995; Latifi *et al.*, 1995).

Although much is known about rhamnolipid gentics, regulation, and biosynthesis, there are still unanswered questions about its function. Rhamnolipid increases degradation rate of hydrocarbons, but how the cell processes hydrocarbon-rhamnolipid complexes is still unknown. The enzyme responsible for the condensation of the two β-hydroxydecanoate molecules has not as yet been isolated. Many researchers

are now working to optimize production of rhamnolipid as a source of rhamnose (Ochsner *et al.*, 1995) and are studying its use in bioremediatoin to increase bioavailability of hydrocarbons sorbed to soil particles (Finnerty, 1994).

2.4.2. Viscosin

P. fluorescens and *P. viscosa* also make an exolipid which is a peptidolipid called viscosin. This compound is unusual in that it possesses antiviral activity (Kochi, 1951). Little is known at this time about its regulation. Its primary function seems to be to promote the spread of bacteria along surfaces colonized by these plant pathogens (Laycock *et al.*, 1991). The structure of viscosin has been elucidated (Neu, 1990). It contains hydrophobic amino acid moieties (L-leucine, D-serine, L-isoleucine, D-valine, and threonine) and a fatty acid.

3. ALTERATION OF LIPIDS IN RESPONSE TO ENVIRONMENTAL CONDITIONS

3.1. Growth Temperature

Most growth temperature studies have been performed on enteric bacteria, but a few investigators have examined temperature response in *Pseudomonas*. *P. aeruginosa*, a mesophile, grows in temperatures ranging from 15–45 °C. Like the enteric bacteria, it conforms to the rules of homeoviscous adaptation, changing membrane lipids of both the inner and outer membranes (Kropinski *et al.*, 1987). As the temperature increases, the percentage of saturated phospholipid fatty acids (dodecanoic, hexadecanoic and octadecanoic acid) increases whereas the percentage of the unsaturated fatty acids, hexadecenoic and octadecenoic acid, decreases. The fatty acids found in Lipid A of the outer membrane also change as temperature increases. Dodecanoic acid, hexadecanoic acid, and 3-hydroxydodecanoic acid increase whereas 3-hydroxydecanoic and 2-hydroxydodecanoic acid decrease. An increase in the LPS:phospholipid ratio was also noted. This increase in carbon chain length and saturation helps to maintain the proper phase-transition state at increased temperature to maintain normal membrane permeability and to provide an environment suitable for membrane proteins.

Psychrophilic pseudomonads do not conform to the rules of homeoviscous adaptation. When grown in the temperature range of 0–20 °Cor

5–30 °C, these *Pseudomonas* species do not show any significant difference in lipid profiles. *Pseudomonas* sp. E-3 showed a small increase in hexadecenoic acid at 5 °C compared with what was observed at 30 °C, but the increase was not significant (Wada *et al.*, 1987). In another experiment, five psychrophilic *Pseudomonas* strains showed small changes in phospholipid composition as temperature decreased from 20 to 0°C (Bhakoo and Herbert, 1980). Small increases were seen in phosphatidylserine and cardiolipin, a decrease in phosphatidylglycerol was noted, and phosphatidylethanolamine content varied at each temperature for each strain. No significant change in fatty acid composition as noted for these strains. It has been hypothesized that because these organisms are generally exposed to low, constant temperature, they have not developed the ability toadapt their membrane lipids as a function of temperature change.

3.2. Oxygen Tension

Oxygen limitation has not been studied extensively in *Pseudomonas* with respect to lipid composition. However, some early studies have shown that oxygen limitation induces the formation of cyclopropyl fatty acids in *P. denitrificans* (Jaques and Hunt, 1980). Further studies have shown that it is an indirect function of oxygen tension. The production of cyclopropyl fatty acids in *P. denitrificans* is directly related to the state of reduction of components of the respiratory system rather than oxygen tension per se (Jacques, 1981) and therefore is associated with starvation response, rather than strictly oxidative stress.

Exposure of *P. aeruginosa* to hyperbaric oxygen tensions (100% O_2) resulted in the formation of giant colonies (Kenward *et al.*, 1980). These cells showed significant increases in readily extractable lipid, free fatty acid and neutral lipids, and a small increase in total phospholipid content. These cells also showed a significant increase in cardiolipin content and a significant decrease in phosphatidylglycerol content. Phosphatidylethanolamine content remained unchanged. These changes are related to adaptation rather than mutation because subculture in normal air restores the normal phenotype.

3.3. Desiccation

Although not much is known about bacterial response to desiccation, some information is available. *P. aureofaciens* lipid profiles are strongly influenced by moisture and nutrient availability (Kieft *et al.*, 1994). The strain studied showed a marked increase in saturated/

unsaturated fatty acid ratios, an increase in trans unsaturated fatty acid to cis unsaturated fatty acid ratios, and an increase in cyclopropyl fatty acids. These changes in fatty acid profiles coincided with entry into the viable but nonculturable state of the starved, desiccated organisms.

3.4. Nutrient Deprivation

Carbon starvation in *Pseudomonas* generally results in the alteration of lipid profiles and content. Prolonged starvation leads to the generation of minicells, which have proportionally more phopholipid. Although the proportions of specific phospholipids do not change greatly in carbon-starved cells, an increase in cardiolipin has been noted for some strains. The bulk of lipid changes are localized to membrane fatty acids (found in phospholipids and ornithine-amide lipids). The ratio of saturated to unsaturated fatty acids incrases. The bulk of the remaining cis unsaturated fatty acids are converted to trans unsaturated fatty acids and to cyclopropyl fatty acids (unpublished data). Although the reasons for these shifts are not completely understood, it is generally believed that the increased saturation of the fatty acids, couple with modifications of the unsaturated fatty acids, create a membrane with a higher phase-transition temperature. It is believed that a membrane with a higher phase-transition temperature creates a more rigid, less permeable cell envelope capable of maintaining envelope integrity during environmental stress, such as starvation. Nitrate and phosphate limitation have different effects on lipid composition. When both of these nutrients are limiting, but carbon is readily available, many *Pseudomonas* species accumulate carbon storage polymer in the form of polyhydroxyalkoanates (PHA) (Anderson and Dawes, 1990). Many strains can accumulate up to 60% or more of their dry weight in nitrogen and/or phosphate-limited conditions. As mentioned previously, most *Pseudomonas* strains accumulate PHA with either short (C_4) or medium carbon chain lengths (C_8–C_{10}). Medium chain PHA monomer length is generally dictated by the carbon source used for growth.

When starved for phosphate, *P. fluorescens* makes ornithine amide lipids virtually to the exclusion of phospholipids, with no apparent adverse affect on cell function (Abdolrazmah and Minnikin, 1974). The same authors also showed that a magnesium-limited chemostat culture of the same organism produces membranes devoid of any ornithine amide lipids. Phosphate starvation in *P. diminuta* decreases its phospholipid content to 0.3% of the membrane lipids, and acidic and neutral glycolipids make up the bulk of the membrane lipid content.

3.5. Solvent Tolerance

Several studies involving membrane alterations of *P. putida* strains resistant to high concentrations of phenol, toluene, ethanol and other organic solvents have been conducted recently (Heipieper *et al.*, 1992; Pinkart *et al.*, 1995; Weber *et al.*, 1994). Most hydrocarbons are toxic to microorganisms because they partition in the membranes, causing swelling and disorganization of the membranes. This disorganization leads to alteration of the cell's permeability, resulting in the leakage of small molecules from the cell and disruption of protonmotive force (Sikkema *et al.*, 1994, 1995). In the past several years, many *P. putida* strains have been discovered that are resistant to the effects of organic solvents. Although the mechanism for resistance is not understood at this time, several changes in membrane composition have been documented in solvent-tolerant and solvent-sensitive strains. One common response seen in cells exposed to toluene, xylene, and phenol is the formation of *trans*-unsaturated fatty acids (Heipieper *et al.*, 1992; Weber *et al.*, 1994). This response is seen in both solvent-tolerant and in solvent-sensitive *P. putida* strains (Pinkart *et al.*, 1995), indicating that it may be a common initial response to membrane damage. The mechanism for formation of trans-unsaturated fatty acids has not been well characterized, but evidence exists for both *de novo* synthesis of *trans*-unsaturated fatty acids (Guckert *et al.*, 1987), and for isomerization of the intact phospholipid (Heipeiper and de Bont, 1994). Some strains also show an increase in cyclopropyl fatty acids when exposed to xylene, which is also a modification of the intact phospholipid. An increase in saturated fatty acids following exposure to organic solvents has been noted in some strains but not in others. Exposure to ethanol decreases saturated fatty acids in *P. putida* S12. Ethanol also increases C_{18} fatty acids relative to C_{16}, whereas toluene causes an opposite reaction. This unusual response could be caused by inhibition of fatty acid biosynthetic enzymes by ethanol. An increase in hydroxy fatty acids has also been seen in the LPS of *P. putida* Idaho following exposure to xylene (Pinkart *et al.*, 1995). This may be a response of the cell to alter outer membrane permeability because strain was shown to have an increase resistance to difloxacin, a hydrophobic antibiotic with the same hydrophobic index as xylene.

3.6. Antibiotic Resistance

Some *Pseudomonas* strains have developed antibiotic resistance through membrane alterations. *P. aeruginosa* strains with increased

amounts of KDO and Lipid A were found to be much less permeable to hydrophilic quinolones and had much higher resistance to these antibiotics (Michea-Hamzepour *et al.*, 1991). In an experiment comparing four *P. aeruginosa* strains with respect to lipid content and antibiotic susceptibility, it was found that strains with a higher lipid content were considerably more resistant to ampicillin than strains containing less lipid (Norris *et al.*, 1985).

4. SUMMARY

Although much research has been conducted on the lipid composition of *Pseudomonas*, several areas of study still need attention. No information is yet available about the genetics or regulation of phospholipid or fatty acid biosynthesis in *Pseudomonas*. The enzymology of trans-unsaturated fatty acid formation has not been elucidated. The mechanism for maintenance of membrane integrity in solvent tolerant organisms is still unclear. Studies are currently underway to examine the possibility of using *Pseudomonas* to make copolyesters of PHB and medium chain length PHA (Lee *et al.*, 1995). There is still much to be learned about the function of rhamnolipid in bioavailability. These probelms provide investigators of *Pseudomonas* with several opportunities for study in the future.

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