Occurrence and Patterns of Waxes in Neisseriaceae

By K. BRYN AND E. JANTZEN

National Institute of Public Health, Postuttak, Oslo 1, Norway

AND K. BØVRE

Institute of Medical Biology, University of Tromsø, Tromsø, Norway

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Forty-five strains classified in the family Neisseriaceae were analysed for wax esters by gas-liquid chromatography. The amounts and types of waxes varied between the taxa. Waxes were not detected in 16 strains of 'true neisseriae' (genus *Neisseria*) or in two strains of *Kingella*, but they were found in all 'false neisseriae', in all species of *Moraxella* except *Moraxella phenylpyrouvica*, in five out of 10 strains of *Acinetobacter*, and in all strains of a group of psychrophilic, oxidase-positive organisms. The chain lengths of the wax esters ranged from C₂₄ to C₄₂, with C₃₆ predominating. In all taxa, esters with even numbers of carbon atoms constituted 70 to 100 % of the total. Saturated, mono-unsaturated and di-unsaturated waxes were found. *Acinetobacter* strains were characterized by large amounts (30 to 98 %) of di-unsaturated wax esters; such waxes did not exceed 8 % in the 'false neisseriae' or *Moraxella* spp. Waxes of strains belonging to the psychrophilic, oxidase-positive group generally resembled those found in *Moraxella*. Wax esters with odd numbers of carbon atoms were abundant in *M. lacunata* (29 %), *M. atlantae* (15 %) and in the psychrophilic group (19 to 28 %); long-chain esters (C₄₀ or above) were characteristic of *M. atlantae* (30 %) and one strain of *M. osloensis* (26 %).

INTRODUCTION

True waxes, i.e. simple esters of fatty alcohols and fatty acids, have only rarely been reported in bacteria. They have been detected in *Corynebacterium* spp. grown on hydrocarbon (Bacchin, Robertiello & Viglia, 1974) and also in *Acinetobacter* (Gallagher, 1971).

During investigations on the fatty acids of strains of *Neisseria*, *Moraxella* and *Acinetobacter* (Jantzen *et al.*, 1974*b*, 1975), unknown compounds, later identified as fatty alcohols released from waxes, were observed. The aim of the present investigation was to survey the occurrence and distribution of true waxes within the heterogeneous family Neisseriaceae (Henriksen, 1973) using gas chromatography.

METHODS

Organisms and cultivation. The 45 strains examined (Table 1) represent a number of taxa within the family Neisseriaceae. The 'true neisseriae' (genus *Neisseria*: Henriksen & Bøvre, 1968*b*; Bøvre & Holten, 1970) examined were: *Neisseria meningitidis* B8152/66, P22 cp⁻ and M1; *N. gonorrhoeae* ATCC19424 and U562/71; *N. flavescens* ATCC13120; *N. flava* ATCC14221; *N. subflava* ATCC19243; *N. perflava* ATCC10555; *N. elongata* M2, 7823/71 and 8554/71; *N. sicca* ATCC9913; *N. lactamica* ATCC23970; *N. mucosa* subsp. *mucosa* ATCC19696; *N. cinerea* 159/62. It has been proposed that the 'false neisseriae' (*N. catarrhalis*, *N. ovis* and *N. caviae*) be transferred to the genus *Moraxella* (Henriksen & Bøvre, 1968*b*) or to *Branhamella* gen.nov. (only for *N. catarrhalis*; Catlin, 1970). However, the name *Neisseria* is retained while this taxonomic problem is being re-examined. The formerly named *Moraxella kingae* has been transferred to *Kingella* gen.nov. in the family Neisseriaceae (Henriksen & Bøvre, 1968*a*, 1976; Bøvre, Henriksen & Jonsson, 1974*b*). The 'New

Table 1. *Wax contents of some Neisseriaceae strains*

Organisms were grown in broth at 33 °C with the exception of strains 31, 39, 40 and 41 which were grown at 22 °C. The 'true neisseriae' examined are detailed in Methods. Wax esters were extracted with chloroform/methanol/water, purified by chromatography on silicic acid and analysed by g.l.c. Strains 39–42 are all oxidase positive.

	Taxon	Strain	Wax [mg (g dry wt bacteria) ⁻¹]	Di-unsaturated wax (% of total)
1–16	'True neisseriae'		—	—
17	<i>Neisseria catarrhalis</i>	NeII	15.0	< 1.0
18	<i>N. ovis</i>	199/55	5.2	< 1.0
19	<i>N. caviae</i>	NCTC10293	2.8	2.6
20	<i>Moraxella nonliquefaciens</i>	NCTC7784 N-a	5.8	< 1.0
21	<i>M. bovis</i>	4 N-b	8.3	< 1.0
22	<i>M. lacunata</i>	ATCC17967	2.3	< 1.0
23	<i>M. osloensis</i>	5873	0.3	8.2
24	<i>M. osloensis</i>	T3	1.8	1.1
25	<i>M. atlantae</i>	A1922	2.0	4.8
26	<i>M. phenylpyrouvica</i>	2863	—	—
27	<i>M. phenylpyrouvica</i>	ATCC17958	—	—
28	<i>M. phenylpyrouvica</i>	752/52	< 0.1	—
29	<i>Acinetobacter</i>	8	1.3	49.8
30	<i>Acinetobacter</i>	BD4	1.8	63.6
31	<i>Acinetobacter</i>	MJT/F4/5,7	4.7	98.0
32	<i>Acinetobacter</i>	ATCC15149	0.5	37.2
33	<i>Acinetobacter</i>	ATCC17959	0.2	30.3
34	<i>Acinetobacter</i>	ATCC17903	—	—
35	<i>Acinetobacter</i>	ATCC17906	—	—
36	<i>Acinetobacter</i>	ATCC17924	—	—
37	<i>Acinetobacter</i>	ATCC17977	—	—
38	<i>Acinetobacter</i>	ATCC17985	—	—
39	Psychrophilic strain	NCMB200	4.3	3.7
40	Psychrophilic strain	NCMB307	0.2	34.3
41	Psychrophilic strain	MJT/F5/199A	0.4	< 1.0
42	Psychrophilic strain	A348	0.2	4.4
43	<i>Kingella kingae</i>	4177/66 N-a ATCC23330	—	—
44	<i>K. indologenes</i>	ATCC25869	—	—
45	' <i>Moraxella urethralis</i> '	WM6	—	—

Moraxella' Bijsterveld (Bijsterveld, 1970; Bøvre *et al.*, 1974a) is now named *Kingella indologenes* (Snell & Lapage, 1976), and the tentatively named *M. urethralis* (Lautrop, Bøvre & Frederiksen, 1970) forms a separate taxonomic group. Most of the test strains have been studied in earlier taxonomic investigations (Bøvre *et al.*, 1972, 1974a, 1976, 1977; Frøholm *et al.*, 1972; Jantzen *et al.*, 1974b, 1975, 1976). The additional strains of *N. flava*, *N. subflava*, *N. perflava*, *N. mucosa* and *N. lactamica* were obtained from Dr E. Holten, Kaptein W. Wilhelmsen og Frues Bakteriologiske Institutt, University of Oslo, Norway, and strain MJT/F5/199A from Dr M. J. Thornley, University of Cambridge.

The strains were cultivated in a rotary shaker operating at 170 rev. min⁻¹ at 33 °C, except three psychrophilic, oxidase-positive strains (NCMB200, NCMB307, MJT/F5/199A) and *Acinetobacter* MJT/F4/5,7 grown at room temperature (about 22 °C), and *N. gonorrhoeae* U562/71 grown on blood agar. Flasks (2 l) containing 400 ml Mueller-Hinton Broth (Difco) and 0.5 % (w/v) yeast extract (Difco) were inoculated with bacteria grown in the same medium. After 20 h, organisms were harvested by centrifugation (7000 g, 10 min, 4 °C) and washed twice with distilled water. Lyophilized samples were stored under nitrogen at -20 °C.

Chemicals. Solvents of *pro analysis* grade were distilled before use. Standards of fatty acid esters and alcohols were obtained from Applied Science Laboratories, Pennsylvania, U.S.A., and wax esters from Sigma.

Extraction and fractionation of lipids. Lipids were extracted by a procedure modified from Bligh & Dyer (1959). Dry organisms (100 mg) were extracted (1 h at room temperature) twice with 32 ml chloroform/methanol/water (5:8:3, by vol.). Chloroform (44 ml) and water (12 ml) were added to the combined extracts and, after centrifuging, the lower phase was recovered, washed twice with 10 ml chloroform/methanol/water

(3:48:47, by vol.), and the solvents were removed on a rotary evaporator. The residue was dissolved in chloroform/methanol (2:1, v/v), flushed with N_2 and held at $-20^\circ C$.

The lipids were fractionated by chromatography on silicic acid (100 mesh; activated at $110^\circ C$ for 1 h; Mallinkrodt, St Louis, U.S.A.). Lipid extracts were adsorbed on silicic acid (50 mg) and applied to columns (0.5 g silicic acid in hexane). Non-polar substances were eluted with 5 ml hexane and waxes were then eluted with 5 ml hexane/chloroform (7:3, v/v).

Thin-layer chromatography (t.l.c.). Wax fractions were purified by preparative t.l.c. on silica gel F₂₅₄ (0.5 mm; Merck) in hexane/diethyl ether (9:1, v/v) (Mangold, 1967). After detection under ultraviolet light a zone with an R_f value corresponding to authentic cetyl palmitate (wax C_{16:0}-C_{16:0}) was scraped off the plate, and waxes were eluted with chloroform. For analytical t.l.c. of waxes, 0.2 mm plates were used and waxes were detected by sulphuric acid charring.

Gas-liquid chromatography (g.l.c.). Wax esters were analysed on a Hewlett-Packard 5710 chromatograph equipped with a glass column (200 × 0.2 cm) with 10 % SP2100 (methyl silicone) on 100/120 mesh Supelco-port (Supelco, Pennsylvania, U.S.A.). A temperature gradient from 250 to 340 °C increasing at 1 °C min⁻¹ was used, with a carrier gas (N_2) flow rate of 20 ml min⁻¹, injection port temperature of 300 °C and flame ionization detector temperature of 350 °C. Peak areas were recorded by a Hewlett-Packard 3370-B digital integrator, and response factors of all wax esters were assumed to be identical to that of C_{23:0} methyl ester which was used as an internal standard.

Wax constituents, i.e. fatty alcohols and fatty acids (as methyl esters), were analysed on the non-polar SP2100 column, increasing from 150 to 250 °C at 2 °C min⁻¹. In addition a polar column, 10 % SILAR-10 C (cyanopropyl silicone) on 100/120 mesh Gas-Chrom Q (Applied Science Laboratories) was used, increasing from 120 to 250 °C at 2 °C min⁻¹.

Identification of wax esters. Wax esters were primarily identified by comparing their retention times on isothermal g.l.c. at 300 °C with those of authentic esters. Fatty acids and alcohols were identified by comparing their retention times with those of commercial standards. The identity of alcohols was verified by determining their retention times before and after conversion to trifluoroacetyl (TFA) derivatives and their extractability into hexane at pH 7.

Wax esters were cleaved by saponification with 2 ml 2 M-KOH/ethanol (1:1, v/v) for 2 h at 100 °C (Dittmer & Wells, 1969). After adjusting to pH 7 with 4 M-HCl, the alcohols were extracted with hexane (2 × 3 ml), the combined hexane phases were washed with 1 ml water and, after concentration under a stream of N_2 , the hexane solution was analysed by g.l.c. before and after forming TFA derivatives. Fatty acids were subsequently extracted with hexane (2 × 3 ml) after acidifying the aqueous phase to about pH 2 with 2 M-HCl. The solvent was removed and methyl esters were formed by treatment with 1 ml BCl_3 (14 %, w/v) in methanol (Merck) at 100 °C for 10 min. After adding 1.5 ml aqueous saturated NaCl, the fatty acid methyl esters were extracted twice with 3 ml hexane/chloroform (4:1, v/v). (Phase separation was aided by centrifuging at 500 g.) The organic phase was evaporated to dryness by N_2 and redissolved in hexane before g.l.c. analysis.

Alternatively wax esters were cleaved by methanolysis using 2 M-HCl in methanol at 85 °C overnight. Reactants were removed by N_2 and the mixture of alcohols and fatty acid methyl esters was dissolved in hexane and analysed by g.l.c.

TFA derivatives were formed by heating to boiling for 5 min with equal volumes of trifluoroacetic anhydride (Merck) and acetonitrile (Merck).

The linear relationship between the logarithms of retention times and chain lengths among homologous series of compounds was used for primary identification of uncleaved wax esters as well as for the constituent fatty alcohols. Further identification of the alcohols was achieved by partition between hexane and acetonitrile at 20 °C (Jantzen *et al.*, 1974*a, b*), with methyl palmitate as an internal standard (Berezkin, Loschilova & Pankov, 1975). The identity was confirmed by mass spectrometry (m.s.), using a Varian MAT CH 7 g.l.c.-m.s. instrument, operating at 70 eV with an electron current of 300 μA , an accelerating voltage of 3 kV, a molecule separator temperature of 250 °C and an ion source temperature of 190 °C.

Mass spectra of wax alcohols and g.l.c. profiles of wax esters or their hydrolysates from several strains have been deposited with the British Lending Library, Boston Spa, Yorkshire LS23 7BQ, as Supplementary Publication No. SUP 28007 (8 pages). Copies may be obtained from the BLL on demand. Where possible, requests should be accompanied by prepaid coupons (held by many university and technical libraries and by the British Council).

RESULTS

Monitoring of lipid fractions by t.l.c.

Extracted lipids of three 'true neisseriae', two 'false neisseriae', two *Moraxella* strains and one *Acinetobacter* strain were fractionated on a silicic acid column. When analysed by t.l.c., one major spot was observed in the hexane/chloroform (7:3, v/v) fractions from all strains

Fig. 1. Typical g.l.c. wax ester profiles of strains within the family Neisseriaceae. For details, see Methods. In the wax designations, the first number indicates the number of carbon atoms, and the second, the number of double bonds. IS, Internal standard (methyl tricosanoate, $C_{23:0}$).

except the three 'true neisseriae'. This t.l.c. spot had an R_F value corresponding to the authentic wax ester cetyl palmitate. More than 97 % of the wax material was found in this hexane/chloroform fraction, as judged by g.l.c. Further purification of the wax fraction by preparative t.l.c. before g.l.c. was not necessary.

Thus, chromatography of extractable lipids on a short silicic acid column provided an almost pure wax fraction in a quantitative yield, well suited for a comparative g.l.c. study of waxes of Neisseriaceae.

Characterization of waxes by g.l.c.

When wax fractions were analysed by g.l.c., doublet or triplet peaks were commonly observed (Fig. 1). Isothermal g.l.c. at 300 °C confirmed that these corresponded to three homologous series of wax esters. Comparison with the authentic esters $C_{14:0}$ – $C_{14:0}$, $C_{16:0}$ – $C_{16:0}$ and $C_{18:0}$ – $C_{18:0}$ showed that the series with highest retention times corresponded to normal saturated esters. The partial resolution of the two other homologous series suggested that these were mono- and di-unsaturated esters (Haahti, 1961). The retention time differences between these series and the saturated series corresponded to the differences between saturated and mono- and di-unsaturated fatty acid methyl esters. Furthermore, cleavage of ester bonds of wax samples revealed the expected degree of unsaturation in the resulting fatty acids and alcohols.

To obtain more detailed information about the composition of wax esters, they were hydrolysed and the constituent fatty acids and alcohols were analysed by g.l.c. When no components with an odd number of carbon atoms were present, the alcohols and fatty acids could be analysed together in a single run. Two homologous series of alcohols were found, corresponding to normal saturated and mono-unsaturated alcohols. Standards of the latter were not available but the retention times (relative to those of saturated alcohols) clearly indicated mono-unsaturation.

In addition to the g.l.c. retention characteristics, the identity of the mono-unsaturated alcohols was supported by the partition behaviour in the two-phase solvent system hexane/ acetonitrile (Jantzen *et al.*, 1974*a*). Standard alcohols, as well as those derived from waxes of *Acinetobacter* and *Moraxella* strains, showed a fivefold increase in their relative partition coefficients after conversion to their TFA derivatives. Mono-unsaturated compounds had coefficients that were about 40 % lower than those for saturated compounds; this was true for free alcohols, for TFA derivatives of alcohols, and for standard fatty acid methyl esters.

Further evidence on the identity of mono-unsaturated alcohols was obtained by g.l.c.-m.s. As expected for a long-chain alcohol, the m.s. fragmentation pattern of the unknown C_{18} alcohol from wax of *Acinetobacter* MJT/F4/5,7 showed the characteristic fragments m/e 250 ($M - 18$, loss of water) and m/e 222 ($M - 46$, loss of water and C_2H_4), and otherwise resembled the pattern of an unsaturated alkane (Budzikiewicz, Djerassi & Williams, 1964). The m.s. fragmentation pattern was nearly identical to that reported for oleyl alcohol, $C_{18:1}$ (Stenhagen, Abrahamsson & McLafferty, 1974). The fragments of m/e $M - 18$ and $M - 46$ also occurred in patterns of standard saturated alcohols, and in the $C_{16:1}$, $C_{16:0}$ and $C_{18:0}$ alcohols from *Acinetobacter* wax.

Wax patterns

Although wax was not detected in 'true neisseriae', in the two strains of *Kingella* or in '*M. urethralis*', it occurred in all 'false neisseriae' and unequivocal *Moraxella* species [0.3 to 15 mg (g dry wt bacteria) $^{-1}$], with the exception of *M. phenylpyruvica*, in five out of 10 *Acinetobacter* strains (0.2 to 4.7 mg g $^{-1}$), and in the four strains of the psychrophilic oxidase-positive group (0.2 to 4.3 mg g $^{-1}$) (Table 1). The total extractable lipids constituted between 5 and 12 % of the bacterial dry weights, and the proportion of wax esters, when present, was about 10 % (range 5 to 30 %).

Of the individual wax esters, $C_{36:1}$, $C_{36:0}$, $C_{34:1}$ and $C_{34:0}$ were abundant in all strains except one *Acinetobacter* strain (Table 2). Waxes with even numbers of carbon atoms predominated, accounting for more than 70 % in all strains and over 90 % in most. No strains contained all the wax esters observed (C_{24} to C_{44}) but waxes of chain lengths C_{32} , C_{34} and C_{36} were always present.

The wax alcohol pattern (Table 3) confirmed that provided by the parent waxes. Thus, alcohols with even numbers of carbon atoms predominated, with chain lengths about half of the wax ester chain lengths.

In all strains of the 'false neisseriae'/*Moraxella* group, far smaller amounts of di-unsaturated wax esters (0 to 8 %) or unsaturated alcohols (0 to 16 %) were found than in *Acinetobacter*; the five wax-containing *Acinetobacter* strains possessed, respectively, 30 to 98 % and 56 to 95 % of these compounds. The psychrophilic oxidase-positive strains, except NCMB307, resembled the 'false neisseriae'/*Moraxella* group in that they had small amounts of di-unsaturated wax esters (0 to 5 %) and unsaturated wax alcohols (5 to 13 %). Strain NCMB307 showed a higher degree of unsaturation in these compounds (34 and 30 %, respectively), but even this strain showed a closer similarity to the 'false neisseriae'/*Moraxella* group than to *Acinetobacter* (Table 4).

Several species-specific features were evident (Table 2, Fig. 1). A comparatively simple wax pattern was found in *N. catarrhalis*, *N. ovis*, *M. nonliquefaciens* and *M. bovis*, characterized by small amounts (less than 1 %) of di-unsaturated waxes and of waxes with odd

Table 4. *Wax alcohol patterns of Neisseriaceae*

Values recorded are the ranges of relative frequencies (% w/w), based on the data of Table 3. *Moraxella phenylpyrouvica* has been omitted. Alcohols are designated as in Table 2.

Alcohol	'False neisseriae'/ <i>Moraxella</i>	<i>Acinetobacter</i>	Psychrophilic group
16:1	0-3	9-50	0-2
16:0	4-30	2-25	4-20
17:0	0-14	0-1	3-11
18:1	0.1-4	16-61	4-13
18:0	44-89	2-24	31-78
19:0	0-3	0	4-6
Total unsaturated	0.1-16	56-95	5-30
Total with odd number of C	0-19	0-2	10-18
16:1 + 16:0	4-34	27-51	4-20
18:1 + 18:0	46-89	29-64	44-81

numbers of carbon atoms. The most common wax esters ($C_{36:1}$, $C_{36:0}$, $C_{34:1}$ and $C_{34:0}$) predominated in these species and waxes with chain lengths above C_{38} did not occur. The wax distribution differed somewhat in *M. osloensis* and *M. atlantae* in which C_{40} to C_{44} esters were found, together with a higher level (2 to 12 %) of waxes with odd numbers of carbon atoms and a concomitant high level (2 to 14 %) of alcohols with odd numbers of carbon atoms; long-chain alcohols (C_{20} or above) were also abundant. Wax patterns of *N. caviae* and, in particular, *M. lacunata* were more complex. The former had more di-unsaturated waxes (2.6 %) and waxes with odd numbers of carbon atoms (8.2 %) than were found in *N. catarrhalis* and similar species. *Moraxella lacunata* differed in its broad range of chain lengths (C_{26} to C_{40}), its abundance of waxes with odd numbers of carbon atoms (27.8 %) and the accompanying high level of alcohols with odd numbers of carbon atoms (19 %), especially C_{17} (14 %).

Di-unsaturated fatty acids or alcohols have not been found in any wax examined, although the fatty acid $C_{18:2}$ has been found in whole cells of most strains of Neisseriaceae (Jantzen *et al.*, 1974*b*, 1975).

DISCUSSION

Waxes occur in a variety of living organisms, e.g. plants (Kolattukudy, 1966), insects (Tulloch, 1971), birds (Bertelsen, 1973; Jacob, 1975) and mammals (Spencer & Tallent, 1973) including man (Haahti, 1961). This wide distribution apparently does not usually include bacteria, although several complex 'wax' compounds are found in the lipid-rich mycobacteria and in *Corynebacterium diphtheriae* (Asselineau, 1966; Goren, 1972). Similar compounds have been reported in *Nocardia* (Lederer, 1967), but true waxes have only recently been demonstrated in bacteria. Although *Corynebacterium* spp. grown on hydrocarbon are known to accumulate waxes (Bacchin *et al.*, 1974), only *Acinetobacter* has been reported to possess true waxes after growth on conventional bacteriological media. Gallagher (1971) identified a wax fraction obtained from three acinetobacters and saponification gave alcohols which were mostly unsaturated (74 to 93 %). Similar results were reported by Russell (1974) for a Gram-variable strain with some resemblance to *Acinetobacter*. Our results on the composition of waxes in *Acinetobacter* agree well with these earlier reports, though only five out of the 10 strains examined possessed wax esters.

In addition to the occurrence of wax esters in the oxidase-negative *Acinetobacter*, we have now established that waxes also occur in oxidase-positive species of Neisseriaceae. This is taxonomically interesting as it may indicate some relation between the two groups, corresponding to genetic findings (Bøvre, 1967). In spite of this similarity, however, wax patterns differed markedly between *Acinetobacter* and the oxidase-positive taxa of Neisseriaceae, as

high levels of di-unsaturated wax esters appeared to be a characteristic feature of wax from the oxidase-negative organisms. This chemotaxonomic difference is in accordance with earlier findings based on genetic relationships (Bøvre *et al.*, 1972, 1977) and fatty acid analysis (Jantzen *et al.*, 1975).

The lack of waxes in 'true neisseriae', which distinguishes this group from 'false neisseriae' and *Moraxella*, and the similarity in wax patterns between the two latter taxa, are also in good agreement with genetic and phenotypic affinities (Henriksen & Bøvre, 1968*b*; Jantzen *et al.*, 1974*b*). The presence of wax esters in the oxidase-positive psychrophilic group, and the fact that the wax pattern of this taxon is clearly closer to that of *Moraxella* than to *Acinetobacter*, indicates a taxonomic proximity between the two former groups, in keeping with fatty acid analysis and genetic data (unpublished results).

Two *Moraxella* species differ from the others with respect to wax content (Table 1). *Moraxella phenylpyrouvica* seems to be devoid of wax esters since only marginal amounts could be detected in one of the three strains examined. This observation is supported by an independent study on whole-cell fatty acid and alcohol composition of 11 strains of *M. phenylpyrouvica* (Bøvre *et al.*, 1976) in which no alcohols could be recognized. In *M. osloensis*, small amounts of waxes may be present. In a survey of 27 new clinical isolates of *M. osloensis* (Bøvre *et al.*, 1977) C_{18:0} alcohol was detected in the lipids of all strains, but in the same study, no wax alcohols were detected in the type strain A1920.

'*Moraxella urethralis*' and the genus *Kingella* differ from *Moraxella* in that they lack waxes, a result in accordance with earlier data (Jantzen *et al.*, 1974*b*; Lautrop *et al.*, 1970; Bøvre *et al.*, 1974*a*).

As strain MJT/F5/199A contained less than 1 % of di-unsaturated wax and less than 5 % unsaturated alcohols, it should not be regarded as an *Acinetobacter*, in keeping with its oxidase-positive character. However, Thorne, Thornley & Glauert (1973) have studied this strain and reported that only unsaturated wax alcohols were present. This discrepancy may have originated from differences in cultural conditions, which are known to influence the content and composition of bacterial lipids (Vácz, 1973), though preliminary data indicate that wax patterns are grossly conserved when bacteria are grown on other media, including blood agar.

An effect of growth temperature on wax composition has been observed. When *Acinetobacter* strain 8 was grown at 33 and 37 °C, and the psychrophilic strain A348 at 22 and 33 °C, the wax contents were, respectively, two- and threefold greater at the higher temperature. Furthermore, the proportions of di-unsaturated wax esters decreased slightly at the higher temperature for both strains. This was not unexpected since the degree of saturation of bacterial lipids often increases with the growth temperature (Vácz, 1973).

The apparent location of waxes in the outer membrane of strain MJT/F5/199A (Thorne *et al.*, 1973) seems to indicate that waxes, like other lipids, serve as structural components of bacterial membranes. Further investigations are necessary to verify this, and to explore the extent to which waxes occur in bacteria. The presence of fatty alcohols in the Gram-negative *Serratia marcescens* (Kates, Adams & Martin, 1964) and *Escherichia coli* (Naccarato *et al.*, 1972, 1974) and in the Gram-positive *Bacillus cereus* (Kates, Kushner & James, 1962), indicates that true waxes may be more widely distributed among bacteria than has previously been recognized.

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