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Similarities Between *Hyphomicrobium* and *Nitrobacter* with Respect to Fatty Acids

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Vaccenic acid (11-18:1) accounted for 92% of the fatty acids in the extractable lipids of log-phase *Nitrobacter* and *Hyphomicrobium*. During the stationary phase, both genera formed a 19-carbon cyclopropane fatty acid which increased in proportion to a decrease in the amount of vaccenic acid.

Microorganisms generally have several different fatty acids in their lipids. However, two instances have been reported which show that certain photosynthetic bacteria synthesize lipids in which one fatty acid accounts for 90% or greater of the fatty acid composition. Park and Berger (9) found that about 90% of the fatty acids in *Rhodomicrobium vannielii* is vaccenic acid (11-18:1). Some strains of *Rhodopseudomonas* (11) make lipids containing this same or higher percentage of vaccenic acid.

The fatty acid composition of the nonphotosynthetic counterpart of *Rhodomicrobium*, namely *Hyphomicrobium* NQ521, was investigated along with the autotrophic nitrifying organisms *Nitrobacter agilis* (ATCC 14123) and *N. winogradskyi*. *Nitrobacter* were grown in completely inorganic medium containing 20 mM nitrite as the energy source. Nitrite consumption was used as an index of growth (10). *Hyphomicrobium* NQ521 was grown in a minimal salts medium with 0.5% methanol added as the source of carbon. Growth of *Hyphomicrobium* was measured using a combination of turbidity and protein determinations (7). All cultures were batch cultures shaken at 30 C.

Table 1 shows the fatty acid composition of the chloroform-methanol-extractable (3) lipids of two species of *Nitrobacter* and of *Hyphomicrobium* NQ521 growing in the log phase. The data are strikingly similar both qualitatively and quantitatively. The spectrum of fatty acids was very restricted; the two *Nitrobacter* species were identical and *Hyphomicrobium* differed only slightly from *Nitrobacter*. Of particular interest was the predominance in both genera of the component identified as octadecenoic acid (18:1), and this component was characterized further. The octadecenoic acid from all species had the same retention time on gas-liquid chromatography, and catalytic hydrogenation converted it to stearic acid (18:0). Chemical oxidation with permanganate and periodate (6) and reductive ozonolysis of the octadecenoic acid established its structure as vaccenic acid (11-18:1).

It was observed that both genera formed an additional lipid component as they entered the stationary phase of growth. The new component had a retention time identical to that of a 19-carbon cyclopropane fatty acid. This tentative identification was corroborated by bromination (2). Figure 1 demonstrates the variations in fatty acid composition that accompany the aging of the culture. As *Nitrobacter* was grown in these experiments, the specific source of energy, nitrite, was consumed completely after 5 to 6 days of growth, resulting in the onset of the stationary phase. The 16:0, 16:1, and 18:0 fatty acids remained constant throughout the stationary phase at the same levels as reported in Table 1 for log-phase cells. The proportion of vaccenic acid, however, dropped progressively from 92 to 56%. Concomitant with the initial decrease in 18:1 in early stationary growth was the appearance of the 19-carbon cyclopropane component, which then increased at almost exactly the same rate as the 18:1 decreased. The data indicate clearly that a 19-carbon cyclopropane component was formed by *N. agilis* during the stationary growth phase at the expense of part of its 18:1 component. *Hyphomicrobium* and *N. winogradskyi* also make the cyclopropane fatty acid but they both make less of it and at a slower rate than does *N. agilis*. These results suggest that the 19:1 component reported by Blumer et al. (1) as one of the two major fatty acids of *N. agilis* probably was a cyclopropane

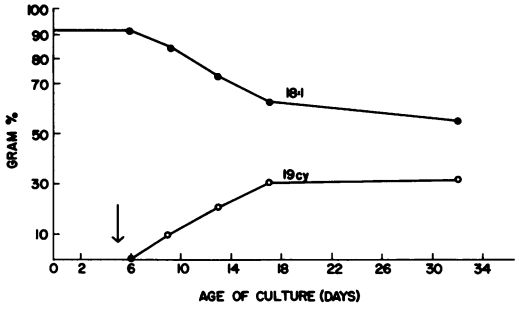

FIG. 1. *Variation in the fatty acid composition of N. agilis during the growth cycle. Arrow indicates nitrite depletion. Symbols: octadecenoic acid, ●; 19-carbon cyclopropane acid, ○.*

TABLE 1. *Per cent fatty acid composition of the chloroform-methanol-extractable lipids in log-phase cells of Nitrobacter and Hyphomicrobium*

Organism	Per cent fatty acid					
	16:0	16:1	17:0	18:0	18:1	19:0
Nitrobacter agilis	6	<1	0	<1	92	0
N. winogradskyi	6	<1	0	<1	92	0
Hyphomicrobium NQ521	2	TR[a]	<1	5	92	TR

[a] Trace present.

fatty acid.

Preliminary results with several *Nitrobacter* isolates from Minnesota soils show this same high percentage of octadecenoic acid in their extractable lipids. We have also found that *Nitrobacter* contains phosphatidyl choline as do several photosynthetic bacteria (5, 8) and *Hyphomicrobium* NQ521 (4).

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