Short communication

The use of the analytical profile index in the identification of activated sludge bacteria : Problems and solutions

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Abstract

The accuracy of activated sludge isolate identification by the analytical profile index (API) 20E system was evaluated relative to conventional biochemical tests. It was found that the API test gives reliable identification provided inoculi are in the logarithmic growth phase, fermentation tests are checked 24 h after the first reading, and a standard oxidase test is performed as a control to that of the API system. Failure to take such precautions may give inappropriately high *Acinetobacter* counts for activated sludge.

Introduction

Bacterial enumeration by way of counting colony forming units (CFU) using selective growth media, is at present one of the methods used to determine the bacterial population structure of activated sludge (Buchan, 1983; Lötter and Murphy, 1985).

This method may not provide a true representation of the population composition, as the disruption of the sludge flocs by sonication may not yield single viable cells uniformly and selective pressures are never completely absent in microbial growth media. In some aquatic ecosystems results from this method could represent between 1% and 10% (or less) of the cell number enumerated by direct microscopic counts (Cloete and Steyn, 1988). Despite this problem, isolates obtained by this method are commonly used as an indication of the groups of microorganisms present in the system.

Previous researchers have found that *Acinetobacter* often represents a large portion of the bacteria from the aerobic zone of biological nutrient removal plants, when enumerated by this method. For example, Hart and Melmed (1982) estimated *Acinetobacter* at 56% to 66% of the total population, Buchan (1983) reported 48% to 66%, Lötter (1985) 56% to 66%, Lötter and Murphy (1985) ca. 60% to 70% and Kerdachi and Healey (1987) 73%. The samples in these reports were taken from plants receiving settled municipal waste water. Lötter *et al.* (1986) reported 90% *Acinetobacter* in sludges sampled from a laboratory-scale Bardenpho system receiving acetate as the sole carbon source.

In each of the above-mentioned cases, the analytical profile index (API) 20E identification system (Analytlab Products, 1977) was used to identify the bacteria. The accuracy of identification by the API 20E system was apparently not examined.

The aim of this study was to evaluate the accuracy of isolate identification by the API 20E system. To achieve this, isolates were identified by the API 20E system and conventional biochemical tests.

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Materials and methods

Activated sludge sample

Mixed liquor samples were taken from the primary aerobic zones of the modified Bardenpho plants at Northern Works and Goudkoppies (Johannesburg) and chilled during transit. Analysis commenced immediately upon arrival at the laboratory.

Isolation of bacteria

The activated sludge samples were prepared and diluted as described by Lötter and Murphy (1985). A sample (0,1mℓ) of each dilution was plated on GCY agar (Pike *et al.*, 1972). After aerobic incubation for 5 d at 20°C, plates containing approximately 100 colonies (10^{-6} or 10^{-7} dilution) were used to isolate colonies for identification. The colonies selected for identification were replated on GCY agar. After incubation at 35°C for two days, these cultures were subjected to the identification tests.

Identification of bacteria

Initially, the cultures were Gram stained. Gram positive cultures were counted and discarded. Yeasts and moulds were also counted and discarded. The Gram negative bacteria were examined for colony morphology and pigmentation.

The Gram negative organisms were subjected to the following conventional biochemical tests in tubes : firstly, the Hugh-Leifson test for oxidative/fermentative metabolism (Cruickshank *et al.*, 1975) with glucose or sucrose as carbon source; secondly, oxidative isolates were tested for catalase, oxidase and β-galactosidase (ONPG broth) reaction (Cruickshank *et al.*, 1975). These isolates were also tested for motility in 0,4% nutrient agar and for the presence of flagella according to the staining technique decribed by Mayfield and Inniss (1977).

In addition to the above tests, API 20E strips were inoculated and the tests performed and recorded according to the instructions of the manufacturer.

TABLE 1
COMPARISON BETWEEN THE IDENTIFICATION OF ISOLATES FROM GOUDKOPPIES MIXED LIQUOR USING API 20E SYSTEM OR CONVENTIONAL TESTING OF BIOCHEMICAL PROPERTIES

Identification	Number of colonies expressed as a percentage (%) of total colonies	
	API 20E test	Conventional test
Gram positive	14	14
Gram negative	86	86
- Facultatively anaerobic	19	28
- Strictly aerobic	67	59
- *Pseudomonas*	15	21
- *Moraxella*	4	12
- *Acinetobacter*	18	6
- *Flavobacterium*	4	1
- *Alcaligenes*	22	18
- *Achromobacter*	4	1

TABLE 2
COMPARISON OF MICROBIAL POPULATIONS OF SLUDGE SAMPLES FROM NORTHERN WORKS AND GOUDKOPPIES USING API 20E SYSTEM OR CONVENTIONAL TESTING OF BIOCHEMICAL PROPERTIES

Identification	Number of colonies expressed as a percentage (%) of total colonies		
	Northern Works (Conventional)	Goudkoppies (Conventional)	Goudkoppies (API 20E)
Gram positive	11	12	11
Gram negative	89	82	89
- Facultatively anaerobic	36	42	48
- Strictly aerobic	53	40	41
- *Pseudomonas*	21	16	14
- *Moraxella*	18	6	6
- *Acinetobacter*	5	10	11
- *Flavobacterium*	2	4	5
- *Branhamella*	5	0	0
- *Alcaligenes*	2	4	5
Yeasts and moulds	0	6	0
Total count on GCY	$2,3 \times 10^7$	$5,1 \times 10^6$	$5,6 \times 10^6$

Results and discussion

Comparing the two identification methods, it is clear that significant differences exist in the numbers of facultatively anaerobic and strictly aerobic Gram negative bacteria as well as in the relative counts for *Pseudomonas, Moraxella, Acinetobacter* and *Alcaligenes* (Table 1).

The difference in numbers of the facultatively anaerobic and strictly aerobic Gram negative bacteria was mainly attributable to the Hugh-Leifson test. Although the API system uses glucose as fermentable carbohydrate, sucrose was used in the test tube in this instance. Contrary to what might be expected on the basis of the carbohydrate alone (Lawson and Tonhazy, 1980), the number of fermentative isolates was less for the API system.

This could be as a result of the small inoculation used in the API 20E test and results should be checked 24 h after the first reading to allow for any delay in the fermentation reaction. Care should also be taken that cultures inoculated in the API tubes are in the logarithmic phase.

The differences between *Pseudomonas, Moraxella* and *Acinetobacter* can be attributed to inaccuracy in the oxidase test as performed when using the API system. Certain isolates were identified as *Acinetobacter* (which is oxidase negative) whilst rendering a positive oxidase result on agar plates. This probably indicates that the amount of growth used for the oxidase test in the API system is too small to give accurate results.

Taking the above-mentioned precautions into account, the API 20E system was used to identify isolates in a second sample from Goudkoppies. In this instance the oxidase test was performed separately using growth on agar plates. These results compared well with previous identification studies of samples from Northern Works and Goudkoppies performed using conventional biochemical tests (Table 2).

Conclusions

The results of this study show that the API 20E test may be used to give reliable identification, providing the following precautions are taken:

- Fermentation tests must be checked 24 h after the first reading and inoculi must be in the logarithmic growth phase.
- The API oxidase test should be supplemented by a standard oxidase test (Cruickshank *et al.*, 1975)

It is clear that for the samples considered, *Acinetobacter* did not constitute as high a percentage of the culturable microorganisms in activated sludges from biological nutrient removal plants as that reported elsewhere *(inter alia* Buchan, 1983; Lötter and Murphy, 1985). Since the API 20E system has been widely used in the past, it is possible that inappropriately high *Acinetobacter* percentages could have arisen due to the unreliable API oxidase test.

In the light of this study and other reports (*inter alia* Brodisch and Joyner, 1983; Kerdachi and Roberts, 1985; Lötter and Murphy, 1985) of the occurence of *Pseudomonas, Moraxella* and *Alcaligenes* in activated sludge samples from biological phosphorus removal plants, designation of *Acinetobacter* as the genus principally responsible for P release and P removal (Wentzel *et al.*, 1986) may not have been fully justified. *Pseudomonas, Moraxella* and *Alcaligenes* could also play a major role. Since these genera have the metabolic features *(Bergey's Manual of Systematic Bacteriology,* Volume 1, 1984) required by the model of Wentzel *et al.* (1986), the model need not be applied exclusively to *Acinetobacter.*

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Wetzel, RG (1975) *Limnology*. WB Saunders Company, Philadelphia. 324.