The Saccharolytic Activity of *Acinetobacter lwoffii* and *A. anitratus*

By A. HENDERSON

Research Department, Glasgow Royal Maternity Hospital,
Glasgow, Scotland

(Accepted for publication 26 June 1967)

SUMMARY

The activity of 75 strains of *Acinetobacter* (16 strains of *A. lwoffii* and 59 strains of *A. anitratus*) against carbohydrates is described. It is concluded that no firm distinction can be made between these species which represent extreme ends of a group of organisms possessing widespread but variable activity.

INTRODUCTION

This paper deals with the saccharolytic properties of *Acinetobacter lwoffii* and *A. anitratus*.

METHODS

Bacteria used. These were 75 strains of the genus *Acinetobacter*, 15 strains from type culture collections and 60 wild strains. Their sources and designations were as follows. *A. lwoffii*: *Mima polymorpha* ATCC9957; *Moraxella lwoffii* Inst. Pasteur 5382, 53116, A162; *A. lwoffii* 12 wild strains.

A. anitratus: *Acinetobacter anitratus* NCTC7250, 7412, 7422, 7461, 7844, 9427, 10292; *Moraxella glucidolytica* Inst. Pasteur 5367, 5497; *Neisseria winogradskyi* Inst. Pasteur Paris; *Acinetobacter anitratus* 49 wild strains.

The wild strains were allotted to the species *A. anitratus* or *A. lwoffii* on the basis of acid production or failure to produce acid from any carbohydrate in peptone water.

Acid production in carbohydrates was detected by two methods. Method A, the carbohydrates were used at 1 % (w/v) in peptone water; the indicator was acid fuchsin. Inoculation was from an 18 hr peptone water culture. Method B, the carbohydrates were used at 1 % (w/v) in the medium of Smith, Gordon & Clark (1952) without agar; the indicator was bromocresol purple. Inoculation was from an 18 hr culture on nutrient agar.

The utilization of carbohydrates as sole carbon source was detected in the mineral salt medium of Chan (1964). The carbohydrates were used at 0.1 % (w/v); the nitrogen requirements were satisfied by the potassium nitrate. Inoculation was from a nutrient agar colony emulsified in autoclaved tap water (method C).

Reduction of nitrate to nitrite was tested in the medium of Jyssum & Joner (1965), by using the reagents of McLean & Henderson (1966).

In all cases, cultures were incubated at 37° for 7 days.

RESULTS

The bacteria received as *Mima polymorpha*, *Moraxella lwoffii* or the wild strains regarded as *Acinetobacter lwoffii* (16 all told) did not produce acid from any carbohydrate when tested by method A (6 of 6 strains) or B (5 of 5 strains) and did not utilize carbohydrate by method C (9 of 9 strains).

The bacteria (59 strains) received as *Acinetobacter anitratus*, *Moraxella glucidolytica*, *Neisseria winogradskyi* or the wild strains regarded as *A. anitratus* produced acid readily from pentoses and hexoses, but not from higher carbohydrates, alcohols or glycosides when tested by method A (59 strains). When tested by method B (29 strains) a wider spectrum of activity was noted, extending through the disaccharides to the trisaccharides which were split by 2 strains. When tested by method C (28 strains), 8 strains utilized arabinose and xylose, 4 utilized arabinose but not xylose, and the remainder did not utilize any carbohydrate tested. These results are summarized in Table 1.

Table 1. *Acinetobacter* strains: acid production from carbohydrates (methods A and B) or utilization of carbohydrates (method C)

	Method A		Method B		Method C	
	<i>A. lwoffii</i> 16	<i>A. anitratus</i> 59	<i>A. lwoffii</i> 5	<i>A. anitratus</i> 29	<i>A. lwoffii</i> 9	<i>A. anitratus</i> 28
Number tested ...						
Acid production from or utilization of						
Pentoses						
Arabinose	0	45	0	27	0	14
Rhamnose	0	3	0	2	0	0
Xylose	0	49	0	28	0	10
Hexoses						
Fructose	0	3	0	17	0	0
Galactose	0	21	0	28	0	0
Glucose	0	45	0	28	0	0
Mannose	0	19	0	28	0	0
Sorbitose	0	0	0	11	0	0
Disaccharides						
Cellobiose	0	0	0	23	0	0
Lactose	0	0	0	14	0	0
Maltose	0	0	0	0	0	0
Melibiose	0	0	0	10	0	0
Sucrose	0	0	0	2	0	0
Trehalose	0	0	0	0	0	0
Trisaccharides						
Melizitose	0	0	0	0	0	0
Raffinose	0	0	0	2	0	0

All the 34 strains tested, including 5 of *Acinetobacter lwoffii* and 29 of *A. anitratus*, produced urease. Of the same 34 strains grown in the medium of Jyssum & Joner (1965), 5 strains of *A. anitratus* but no strain of *A. lwoffii* reduced nitrate to nitrite.

DISCUSSION

Acinetobacter lwoffii is usually recognized by its inactivity towards carbohydrates in contrast to *A. anitratus* which attacks them. At the outset of this work it was thought

that the apparent inactivity of *A. lwoffii* might be due to neutralization of acid produced from the carbohydrate by ammonia produced from the peptone when the organisms were grown in peptone water sugars. Elimination of the peptone failed in my hands to reveal saccharolysis by *A. lwoffii* but allowed the *A. anitratus* group to show a wider range of activity. On the other hand, 14 of 28 strains of *A. anitratus* did not utilize carbohydrate as sole carbon source for growth, so resembling the *A. lwoffii* strains. Also both groups readily utilized 2,3-butanediol as carbon source for their isolation from earth, and it is now known that both species produce urease uniformly (Henderson, 1967) when tested by the method of Elek (1946).

A difficulty in dividing the species has been the hitherto apparent inactivity of the organisms on the substrates usually employed; however, Fewson (1967) recorded the activities of one strain on 150 of 450 compounds. Mitchell & Burrell (1964) suggested that although the species within the 'Mima-Herellea' group may be distinguishable, they are closely linked serologically. The investigations of other workers have led to the conclusion that the large number of species hitherto recognized can be reduced to two (review by Henderson, 1965). Pintér & Bende (1967) made an Adansonian analysis of 31 strains of *Acinetobacter lwoffii* and 8 of *A. anitratus*, and while affirming the validity of both species did not find a sharp distinction between them. The results of the present work lead the author to the conclusion that no firm distinction can be made between the two species and that they represent only extreme ends of a group exhibiting widespread but variable activity.

The author thanks Dr H. Girard (Institut Pasteur, Paris) for the strain of *Neisseria winogradskyi*.

REFERENCES

- CHAN, E. C. S. (1964). Morphological aberration of *Arthrobacter globiformis* cells due to biotin deficiency. *J. Bact.* **87**, 641.
- ELEK, S. D. (1946). Rapid identification of *Proteus*. *J. Path. Bact.* **60**, 183.
- FEWSON, C. A. (1967). The growth and metabolic versatility of the Gram-negative bacterium NCIB 8250 (*Vibrio* O 1). *J. gen. Microbiol.* **46**, 255.
- HENDERSON, A. (1965). The *Moraxella lwoffii* group of bacteria; a review. *Antonie van Leeuwenhoek J. Microbiol. Serol.* **31**, 395.
- HENDERSON, A. (1967). Urease activity of *Acinetobacter lwoffii* and *A. anitratus*. *J. gen. Microbiol.* **46**, 399.
- JYSSUM, K. & JONER, P. E. (1965). Growth of *Bacterium anitratum* (B5W) with nitrate or nitrite as nitrogen source. *Acta path. microbiol. scand.* **64**, 381.
- MCLEAN, J. & HENDERSON, A. (1966). Test for the presence of nitrate not involving carcinogenic reagents. *J. clin. Path.* **19**, 632.
- MITCHELL, P. D. & BURRELL, R. G. (1964). Serology of the Mima-Herellea group and the genus *Moraxella*. *J. Bact.* **87**, 900.
- PINTÉR, M. & BENDE, I. (1967). Computer analysis of *Acinetobacter lwoffii* (*Moraxella lwoffii*) and *Acinetobacter anitratus* (*Moraxella glucidolytica*) strains. *J. gen. Microbiol.* **46**, 267.
- SMITH, N. R., GORDON, R. E. & CLARK, F. E. (1952). Aerobic sporeforming bacteria. *Agric. Monogr. U.S. Dep. Agric.* no. 16.