The ortho-nitrophenol (ONPG) test and acid from lactose in Gram-negative genera

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SYNOPSIS The results are given of the ortho-nitrophenol test for beta-galactosidase production (ONPG test) on 588 strains of 123 aerobic species of bacteria, representing 30 genera. Apart from some strains of *Erwinia herbicola* (synonym *Chromobacterium typhiflavum*) and of *Yersinia* spp, these strains were not members of the Enterobacteriaceae, in which family the ONPG test is widely used and well documented. The strains were also tested for acid production from 1, 5, and 10% lactose and the findings are discussed in relation to the ONPG test.

The ortho-nitrophenol test for beta-galactosidase production (ONPG test) has been in widespread use since the value of the test was first reported by Le Minor and Ben Hamida (1962). In lactose-fermenting bacteria the breakdown of lactose to glucose and galactose involves the enzyme beta-galactosidase, but some organisms may give a positive result in the ONPG test yet fail to produce enough acid from lactose to change the indicator under the test conditions. This may be due to lack of permease to allow sufficient lactose to enter the cells, though an adequate number of molecules of beta-galactosidase may diffuse out of the cells to produce the yellow ortho-nitrophenol from the colourless ortho-nitrophenyl-beta-D-galactopyranoside, used as a substrate in the ONPG test. The ONPG test is a much more sensitive test than conventional acid from lactose tests. Moreover, other species can oxidize lactose to lactobionic acid, eg, *Acinetobacter anitratus* and several pseudomonads (Stodola and Lockwood, 1947; Villecourt and Blachère, 1955; Miller and Von Riesen, 1968). Lactobionic acid production does not involve beta-galactosidase and thus such species are ONPG negative and acid from lactose positive.

Most investigations of the ONPG test and acid from lactose tests have been concerned chiefly with Enterobacteriaceae (Szturm-Rubinsten and Piéchaud, 1962, 1963; Lapage and Jayaraman, 1964;

Lubin and Ewing, 1964; Giammanco, 1966; Costin 1966). Some data for both tests for non-enterobacterial species, mainly pseudomonads, have been published by Le Minor and Ben Hamida (1962) and Bülow (1964), while Tacquet, Tison, Polspoel, Roos, and Devulder (1966) found the occasional ONPG positive strain among some species of *Mycobacterium*. In the present work, the range of non-enterobacterial, Gram-negative, aerobic organisms studied in both tests has been extended to 30 genera representing 123 named species and varieties.

Materials and Methods

ORGANISMS

A total of 588 strains have been examined. All, except 30, were NCTC strains and have been routinely checked and characterized by a wide range of tests over the years in the National Collection of Type Cultures. The additional 30 strains were newly isolated strains sent to the NCTC for identification: 10 strains of *Eikenella* (synonym *Bacteroides*) *corrodens* (Hill, Snell, and Lapage, 1970), 10 strains of *Pseudomonas cepacia* (Snell, Hill, and Lapage, 1972), and 10 strains of *P. stutzeri* (Lapage, Hill, and Reeve, 1968).

The strains examined are listed by species names in table I.

ONPG TEST

This was carried out by the method of Lowe (1962, method 2, without benzene or toluene treatment). Results were read up to five days' incubation at optimum temperature, usually 37°C.

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Species	Number of Strains	O-F Reaction Patterns				Positive Acid from Lactose Tests			Positive in ONPG Test	
		Tested	Ferm	Ox	Alk	Nil ¹	1%	5%		10%
<i>Achromobacter citroalcaligenes</i>	1	—	1	—	—	0	1	1	0	
<i>Achromobacter conjunctivae</i>	1	—	1	—	—	0	0	1	0	
<i>Achromobacter haemolyticus</i> var. <i>haemolyticus</i>	1	—	1	—	—	0	1	1	0	
<i>Achromobacter haemolyticus</i> var. <i>alcaligenes</i>	1	—	—	—	1	0	0	NG ²	0	
<i>Achromobacter metalcaligenes</i>	2	—	1	—	1	0	0	1	0	
<i>Achromobacter mucosus</i>	1	—	1	—	—	0	1	1	0	
<i>Achromobacter xylosoxidans</i>	3	—	—	3	—	0	0	NG	0	
<i>Acinetobacter anitratus</i>	18	—	18	—	—	0	15	18	0	
<i>Acinetobacter lwoffii</i>	6	—	5	1	—	0	0	NG	0	
<i>Actinobacillus actinomycetemcomitans</i>	2	—	2	—	—	0	0	0	0	
<i>Actinobacillus equuli</i>	7	7	—	—	—	7	7	0	7	
<i>Actinobacillus lignieresii</i>	6	5	—	—	1	6	6	NG	6	
<i>Actinobacillus</i> spp.	4	4	—	—	—	2	2	NG	4	
<i>Actinobacillus suis</i>	1	1	—	—	—	1	1	NG	1	
<i>Aeromonas formicans</i>	4	4	—	—	—	2	3	0	4	
<i>Aeromonas liquefaciens</i>	4	4	—	—	—	0	0	NG	4	
<i>Aeromonas salmonicida</i>	3	3	—	—	—	1	1	1	3	
<i>Aeromonas shigelloides</i>	3	3	—	—	—	2	2	NG	2	
<i>Alcaligenes denitrificans</i>	1	—	—	1	—	0	0	0	0	
<i>Alcaligenes faecalis</i>	6	—	—	—	6	0	0	NG	0	
<i>Alcaligenes odorans</i>	2	—	—	2	—	0	0	NG	0	
<i>Alcaligenes viscosus</i>	1	—	—	1	—	0	0	0	0	
<i>Alysiella filiformis</i>	1	1	—	—	—	0	0	NG	0	
<i>Bordetella bronchiseptica</i>	11	—	—	6	5	0	0	NG	0	
<i>Bordetella parapertussis</i>	9	—	—	9	—	0	0	NG	0	
<i>Bordetella pertussis</i>	8	—	—	—	8	0	0	NG	0	
<i>Brucella abortus</i>	20	—	—	—	20	0	0	NG	0	
<i>Brucella melitensis</i>	11	—	—	2	9	0	0	NG	0	
<i>Brucella neotomae</i>	4	—	2	—	2	0	0	NG	0	
<i>Brucella ovis</i>	2	—	—	—	2	0	0	NG	0	
<i>Brucella suis</i>	11	—	—	—	11	0	0	NG	0	
<i>Campylobacter bubulus</i>	1	—	—	—	1	0	0	NG	0	
<i>Campylobacter fetus</i>	4	1	—	1	2	1	1	NG	1	
<i>Cardiobacterium hominis</i>	3	3	—	—	—	0	0	NG	0	
<i>Chromobacterium amethystinum</i>	1	—	—	—	1	0	0	NG	0	
<i>Chromobacterium lividum</i>	6	—	6	—	—	0	1	NG	4	
<i>Comamonas percolans</i>	3	—	—	—	3	0	0	NG	0	
<i>Cytophaga</i> sp.	1	—	—	—	1	0	0	NG	0	
<i>Eikenella corrodens</i>	12	—	—	—	12	0	0	NG	0	
<i>Erwinia herbicola</i>	6	6	—	—	—	2	2	5	6	
<i>Flavobacterium meningosepticum</i>	8	—	8	—	—	2	3	0	7	
<i>Gemella haemolysans</i>	3	—	1	—	2	1	1	NG	1	
<i>Haemophilus aphrophilus</i>	4	3	—	—	1	2	3	NG	3	
<i>Haemophilus canis</i>	2	—	—	—	2	0	1	1	1	
<i>Haemophilus gallinarum</i>	1	—	—	—	1	0	0	NG	0	
<i>Haemophilus haemolyticus</i>	2	—	—	—	2	1	1	NG	1	
<i>Haemophilus influenzae</i>	15	—	—	—	15	0	0	NG	0	
<i>Haemophilus parahaemolyticus</i>	1	—	—	—	1	0	0	NG	0	
<i>Haemophilus parainfluenzae</i>	3	—	—	—	3	0	0	NG	0	
<i>Haemophilus paraphrohaemolyticus</i>	3	—	—	—	3	0	0	NG	0	
<i>Haemophilus paraphrophilus</i>	3	—	—	—	3	0	0	NG	3	
<i>Haemophilus suis</i>	4	—	—	—	4	0	0	NG	0	
<i>Haemophilus vaginalis</i>	1	—	—	—	1	1	1	1	1	
<i>Haemophilus</i> sp.	1	1	—	—	—	0	0	NG	0	
<i>Moraxella bovis</i>	3	—	—	—	3	0	0	NG	0	
<i>Moraxella kingii</i>	3	2	—	—	1	0	0	NG	0	
<i>Moraxella lacunata</i>	4	—	—	—	4	0	0	NG	0	
<i>Moraxella liquefaciens</i>	3	—	—	—	3	0	0	NG	0	
<i>Moraxella nonliquefaciens</i>	2	—	—	—	2	0	0	NG	0	
<i>Moraxella osloensis</i>	4	—	—	4	—	0	0	NG	0	
<i>Moraxella phenylpyruvica</i>	3	—	—	3	—	0	0	NG	0	
<i>Moraxella</i> sp.	1	—	—	—	1	0	0	NG	0	
<i>Moraxella saccharolytica</i>	1	1	—	—	—	0	0	NG	0	
<i>Neisseria animalis</i>	1	—	—	—	1	0	0	NG	0	
<i>Neisseria canis</i>	1	—	—	—	1	0	0	NG	0	
<i>Neisseria catarrhalis</i>	4	—	—	2	2	0	0	NG	0	
<i>Neisseria caviae</i>	1	—	—	—	1	0	0	NG	0	

Table I Results of the O-F, acid from lactose, and ONPG tests

¹Ferm = fermentative; Ox = oxidative; Alk = alkali-producing; nil = no reaction. ²NG = no growth.

Species	Number of Strains								
	Tested	O-F Reaction Patterns				Positive Acid from Lactose Tests			Positive in ONPG Test
		Ferm	Ox	Alk	Nil ¹	1%	5%	10%	
<i>Neisseria cinerea</i>	1	—	—	—	1	0	0	NG ^a	0
<i>Neisseria cuniculi</i>	1	—	—	—	1	0	0	NG	0
<i>Neisseria denitrificans</i>	1	1	—	—	—	0	0	NG	0
<i>Neisseria elongata</i>	1	—	—	—	1	0	0	NG	0
<i>Neisseria flavescens</i>	3	—	—	—	3	0	0	NG	0
<i>Neisseria gonorrhoeae</i>	11	—	—	—	11	0	0	NG	0
<i>Neisseria lactamicus</i>	3	—	2	1	—	1	3	NG	3
<i>Neisseria meningitidis</i>	10	—	3	—	7	0	0	NG	0
<i>Neisseria mucosa</i>	3	3	—	—	—	0	0	NG	0
<i>Neisseria pharyngis</i>	1	—	—	—	1	0	0	NG	0
<i>Pasteurella bettii</i>	3	3	—	—	—	0	0	NG	0
<i>Pasteurella haemolytica</i>	10	10	—	—	—	2	2	NG	2
<i>Pasteurella haemolytica</i> var. <i>ureae</i>	9	8	—	—	1	0	0	NG	0
<i>Pasteurella pneumotropica</i>	10	10	—	—	—	0	0	NG	4
<i>Pasteurella multocida</i>	32	32	—	—	—	3	3	NG	3
<i>Pasteurella</i> spp.	10	10	—	—	—	0	0	NG	2
<i>Plesiomonas shigelloides</i>	6	6	—	—	—	5	5	0	5
<i>Pseudomonas acidovorans</i>	2	—	—	1	1	0	0	0	0
<i>Pseudomonas aeruginosa</i>	16	—	16	—	—	0	0	15	0
<i>Pseudomonas alcaligenes</i>	2	—	—	2	—	0	0	0	0
<i>Pseudomonas aminovorans</i>	1	—	1	—	—	0	0	0	1
<i>Pseudomonas atlantica</i>	1	—	—	1	—	0	0	0	1
<i>Pseudomonas aureofaciens</i>	1	—	1	—	—	0	0	1	0
<i>Pseudomonas cepacia</i>	14	—	14	—	—	0	3	10	3
<i>Pseudomonas chlororaphis</i>	1	—	1	—	—	0	0	0	0
<i>Pseudomonas cruciviae</i>	1	—	—	1	—	0	0	0	0
<i>Pseudomonas denitrificans</i>	1	—	1	—	—	0	0	NG	0
<i>Pseudomonas diminuta</i>	1	—	—	1	—	0	0	0	0
<i>Pseudomonas fluorescens</i>	4	—	4	—	—	0	0	4	0
<i>Pseudomonas fragi</i>	1	—	1	—	—	0	0	1	0
<i>Pseudomonas graveolens</i>	2	—	2	—	—	0	2	2	0
<i>Pseudomonas lemonnieri</i>	1	—	1	—	—	0	0	NG	0
<i>Pseudomonas mallei</i>	9	—	—	—	9	0	0	8	0
<i>Pseudomonas maltophilia</i>	7	—	7	—	—	0	0	0	0
<i>Pseudomonas mucidolens</i>	1	—	1	—	—	0	1	1	0
<i>Pseudomonas nigrifaciens</i>	1	—	1	—	—	0	0	0	1
<i>Pseudomonas oleovorans</i>	1	—	1	—	—	0	0	NG	0
<i>Pseudomonas ovalis</i>	2	—	2	—	—	0	0	2	0
<i>Pseudomonas pavonacea</i>	1	—	—	1	—	0	0	0	0
<i>Pseudomonas pseudomallei</i>	13	—	13	—	—	1	11	13	0
<i>Pseudomonas putrefaciens</i>	4	—	4	—	—	0	0	0	0
<i>Pseudomonas rubescens</i>	1	—	—	1	—	0	0	0	0
<i>Pseudomonas stutzeri</i>	13	—	13	—	—	0	0	2	0
<i>Pseudomonas syncyanea</i>	1	—	1	—	—	0	0	NG	0
<i>Pseudomonas synxantha</i>	1	—	1	—	—	0	0	1	0
<i>Pseudomonas testosteroni</i>	1	—	—	1	—	0	0	0	0
<i>Pseudomonas</i> spp. <i>achromogenic</i>	5	—	5	—	—	0	0	1	0
<i>Ramibacterium pseudoramosum</i>	1	—	—	—	1	0	0	NG	0
<i>Simonsiella crassa</i>	1	1	—	—	—	0	0	NG	0
<i>Spirillum serpens</i>	1	—	—	—	1	0	0	NG	0
<i>Streptobacillus moniliformis</i>	1	—	—	1	—	0	0	NG	0
<i>Vibrio alcaligenes</i>	3	—	—	—	3	0	0	0	1
<i>Vibrio alginolyticus</i>	1	—	—	—	1	0	0	0	0
<i>Vibrio cholerae</i>	15	15	—	—	—	15	15	0	15
<i>Vibrio eltor</i>	12	12	—	—	—	12	12	0	12
<i>Vibrio metschnikovii</i>	1	1	—	—	—	1	1	NG	1
<i>Vibrio proteus</i>	2	2	—	—	—	2	2	NG	2
<i>Vibrio parahaemolyticus</i>	1	1	—	—	—	0	0	0	0
<i>Vibrio</i> spp.	7	7	—	—	—	3	3	2	7
<i>Yersinia enterocolitica</i>	7	7	—	—	—	1	2	1	2
<i>Yersinia pestis</i>	12	12	—	—	—	0	0	NG	12
<i>Yersinia rodentum</i>	23	23	—	—	—	0	0	NG	23
<i>Zoogloea ramigera</i>	1	—	1	—	—	0	0	0	0

Table I continued

¹Ferm = fermentative; Ox = oxidative; Alk = alkali-producing; nil = no reaction. ^aNG = no growth.

ACID FROM LACTOSE TEST

Three concentrations of lactose were used in the following media.

Medium A (1.0% w/v lactose peptone water)

Peptone (Evans)	2 g
NaCl	1 g
Lactose	2 g
1% w/v Bromcresol purple	0.4 ml
Distilled water	200 ml

Medium B (5.0 w/v lactose peptone water)

Peptone (Evans)	2 g
NaCl	1 g
Lactose	10 g
Andrade's indicator	10 ml
Distilled water	190 ml

Medium C (10.0% w/v lactose agar slopes)

Lab Lemco (Oxoid)	3 g
Peptone (Evans)	5 g
Lactose	100 g
Agar	10 g
Bromcresol purple	0.025 g
Distilled water	1000 ml

Results in all three media were read up to 10 days' incubation at optimum temperature, usually 37°C.

O-F TEST

The method and medium of Hugh and Leifson (1953), modified to give a final concentration of bromthymol blue of 0.006% w/v, was used to determine the oxidative or fermentative breakdown of glucose.

Results and Discussion

The results of the O-F test were recorded as either fermentative, oxidative, alkali-producing, or nil. These results and those for the acid from lactose tests, and the ONPG test are given in table I. All four possible combinations of acid from lactose positive or negative and ONPG positive or negative

were found, acid production from lactose being scored positive if positive by any of the three methods. The numbers of strains in each combination are given in table II, which also gives the numbers of strains in each O-F reaction pattern. No discrepancies were found between our data and those of Le Minor and Ben Hamida (1962) and Bülow (1964) among those taxa studied by those authors and ourselves.

LACTOSE AND ONPG NEGATIVE

The largest proportion of strains (59.5%) were both lactose and ONPG negative and need not be discussed further except to say that they included *Brucella*, *Campylobacter*, *Comamonas*, *Haemophilus*, *Moraxella*, *Neisseria* (except *N. lactamicus*), *Pasteurella*, and several *Pseudomonas* spp. (In this, and subsequent lists, some names may appear more than once as different strains of the same taxon behaved differently.)

LACTOSE AND ONPG POSITIVE

These comprised 15% of all the strains and were mainly fermentative (see table II): *Actinobacillus* spp., *Aeromonas* spp., *Campylobacter fetus*, *Erwinia herbicola* (synonym: *Chromobacterium typhiflavum*, an enterobacterial species, Graham and Hodgkins, 1967), *C. lividum*, *Flavobacterium meningosepticum*, *Gemella haemolysans*, some *Haemophilus* spp., *Neisseria lactamicus*, exceptional strains of *Pasteurella multocida*, *Plesiomonas shigelloides*, *Pseudomonas cepacia* (exceptional strains), and *Yersinia enterocolitica*.

LACTOSE POSITIVE, ONPG NEGATIVE

These comprised 14% of all the strains. Two were fermentative (exceptional strains of *Pasteurella haemolytica*), but all the remainder were oxidative: 58 strains of *Pseudomonas* spp, 18 strains of *Acinetobacter anitratus*, and five strains of *Achromobacter* spp. These 81 strains were all positive on the 10% lactose slopes and 32 of them also positive in the 5% lactose broths but only one positive result was obtained in the 1% lactose broths. It is interesting

Result for		O-F Reaction Pattern				Totals
Lactose	ONPG	Ferm. ¹	Ox.	Alk.	Nil.	
+	+	72	10	1	6	89 (15%)
+	-	2	81	0	0	83 (14%)
-	+	53	9	1	5	68 (11.5%)
-	-	90	51	54	153	348 (59.5%)
Totals		217	151	56	164	588

Table II Numbers of strains in the four combinations of acid from lactose and ONPG results and the four O-F reaction patterns.

¹Ferm. = fermentative; Ox = oxidative; alk = alkali producing; nil = no reaction.

to note that of the 18 *Acinetobacter anitratus* strains studied here, 15 were positive in the 5% lactose broths as well as on the 10% lactose slopes. Thus it would appear that in this set of 81 strains, the 5% lactose broth test, together with a negative ONPG test result, is indicative of the lactobionic acid pathway.

LACTOSE NEGATIVE AND ONPG POSITIVE

These comprised 11.5% of all the strains. They included *Actinobacillus* spp, *Aeromonas* spp, *Chromobacterium typhiflavum* (one strain), *C. lividum*, *Flavobacterium meningosepticum*, *Haemophilus paraprophilus*, *Pasteurella pneumotropica*, a few *Pseudomonas* spp (including *P. atlantica* which has a DNA base composition of 55% GC, atypical of the genus), *Vibrio alcaligenes*, *Yersinia pestis*, and *Y. rodentium* (synonym: *Pasteurella pseudotuberculosis*).

In conclusion, although the majority of the strains tested were both lactose and ONPG negative, the numbers found in the other three categories demonstrate the value of these tests together in the identification and differentiation of non-fermentative and fermentative bacterial genera in addition to those of the family Enterobacteriaceae.

The authors wish to pay tribute to the late Dr K. J. Steel for his work on an earlier, unpublished study of the same nature as this present paper.

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