BIOCHEMICAL TESTS FOR BACTERIAL
CHARACTERIZATION. 7. GLUCONATE TEST.

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For characterizing Pseudomonas aeruginosa, Haynes (1951) described a medium in which the conversion of potassium gluconate to potassium 2-ketogluconate was detected by the appearance of reducing substances. Shaw and Clarke (1955) used a modification of this medium in their study of Providence cultures and developed from the test a micro-method using heavy suspensions of living organisms. These tests were said to be useful in taxonomic work on the klebsiella group, but in my hands the microtest did not always give the same result as the culture growing on the gluconate medium. This paper records experiments made to find the optimal conditions for the microtest, and also describes a microtest suitable for routine use in taxonomic work.

Methods

Media. A simple Lemco agar (LA) medium was made up as follows: Lemco, 10 g.; peptone, 10 g.; NaCl, 5 g.; water, 1 litre; pH adjusted to 7.6; sterilized at 10 lb. pressure for 10 minutes. To this base (LA) might be added 1 per cent glucose (GA) or other carbohydrate.

Suspensions. Strains were grown on LA and on GA at 30° C. for 18-24 hours; growth was washed off with physiological saline, spun, and the deposit resuspended in distilled water. Suspensions made from growth on Lemco agar (LA) are identified in this paper by the designation LAS, those made from glucose agar (GA) by GAS. Some klebsiella strains were so mucoid that the cells would not pack on centrifugation; in these instances as much as possible of the supernatant was removed and the cells were resuspended in the remaining syrupy fluid. To compensate for this inability to concentrate, these lighter suspensions were added in greater volume in the microtests. For strains that packed well on centrifugation, 0.75 ml. water was added to the deposit from one slope in a 6 x 1/2 inch tube.

Solutions. Gluconate solutions were made either by dissolving potassium gluconate crystals or by adjusting a 1 per cent gluconic acid solution to the required pH value by adding normal potassium hydroxide (referred to as 1 per cent gluconate solutions).

Buffers. McIlvaine's buffer solutions were used in most experiments on the gluconate test. Sørensen's phosphate buffers were used in other tests (such as that for acetoin) made at the same time.

Gluconate test. Conditions of the test are shown at the head of each table. After incubation of the gluconate and suspension mixtures 0.1 ml. Benedict's qualitative reagent was added to each tube, and tubes were placed in a water-bath at 100°C. for 5 minutes. A positive test showed reduction of the reagent.

Experimental

The mucoid nature of many klebsiella organisms prevented their complete sedimentation on spinning; and it was necessary to find whether variation in suspension concentration materially affected the result of the gluconate test. Six nonmucoid strains were grown on LA and on GA, and the centrifuged cells were resuspended in distilled water. Twofold dilutions of each suspension were made in water, and each tested in duplicate by the gluconate microtest described by Shaw and Clarke (1955). The results of this experiment (Table 1) showed that, in general, the test was not particularly sensitive to changes in suspension concentration. Strains No. 2794 and No. 5055 gave unexpected results in that the weaker suspensions from glucose agar (GA) were positive, while the more concentrated suspensions were negative.

The medium on which the strain had been grown seemed to have a variable effect on this test; in this experiment suspensions of two strains were positive when grown on LA and only feebly positive from GA. In later experiments with a slightly modified method, GAS of these strains gave positive results. Three strains, NCTC 204, 3263, and 5056, were positive only in GAS. This association of a positive gluconate test with growth on GA was analogous to the VP microtest in which most VP-positive strains are negative when tested as suspensions from LA. Clarke and Cowan (1952) showed that

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Table 1. Effect of (a) suspension concentration and
(b) growth medium on the gluconate microtest.

(1% K. gluconate, 0.04 ml.; 0.025 M-phosphate
buffer, pH 6.8, 0.04 ml.; suspension, 0.04 ml.)

Strain No.	Suspension	Undiluted	Suspension dilution		
			1/2	1/4	1/8
204	LAS	-	-	-	-
	GAS	+	+	+	+
2794	LAS	+	+	+	+
	GAS	-	-	-	+
3263	LAS	-	-	-	-
	GAS	+	+	+	±
5054	LAS	-	-	-	-
	GAS	-	-	-	-
5055	LAS	+	+	+	+
	GAS	-	-	±	±
5056	LAS	-	-	-	-
	GAS	+	+	+	+

LAS = suspension made from growth on Lemco agar.

GAS = suspension made from growth on glucose agar.

this was not a glucose adaptation, but an effect of reduction of pH value due to growth in a glucose-containing medium.

Experiments were made to find out whether the pH effect was primarily concerned with the reaction at which the test was made, or with the reaction of the medium on which the culture had been grown. 1 per cent gluconic acid solution was adjusted with N-NaOH to pH values of 4, 5, 6, and 7, and these were used with McIlvaine's buffer solutions of corresponding pH value. Gluconate-suspension mixtures were incubated at 37°C. for varying times. Benedict's reagent was then added and the mixtures were boiled.

The results of this (Table 2) and similar experiments showed that for most strains the optimal pH value for the test was 4, but for a few strains (see Table 4) the optimum was 6. The effect of the medium was found by adjusting the pH value; agar could not be used throughout the range as it failed to solidify at the lower pH values.

Table 2. Effect of (a) pH value and (b) duration of reaction on the gluconate microtest.

(1% gluconate solutions, 0.06 ml.; buffer, 0.06 ml.; suspensions, 0.04 ml. Test organism, NCTC 9132)

Suspension	Reaction time (hours)	pH value of gluconate solution			
		4	5	6	7
LAS	1	-	-	-	-
GAS	1	+	+	-	-
LAS	2	-	-	-	-
GAS	2	++	+	+	-
LAS	4	+	-	-	-
GAS	4	++	+	+	-
LAS	22	++	-	-	-
GAS	22	+	+	+	-

Table 3. Effect of growth medium on the gluconate microtest.

(Strain 9132 grown on LA and GA of pH 7.6 and on acid broth (AB) at pH 5. Gluconate solution at pH 4.)

Time of test (hours)	Suspensions		
	LAS	GAS	ABS
1	-	+	++
2.5	-	++	++
5	+	++	++

LAS = suspension from growth on LA at pH 7.6.

GAS = suspension from growth on GA initially at pH 7.6.

ABS = suspension from growth on Lemco broth initially at pH 5.

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Table 4. Effects of variation in pH value of (a) medium in which cultures were grown and (b) gluconate solutions in the test.

(McIlvaine buffer solutions were of the same pH values as the gluconate solutions. Tests were made after incubation for 6 hours.)

Strain No.	Medium	pH	pH of gluconate solution			
			4	5	6	7
9162	LB	5	+	+	-	-
	LB	6	+	+	-	-
	LB	7	+	+	+	-
	GB	7	+	+	+	+
8813	LB	5	+	++	++	+
	LB	6	+	++	+	-
	LB	7	+	++	+	-
	GB	7	+	+	+	-
8168	LB	5	-	-	-	-
	LB	6	-	-	-	-
	LB	7	-	-	-	-
	GB	7	-	-	+	+
9665	LB	5	-	-	+	-
	LB	6	-	-	+	-
	LB	7	-	-	+	-
	GB	7	+	+	+	+

LB = Lemco broth. GB = Lemco broth + 1 per cent glucose.

Cultures were grown in Lemco broth at pH 5 and the tubes were placed at an acute angle in the incubator so that there was a large air surface. The same strain was grown on LA (pH 7.6) and on GA (pH 7.6). Table 3 shows that growth in an acid medium had the same effect as growth in a glucose medium, and from this it can be concluded that the gluconate test is not dependent on adaptation to glucose. However, the effect of variation of pH value both of the medium and of the test is complex, as is shown by the results presented in Table 4.

In each test the suspension was mixed with gluconate solution and buffer and the mixture kept in a water-bath at 37°C for a definite time before Benedict's reagent was added. Table 5 shows the effect of varying the duration of the test; most strains resembled No. 9162 in giving a stronger reaction (i.e. showing more complete reduction of the Benedict's reagent) after 24 hours, but as this type of reaction was not invariable it was necessary to carry out the test on a representative collection of strains.

Table 5. Effect of time factor on the gluconate test.

(1% gluconate solution, pH 4, 0.06 ml.; McIlvaine buffer, pH 4, 0.06 ml.; suspension, 0.04 ml.)

Strain No.	Time of test (hours)	Suspension			
		LBS 5	LBS 6	LBS 7	GBS 7
9162	2	+	+	-	+
	4	+	+	+	+
	6	+	+	+	+
	24	++	++	++	++
9665	4	-	-	-	-
	6	-	-	-	+
	24	-	-	-	-

LBS = suspension from growth in Lemco broth; figure represents pH value of the medium before inoculation.

GBS 7 = suspension from growth in Lemco broth + 1 per cent glucose at pH 7.

A series of 109 strains (92 of these being Aerobacter or Klebsiella spp.) was tested, using both LAS and GAS suspensions, gluconate solutions of pH 4 and of pH 6, and incubating the mixtures for 4 and 24 hours at 37°C. The results of this trial showed that 62 strains were gluconate-positive in one or more combinations of pH and time, and that GAS tested after 4 hours at pH 4 gave the greater number of positives (Table 6). Further analysis (Table 7) showed that LAS suspensions were not positive when GAS were negative, and that some strains were only positive under certain pH-time combinations.

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Table 6. Results of gluconate microtests on 92 strains of Aerobacter and Klebsiella cultures; tests made at different pH values and after varying intervals.

pH of test	Reaction time (hours)	No. positive	
		LAS	GAS
4	4	29	55
4	24	38	52
6	4	3	22
6	24	3	16

LAS = suspensions made from growth on Lemco agar.

GAS = suspensions made from growth on glucose agar.

Table 7. Analysis of positive gluconate results.

Total number positive	62 strains
Positive only at 4 hours	5 "
Positive only at 24 hours	5 "
Positive only with LAS	0 "
Positive only with GAS	23 "
Positive only at pH 4	33 "
Positive only at pH 6	6 "
Positive only at pH 4 and 4 hours	1 "
Positive only at pH 6 and 4 hours	1 "
Positive only at pH 6 and 24 hours	5 "

It is clear from Table 7 that the gluconate microtest cannot be a single tube test; it must be a series of tests, exploiting the combinations of pH value and duration of incubation found most useful. It is suggested, therefore, that the gluconate microtest should be performed in duplicate at pH 4 and 6, and tests made after incubation for 4 and 24 hours. Two gluconate solutions are required; 1 per cent gluconic acid is adjusted with N-NaOH or N-KOH to pH 4 and pH 6. The test mixture consists of gluconate solution, 0.06 ml.; McIlvaine buffer solution of the same pH value, 0.06 ml.; suspension (GAS), 0.04 ml.; control tubes of water, 0.06 ml.;

and GAS suspension, 0.04 ml. are included. The mixtures are incubated at 37°C and after 4 hours two sets of tubes (one test and one control at pH 4 and another pair at pH 6) are withdrawn and 0.1 ml. Benedict's reagent is added to each tube. All four tubes are placed in a water-bath at 100°C for 5 minutes. Reduction of the Benedict's reagent indicates a positive gluconate test. The other sets of tubes are tested by adding the Benedict's reagent after incubation at 37°C for 24 hours.

DISCUSSION

The gluconate microtest, carried out at two pH levels and after different reaction times, gave results which were easy to read and were readily reproducible. The microtest, however, did not give exactly the same results as the test with growing cultures used by Shaw and Clarke (1955). The same strains were examined by Miss C. Shaw, using cultures growing in a gluconate medium, and by me using the microtest described here. An unexpected discrepancy was found in strains which, in the microtest, were positive only with suspensions from growth on glucose agar (GAS); these strains were negative when grown in the gluconate medium. It remains to be seen whether the strains behaving in this way fall into one group, and whether different results in the macro- and microtests have any taxonomic significance.

SUMMARY

1. A microtest was developed to detect the ability of bacterial suspensions to convert potassium gluconate to potassium 2-ketogluconate.
2. Suspensions prepared from growth on glucose agar, or Lemco broth at pH 5, gave more positive results than suspensions from Lemco agar or broth at pH 7-7.6.
3. Most suspensions were more active at pH 4 than at pH 6, but there were enough exceptions to justify carrying out the test at both pH values.
4. Tests made after 24 hours of incubation gave stronger

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reactions than those after 4 hours; a few strains were positive only when tested after 4 hours.

5. The microtest proposed should be made with (a) suspensions from growth on glucose agar, (b) at both pH 4 and 6 and (c) after 4 and 24 hours of incubation at 37°C.

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