BACTERIAL OXIDATION OF ARSENITE

I. DESCRIPTION OF BACTERIA ISOLATED FROM ARSENICAL CATTLE-DIPPING FLUIDS

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Summary

From arsenical cattle-dipping fluids from Queensland, in which spontaneous oxidation of arsenite to arsenate had occurred, 15 strains of bacteria were isolated which are able to bring about oxidation of arsenite in culture media. Their characteristics, their taxonomy, and some factors which influence their arsenite-oxidizing activity are described. The strains share many common features, but do not form a homogeneous group. They are regarded as constituting five species, of which three belong to the genus *Pseudomonas,* and one to the genus *Xanthomonas,* in the family Pseudomonadaceae; the remaining species is allotted to the genus *Achromobacter,* in the family Achromobacteriaceae.

I. Introduction

Spontaneous oxidation of arsenite to arsenate in cattle-dipping fluids was first described by Brünnich (1909) in Queensland. It was subsequently reported in the U.S.A. by Fuller (1911) and Chapin (1915), and in South Africa by Williams (1913). The observations of those workers, and by Brünnich and Smith (1914), that it could be prevented or arrested by disinfectants of the coal-tar series, suggested a causal relationship with bacterial growth. Finally, at Onderstepoort, South Africa, Green (1918a) isolated from a dipping fluid a bacterium, provisionally named *Bacillus arsenoxydans,* which in pure cultures was able to bring about oxidation of arsenite to arsenate. Appearing in a yearly publication mainly devoted to animal diseases, this important and detailed work apparently escaped the notice of general microbiologists. The bacterium was eventually lost, and it is therefore no longer available for study. This appears to be the only record of arsenite-oxidizing bacteria until the author's preliminary note (Turner 1949).

In 1944, when the investigations to be described were begun, the cause of spontaneous oxidation of arsenical cattle-dipping fluids in Queensland had not been determined, although empirical methods existed for overcoming it (see Turner 1943). It was therefore decided to examine partly "oxidized" cattle-dipping fluids from Queensland for the presence of arsenite-oxidizing bacteria. If they were found, it was hoped that the study of their characteristics and mode of action might lead to more specific means of controlling the deterioration. Eventually, 15 strains of arsenite-oxidizing bacteria were isolated, and their general features were briefly described in the preliminary note referred to.

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A more complete description of the bacteria, and of the enzymic mechanism concerned in oxidation of arsenite, is now presented in a series of papers.

This first paper of the series describes the isolation of arsenite-oxidizing bacteria from oxidized arsenical cattle-dipping fluids from various parts of Queensland, and discusses their characteristics, their taxonomy, and some factors which influence their activity.

II. METHODS

(a) Temperature of Incubation

Unless otherwise stated, 25°C.

(b) Enrichment Media

In the early experiments, the enrichment medium of Green (1918a) was used. For the main part, a medium referred to briefly as KA3 was devised. This consists of the citrate-ammonium medium of Koser (1923), modified by the addition of 0·26 per cent. (= 0·02M) sodium meta-arsenite; it is adjusted to pH 8·1 before autoclaving, and is moderately buffered over the range pH 4·5 to 7·5 by the phosphoric and citric acid systems.

(c) Isolation Procedure

One ml of the sample was sown into 200 ml of KA3 in a 500-ml erlenmeyer flask. Progress of oxidation was followed by means of iodometric titration. When oxidation was well advanced, or was complete, the enriched cultures were stroked on nutrient agar, usually without a second enrichment. Representative colonies were sown into a presumptive test medium, by means of which arsenite oxidizers were quickly recognized. It consists of KA3 with 0·0032 per cent. bromothymol blue as indicator, adjusted to pH 7·0, and distributed in 5-ml amounts in 6 by ⅝ in. test-tubes. Inoculated tubes were incubated almost horizontally, in order to increase the surface area. The test is based upon the fall of pH which accompanies the oxidation of arsenious acid (pK = 9·22) to arsenic acid (pK$_1$ = 2·3); oxidation of arsenite is accompanied by a change from faint blue to yellow, whereas strains not able to oxidize arsenite usually cause a deepening of the blue tint.

(d) Fermentation Tests

These were carried out in peptone solution, adjusted to pH 7·0 and containing 1 per cent. Andrade's indicator; 1 per cent. of test substance, separately sterilized by filtration, was added aseptically. Incubation was continued for 21 days. Micro-fermentation tests were also carried out by the procedure of Cowan (1953), modified by the inclusion of 0·017M phosphate buffer pH 7·4. Cell suspensions for this were harvested from young cultures on Cotton's potato-infusion beef-extract glucose glycerol peptone agar (PA), prepared as described by Mitchell and Moore (1941), which gives a high yield of cells. Some tests were also carried out by the procedure of Hugh and Leifson (1953), in order to determine the type of attack on carbohydrates, i.e. whether oxidative or fermentative.

(e) Carbon-source Utilization

The basal medium of Seleen and Stark (1943), adjusted to pH 7·0, was used. Growth was judged by opacity, and acid production was determined by adding a drop of bromocresol purple solution. Incubation was continued for 25 days.

(f) Serological Tests

Antisera were prepared in rabbits. Agglutination tests, carried out with thick suspension on glass plates, were read within 2 min. Antigens for precipitin tests were prepared by modification of Lancefield's method described by Stableforth (1932).

(g) Estimation of Arsenite Oxidation

Arsenite was estimated by iodometric titration. The $AgNO_3$ spot test for arsenate (Feigl 1937) was used as a qualitative test for oxidation, but in dipping fluids contaminated with excreta, or in cultures in KA3, a positive reaction was not obtained until oxidation had proceeded to the extent of 30-50 per cent.

III. RESULTS

(a) Isolation of the Strains

From each of the 15 samples bacteria were isolated which were capable of bringing about oxidation of arsenite to arsenate in suitable culture media. It is assumed that they were responsible for the oxidation of the respective dipping fluids. In the early stages of investigation it was found that samples from the dip at the Cannon Hill Meatworks, Brisbane, yielded only one type of arsenite-oxidizing bacterium which formed on nutrient agar very characteristic colonies. Its unusual appearance was used in recognizing it in a total of 10 samples. It is assumed that it was the only arsenite-oxidizing type present, because all other colonies examined failed to bring about oxidation of arsenite. However, in five samples this bacterium, provisionally referred to as type I, was not present. This led to the recognition of types II, III, IV, and V, of which type II was recovered on two occasions; but in none of the samples was more than the one type found.

(b) Source and Geographical Distribution of the Strains

The samples from which the strains were isolated were allotted serial numbers when received. The date when collected, the location of the dip, the provisional "type" to which the isolated strain was allotted, and the suggested classification and nomenclature of the strains, are set out in Table 1.

In Queensland, October and December correspond to the warmer period of the year and the early "wet season"; June corresponds to the relatively cooler period of the year.

The geographical distribution is clearer from the map (Fig. 1). Type I was found widely between lat. 21° S. and 28° S.; types II and III occurred on the coast near the Tropic of Capricorn; and types IV and V were found under
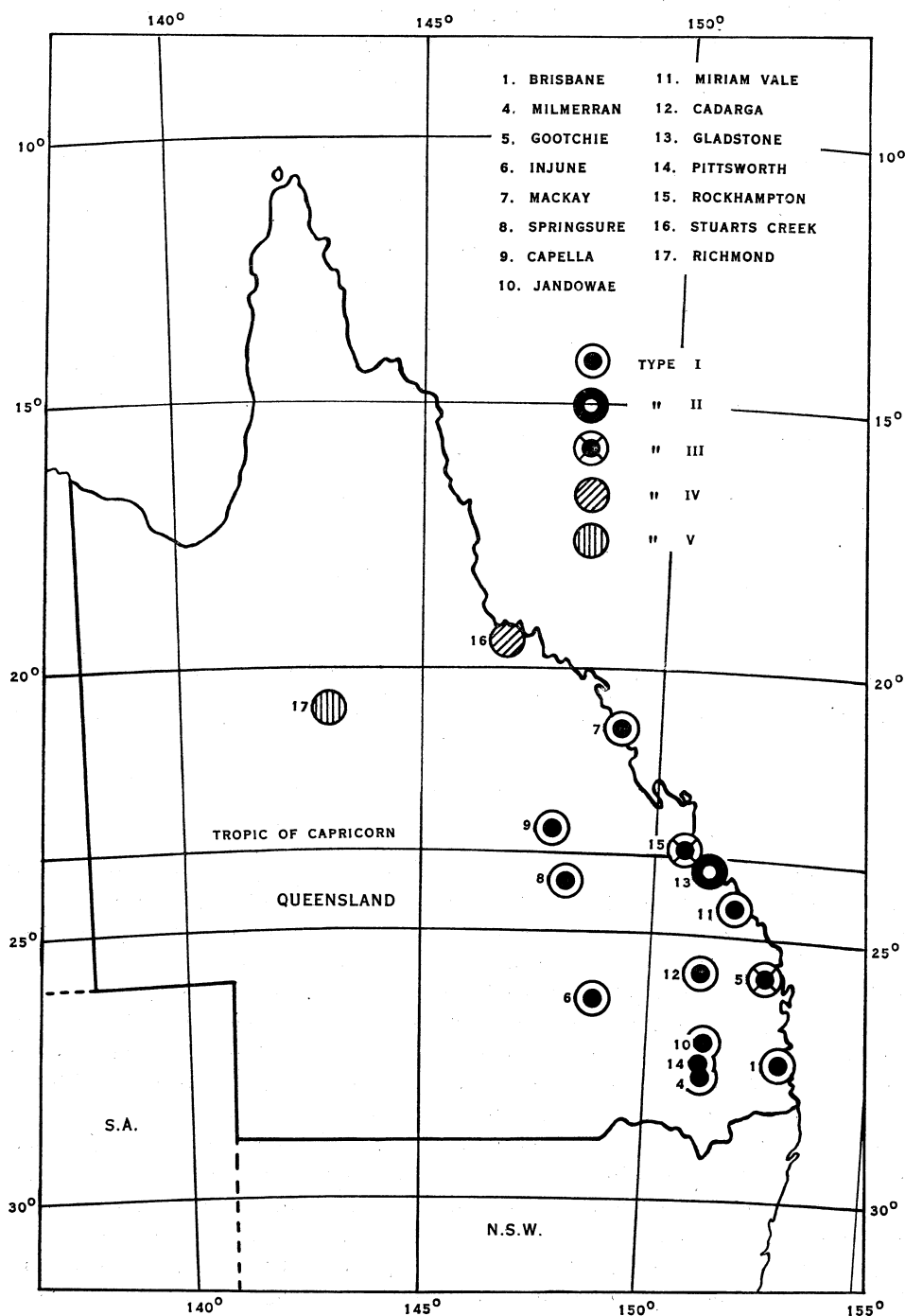
Fig. 1.—Map of Queensland, showing location of the arsenical cattle dips from which samples were obtained, and the provisional "type" to which the strains isolated were assigned.

warm conditions near lat. 20° S., type IV near the coast and type V inland. However, in view of the small number of samples, the distribution may not be significant.

TABLE 1

PARTICULARS OF ARSENITE-OXIDIZING BACTERIA ISOLATED FROM QUEENSLAND ARSENICAL CATTLE-DIPPING FLUIDS

Strain No.	Date Collected	Locality	Provisional "Type"	Designation
1, 2, 3	3.x.1944	Brisbane	I	*Ps. arsenoxydans-unus* sp. nov.
4	3.x.1944	Milmerran	I	*Ps. arsenoxydans-unus* sp. nov.
6	5.vi.1945	Injune	I	*Ps. arsenoxydans-unus* sp. nov.
7	5.vi.1945	Mackay	I	*Ps. arsenoxydans-unus* sp. nov.
8	5.vi.1945	Springsure	I	*Ps. arsenoxydans-unus* sp. nov.
9	5.vi.1945	Capella	I	*Ps. arsenoxydans-unus* sp. nov.
10	5.vi.1945	Jandowae	I	*Ps. arsenoxydans-unus* sp. nov.
11	5.vi.1945	Miriam Vale	I	*Ps. arsenoxydans-unus* sp. nov.
12	5.vi.1945	Cadarga	I	*Ps. arsenoxydans-unus* sp. nov.
14	5.vi.1945	Pittsworth	I	*Ps. arsenoxydans-unus* sp. nov.
5	5.vi.1945	Gootchie	II	*Ps. arsenoxydans-duo* sp. nov.
15	5.vi.1945	Rockhampton	II	*Ps. arsenoxydans-duo* sp. nov.
13	5.vi.1945	Gladstone	III	*Achr. arsenoxydans-tres* sp. nov.
16	8.xii.1947	Stuart's Creek	IV	*Xanth. arsenoxydans-quattuor* sp. nov.
17	8.xii.1947	Richmond	V	*Ps. arsenoxydans-quinque* sp. nov.

(c) Description of the Bacteria

(i) Common Features

Although these bacteria may be separated into several types, they have many similarities. They are all Gram-negative, non-sporulating, aerobic, motile rods. They grow well in the presence of bile salts or of penicillin. They do not produce gas from any attackable carbohydrate; those that attack carbohydrates do this oxidatively and not fermentatively. They do not produce acid from lactose, nor can they utilize it as the sole source of carbon. They do not produce acetylmethylcarbinol. They break down nitrates, produce small amounts of H_2S but not indole, and are catalase-positive. They have a slight, delayed alkalinizing action upon milk. They do not produce turbidity in lecithovitellin solution. They are able to grow well on simple culture media such as Koser's medium, in which the source of nitrogen is the NH_4^+ ion and the source of carbon is citrate or other simple organic source. None grew in the inorganic basal medium of Doudoroff (1940), in which was incorporated 0·02M $NaAsO_2$, when placed in an atmosphere of 80 per cent. CO_2 and 20 per cent. O_2. There was thus no evidence from this experiment to suggest that they can use the energy of oxidation of arsenite for growth, but further investigation is desirable. They grow very luxuriantly on the usual meat-infusion, peptone, or yeast-extract types of culture media, and are therefore typical heterotrophic

microorganisms. They appear to be non-pathogenic to animals: young active cultures in meat-infusion peptone broth did not produce ill effects when injected intramuscularly into guinea pigs in a dose of 1 ml, nor intraperitoneally into mice in a dose of 0·25 ml. None of the strains is haemolytic when grown on the surface of sheep-blood meat-infusion peptone agar.

(ii) *Differential Features*

The differentiation of these strains into "types" was based upon the following characteristics: cell size, type of flagellation, colony form, pigment production, ability to grow anaerobically or autotrophically, temperature requirements for growth, serological reactions, biochemical properties (including pigment production), proteolytic ability, ability to produce acid from the usual range of "fermentation" substances, and ability to utilize various compounds as the sole source of carbon.

Cell size.—The cells of strains 5 and 15 (type II) are larger, particularly in width, than those of the other strains when grown on the same medium such as PA.

Type of flagellation.—Types I, II, IV, and V develop polar flagella, either singly or in tufts, whereas strain 13 (type III) is peritrichially flagellated.

Colony form.—On the surface of infusion peptone agar or of PA, the strains classed as type I form colonies which at first are whitish by reflected light, but develop semi-translucent dirty greyish flecks or streaks; they are very soft and semi-fluid, and readily coalesce to form very large colonies. If cultures in petri dishes are left inverted, the colonies may drip to the lower lid or may run down the side under the influence of gravity. Strain 16 (type IV) also is quite distinctive: its colonies are tough and gelatinous, and they are much wrinkled, more or less concentrically; when more thickly srown, the continuous surface growth is thrown into great irregular folds. Types II, III, and V are less distinctive.

Pigment production.—Strains 5 and 15 (type II) quickly produce strongly fluorescent greenish yellow soluble pigment in most media, and particularly well in the medium of Georgia and Poe (1931). On this medium, the strains grouped as types I, III, IV, and V do not produce pigment. Strain 16 (type IV) produces bright yellowish brown pigmented growth on potato.

Temperature requirements.—Strains 5 and 15 (type II) grow relatively well at low temperatures (3°C), but not at all at 37°C, whereas all other strains fail to grow at 3°C even after 4 wk, but will grow at 37°C. Strain 16 (type IV) is unique, in that it will grow infusion peptone broth even at 44°C.

Fermentation reactions.—Strains 1, 4, 5, 6, 7, 10, 11, 12, 13, 14, and 15 were subjected to these tests in the usual way, with peptone solution as the basal medium; strains 16 and 17, which were isolated subsequently, were not. It was difficult to obtain reproducible results, and it was assumed that production of alkali obscured acidifaction. Similar difficulties with standard fermentation

tests, because of concurrent ammonification, have been recorded by many inves-
tigators (e.g. Stein, Weaver, and Scherago 1942; Orcutt 1943; Seleen and Stark
1943). The main findings were as follows. None of the strains produced gas;
those subsequently grouped under type I (i.e. strains* 1, 4, 5, 6, 7, 10, 11, and
12) all produced an acid reaction with xylose, some with arabinose and
rhamnose, some with glucose and fructose, some with erythritol and adonitol,
but none with any other member of the wide range of test substances; those
grouped under type II (strains 5 and 15) produced an acid reaction with xylose,
arabinose, glucose, and fructose only; and type III (strain 13) did not produce
an acid reaction with any test substance.

Tests for preformed or readily synthesized enzymes were carried out by
the method of Cowan (1953). Although cells harvested from any single
medium, such as PA, may not attack all the substrates which the bacterium is
capable of attacking, the findings under the defined conditions help to differen-
tiate the types. Types III and V were unable to produce acid under these
conditions from any of the extensive range of pentoses, hexoses, disaccharides,
trisaccharides, polysaccharides, and alcohols. Types I, II, and IV produced
acid from the pentoses arabinose and xylose, but not from rhamnose. Acid was
produced from the hexoses as follows: type I—glucose and fructose; type II—
glucose, mannose, and galactose; type IV—glucose, fructose, mannose, and
galactose. Acid was not produced by any of the strains from the trisaccharide
raffinose, or from the polysaccharides (starch, inulin, dextrin, glycogen). Of
the disaccharides (saccharose, maltose, lactose, trehalose) the only one to yield
acid under these conditions was maltose, with type II. Of the alcohols
(methanol, ethanol, glycerol, erythritol, adonitol, mannitol, dulcitol, sorbitol)
only ethanol and dulcitol yielded acid, again with type II. The glucosides
aesculin and amygdalin, but not salicin, yielded acid with type II and with type
IV. The non-carbohydrate inositol did not yield acid with any type.

Tests by the procedure of Hugh and Leifson (1953), in order to determine
the mode of attack upon xylose and on glucose, showed that, when it occurred,
it was strictly oxidative. Types III and V were without action; types I, II,
and IV oxidatively assimilated both sugars, the attack upon glucose by type I
being only slight under those conditions.

Utilization of carbon compounds as a sole C source.—The first series of tests
(Table 2) comprised the extensive list of substances used in the fermentation
tests, together with two phosphorylated hexoses, glucose-1-phosphate and
fructose-1, 6-diphosphate.

Some distinctive features were as follows:

Type I utilized all three pentoses, all the alcohols, and inositol.

Type II could not utilize any alcohol higher than the trihydric glycerol.

Type III was distinctive in its relative inertness towards these substances;
        it could not utilize any saccharide, or any alcohol higher than ethanol.

* Strains 2, 3, and 8 also belonged to this type, but were lost before complete tests
could be made.

Type IV utilized starch and glycogen.

Type V could not utilize any hexose, although it utilized maltose and raffinose.

TABLE 2

CARBON SOURCE UTILIZATION

(a) "Fermentation-test" series

Substance	Type I								Type II		Type III	Type IV	Type V
	Strain								Strain				
	1	4	6	7	10	11	12	14	5	15	13	16	17
Arabinose	++	++	++	++	++	++	++	++	++	++	−	−	−
Xylose	++	++	++	++	++	++	++	+	++	++	−	++	+
Rhamnose	++	++	++	++	++	++	++	++	−	−	−	−	−
Glucose	++	++	++	++	++	++	++	++	++	++	−	++	−
Fructose	+	++	++	++	++	++	++	+	++	++	−	++	−
Mannose	++	++	++	++	+	++	+	++	−	−	−	−	−
Galactose	++	++	++	++	++	++	++	++	++	++	−	++	−
Sucrose	+	+	+	+	−	+	−	−	−	−	−	−	−
Maltose	+	+	−	+	+	+	++	+	+	+	−	++	+
Lactose	−	−	−	−	−	−	−	−	−	−	−	−	−
Raffinose	−	−	−	−	−	−	−	−	−	−	−	−	+
Starch	−	−	−	−	−	−	−	−	−	−	−	++	−
Inulin	+	++	+	+	+	+	+	+	+	+	++	−	+
Dextrin	+	+	+	+	+	+	+	+	+	+	−	++	+
Glycogen	−	−	−	−	−	−	−	−	−	−	−	++	−
Methanol	+	+	+	+	+	+	+	+	+	+	+	+	+
Ethanol	++	++	+	+	+	++	++	++	++	++	++	++	++
Glycerol	+	+	+	+	+	+	+	+	++	++	−	++	++
Erythritol	++	++	+	++	+	++	+	+	−	−	−	−	−
Adonitol	++	++	++	++	++	++	++	++	−	−	−	−	−
Mannitol	+	+	+	+	+	+	+	−	−	−	−	++	+
Dulcitol	+	+	+	+	+	+	+	+	−	−	−	−	−
Sorbitol	+	+	+	+	+	+	+	+	−	−	−	−	−
Amygdalin	+	+	+	+	+	+	+	+	+	+	+	+	+
Salicin	−	−	−	−	−	−	−	+	−	−	−	−	−
Inositol	+	+	+	+	+	++	+	+	−	−	−	−	−
l-Sorbose	+	+	−	−	−	−	−	+	−	−	−	−	−
Trehalose	+	+	−	−	−	+	+	+	−	−	−	−	−
Glucose-1-phosphate	+	+	+	+	+	+	+	+	++	++	−	+	+
Fructose-1,6-diphosphate	+	+	+	+	+	+	+	+	+	+	+	+	+

+ = Weak growth, ++ = strong growth.

The second series of tests (Table 3) included various aliphatic, aromatic, cyclic, and heterocyclic compounds that were available, but it was not possible to carry out systematic tests upon homologous series.

TABLE 3

CARBON SOURCE UTILIZATION

(b) Miscellaneous series

Test Substance	Concentration (%)	Type I								Type II		Type III	Type IV	Type V
		1	4	6	7	10	11	12	14	5	15	13	16	17
Formic acid	1·0	+	+	−	+	+	+	+	+	+	+	+	+	+
Acetic acid	1·0	++	++	++	++	++	++	++	++	++	++	++	++	++
Lactic acid	1·0	++	++	++	++	++	++	+	++	++	++	++	++	++
Oxalic acid	1·0	+	+	+	±	±	±	±	+	+	±	+	+	+
Mucic acid	1·0	+	+	+	+	+	±	+	+	+	+	−	−	++
Tartaric acid	1·0	+	+	+	+	+	+	+	+	++	+	+	+	++
Ascorbic acid	1·0	+	+	+	+	+	+	+	+	±	±	−	+	−
Glycollic acid	1·0	+	+	+	+	+	+	+	±	+	±	++	+	±
Na glycollate starch ether	1·0	+	+	+	+	+	+	+	±	+	±	+	+	±
Succinic acid	1·0	+	+	+	+	+	+	+	+	++	++	+	++	++
Fumaric acid	0·25	++	+	+	+	+	+	+	+	++	++	+	++	++
Malic acid	0·25	+	+	+	+	+	+	+	+	++	++	++	++	++
Aconitic acid	0·25	+	+	+	+	+	+	+	+	++	++	+	+	+
Pyruvic acid	0·5	+	+	+	+	+	+	+	+	++	++	+	++	++
Mandelic acid	0·5	+	+	+	+	+	+	+	+	+	±	+	±	0
Adenylic acid	0·25	+	+	+	+	+	+	+	+	+	+	+	+	+
Butyric acid	0·5	−	−	−	−	−	−	−	−	++	++	++	−	−
Phenol	0·05	+	+	+	+	+	+	+	+	++	++	−	+	+
p-Hydroxyphenyl-acetic acid	0·05	+	+	+	+	+	+	+	+	++	++	+	+	++
Benzoic acid	0·1	−	−	−	−	−	−	−	−	++	++	+	−	−
p-Hydroxybenzoic acid	0·05	+	+	+	+	+	+	+	+	++	++	+	−	±
p-Aminobenzoic acid	0·05	+	+	+	+	+	+	+	+	−	−	+	−	++
o-Aminobenzoic acid	0·05	−	−	−	−	−	−	−	−	−	−	−	−	−
Salicylic acid	0·05	−	−	−	−	−	−	−	−	−	−	−	−	−
p-Cresol	0·05	−	−	−	−	−	−	−	−	++	++	−	−	−
Aniline	0·05	+	+	+	+	+	+	+	+	±	±	±	+	±
Pyridine	0·05	+	+	+	+	+	+	+	+	±	±	+	−	+
α-Picoline	0·05	+	+	+	+	+	+	+	+	±	±	+	−	+
s-Collidine	0·05	+	+	+	+	+	+	+	+	+	−	+	−	+
Pimelic acid	0·05	+	+	+	+	+	+	+	+	+	+	+	±	++
Phthalic acid	0·05	+	+	+	+	+	+	+	±	+	±	+	+	+
Diethyleneglycol	1·0	+	+	+	+	+	+	+	+	±	+	+	+	+

TABLE 3 (*Continued*)

β-Glycerosphosphoric acid	1·0	+	+	+	+	+	+	+	+	+	+	+	+	+
Sodium luminal	0·1	+	+	+	+	+	+	+	+	+	+	+	+	+
Phenylpropionic acid	0·05	−	−	−	−	±	−	−	−	±	±	−	±	±
Uric acid	0·05	+	+	+	+	+	+	+	+	+	+	−	+	+
α-Naphthylacetic acid	0·03	±	±	−	−	−	−	−	−	+	+	−	+	+
cycloHexanol	0·05	+	+	+	+	−	+	+	+	−	−	+	−	−
Methyl-cyclo-hexanone	0·02	+	+	+	+	+	+	+	+	+	+	+	+	+

± = Very slight growth, + = moderate growth, ++ = strong growth, 0 = not done.

Some distinctive features were as follows:

Type II was the most versatile and utilized even *p*-cresol.

Type III could not utilize uric acid or phenol.

Type IV was the least versatile; it could not utilize any of the pyridine or benzoic series but, like types II and V, could utilize phenol.

Type V, like types I and IV, could not utilize butyric acid.

Proteolytic properties.—Strain 16 alone, classed as type IV, liquefies gelatine and also inspissated serum. Strain 17 (type V) causes very slight softening of gelatine, but not liquefaction, near the line of inoculation, after many weeks' incubation at 25°C. The others are completely without action.

Action upon nitrate.—All the strains break down nitrate, but whereas types I, IV, and V produce gas, presumably N_2, N_2O, or both, types II and III reduce it still further to NH_3.

Ability to grow autotrophically.—Types I and II are probably facultative chemosynthetic autotrophs, able to obtain energy for growth by oxidizing H_2 to water when more complex compounds are not available. In an unrepeated experiment, these types grew slowly, whereas types III, IV, and V did not, in the simple inorganic solution of Doudoroff (1940) containing NH_4^+ ion as the N source, when placed in an atmosphere of H_2 (83 per cent.), CO_2 (15 per cent.), and O_2 (2 per cent.).

Serological relationships.—The results of the agglutination tests are set out in Table 4, in which the strains are arranged according to the provisional types. It is evident that (*a*) within type I, strains 1, 4, 11, and 12 form a fairly homogeneous subtype, the other strains constituting distinctive or individual subtypes; (*b*) types II, III, IV, and V are sharply distinguished from each other and from type I; and (*d*) strains 18*L* and 18*S**, isolated from cattle faeces in Melbourne, are antigenically distinctive, but have some cross relationships with types II and V respectively. The agglutinin reactions were confirmed to a con-

* These strains have not been examined otherwise in detail.

TABLE 4

AGGLUTININ AND PRECIPITIN RELATIONSHIPS OF ARSENITE-OXIDIZING BACTERIA

| | | Antisera | | | | | | | | | | | | | | |
| | | Type I | | | | | | | | Type II | | Type III | Type IV | Type V | Melbourne Types | |
Type	Strain	1	4	11	12	10	6	7	14	5	15	13	16	17	18L	18S
I	1	9(+)	9(+)	9(+)	7(+)	—(—)	—(—)	—(—)	1(—)	—(—)	—(—)	—(—)	—	—(—)	—(—)	—(—)
	4	10(+)	10(+)	10(+)	7(+)	—(—)	—(—)	—(—)	—(—)	—(—)	—(—)	—(—)	—	—(—)	—(—)	—(—)
	11	10(+)	10(+)	10(+)	6(+)	3(—)	2(—)	3(—)	1(—)	—(—)	—(—)	—(—)	—	—(—)	1(—)	1(—)
	12	9(±)	9(±)	9(±)	8(+)	—(+)	—(—)	—(+)	—(+)	—(—)	—(—)	—(—)	—	—(—)	2(—)	2(—)
	10	4(—)	3(—)	4(—)	7(—)	9(+)	2(—)	9(—)	4(—)	—(—)	—(—)	—(—)	—	—(—)	2(—)	2(—)
	6	1(±)	1(±)	—(±)	2(+)	1(+)	7(+)	—(+)	—(+)	—(—)	—(—)	—(—)	—	—(—)	—(—)	—(—)
	7	—(—)	—(—)	—(—)	—(—)	—(—)	—(+)	8(+)	9(+)	—(—)	—(—)	—(—)	—	—(—)	1(—)	1(—)
	14	4(—)	4(—)	5(—)	5(+)	—(—)	—(+)	—(—)	9(+)	—(—)	—(—)	—(—)	—	—(—)	3(—)	3(—)
II	5	—(—)	—(—)	—(—)	5(+)	—(—)	—(—)	—(—)	—(—)	10(+)	10(+)	—(—)	—	—(—)	1(—)	1(—)
	15	—(—)	—(—)	—(—)	2(—)	—(—)	—(—)	—(—)	—(—)	9(+)	9(+)	—(—)	—	—(—)	2(—)	2(—)
III	13	—(—)	—(—)	—(—)	—(—)	—(—)	—(—)	—(—)	—(—)	—(—)	—(—)	17(+)		—(+)	1(—)	1(—)
IV	16	—	—	—	—	—	—	—	—	—	—	—	5	—	—	—
V	17	—(—)	—(—)	—(—)	2(—)	—(—)	—(—)	—(—)	—(—)	—(—)	—(—)	—(—)	—	9(+)	9(±)	9(+)
Melbourne types	18L	2(—)	2(—)	—(—)	—(—)	—(—)	—(—)	—(—)	—(—)	6(+)	6(+)	—(—)	—	—(—)	8(+)	4(+)
	18S	—(—)	—(—)	—(—)	—(—)	—(—)	—(—)	—(—)	—(—)	1(—)	1(—)	—(—)	—	10(+)	11(+)	11(+)

The agglutinin titres are expressed as $\log_2$ (titre/10): thus $2 \equiv 20$, $10 \equiv 5120$, etc. The symbols in parentheses refer to the precipitin reaction: $+$ = positive, $\pm$ = weak, $-$ = negative.

siderable extent by the precipitin reactions, indicating that, in general, the same antigenic components are implicated in the two reactions, although some inconsistencies suggest that this is not so in all strains.

Rate of arsenite oxidation in KA3 medium.—During prolonged subculturing, frequently from single colonies, arsenite-oxidizing activity of types II and V decreased to some extent. This was correlated with an increase of cell size in type V, and appears to have been the result of chance selection of mutants. By suitable re-selection of colonies, full activity and normal morphology were restored to type V. Type II used to bring about complete oxidation of 0·02M arsenite in KA3 within 4 days, but now requires about 7 days; possibly re-selection of colonies could restore the original activity.

The present position is summed up in Figure 2, which gives the oxidation curves of the type strains of the five types. The most active is type V.
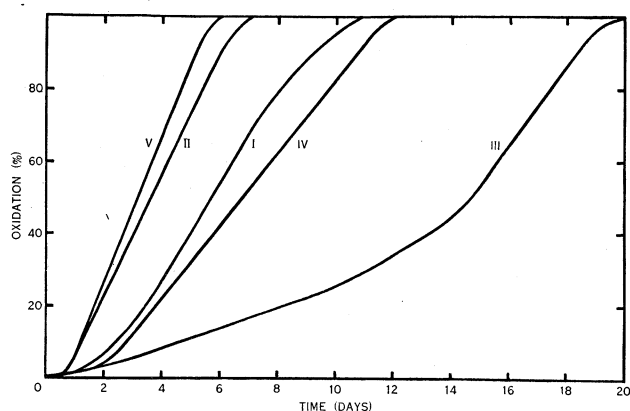
Fig. 2.—Arsenite oxidation curves of the "type strains" of the five species, when incubated with KA3 medium at 25°C.

The differences in arsenite-oxidizing activity in KA3 at 25°C are probably due partly to differences in growth rate. This has not been studied in detail, but it was found that equally dense washed cell suspensions of types I, III, IV, and V harvested from KA3 cultures were equally active when shaken with arsenite.

Influence of temperature upon arsenite oxidation.—Interesting differences in arsenite-oxidizing ability of the types were revealed when incubation was carried out at 37°C instead of at 25°C. Type II will not grow at 37°C, even in nutritionally complex media, but the other four types grow quite satisfactorily. Under these conditions, type I was slightly less active than at 25°C, and required 14 days instead of 11 days for complex oxidation; on the other hand, type III was slightly more active, and at 37°C required 16 days instead of 20 days at 25°C. Types IV and V grew well at 37°C, but failed to oxidize arsenite. Tests with type IV, which is capable of growth even at 44°C, suggested that this is due to temperature sensitivity of the enzyme system: cells

harvested from KA3 cultures grown at 37°C were unable to oxidize arsenite when shaken with it at 25°C, and conversely, cells grown at 25°C were unable to oxidize arsenite when shaken with it at 37°C.

Summary of differential features.—The main differential features between the five types are tabulated in Table 5. Included in this are the corresponding features of *B. arsenoxydans,* as given by Green (1918*a*). The resemblance between it and our type III is evident.

TABLE 5

MAIN DIFFERENTIAL FEATURES OF ARSENITE-OXIDIZING BACTERIA

Criterion	Queensland Bacteria					B.a.*
	I	II	III	IV	V	
gella polar	+	+	−	+	+	−
agella peritrichial	−	−	+	−	−	+
Colonies semi-fluid	+	−	−	−	−	−
Colonies very wrinkled	−	−	−	+	−	−
Pigment fluorescent, greenish yellow	−	+	−	−	−	−
Pigment brownish yellow, water-insoluble	−	−	−	+	−	−
Will grow at 3°C	−	+	−	−	−	0
Will grow at 44°C	−	−	−	+	−	0
Obligate aerobe	+	−	+	+	+	+
Facultative anaerobe	−	+	−	−	−	−
Produces acid from saccharides	+	+	−	+	−	−
Liquefies gelatine and coagulated serum	−	−	−	+	−	−
Breaks down nitrate to N_2	+	−	−	+	+	+
Breaks down nitrate to NH_3	−	+	+	−	−	−
Utilizes, as sole C source, starch and glycogen	−	−	−	+	−	0
Utilizes, as sole C source, maltose, raffinose, dextrin, and inulin, but not hexose	−	−	−	−	+	0
Utilizes, as sole C source, butyric acid	−	+	+	−	−	0
Utilizes, as sole C source, *p*-cresol	−	+	−	−	−	0
Unable to utilize, as sole C source, any saccharide	−	−	+	−	−	0
Facultative chemosynthetic autotroph	+	+	−	−	−	0

B.a. = B. arsenoxydans Green 1918; 0 = information not available.

(iii) *Taxonomy*

All the strains clearly fall into the class Schizomycetes Nägeli 1851, order Eubacteriales Buchanan 1917, sub-order Eubacteriineae Breed, Murray, and Hitchens 1944; all except strain 13 clearly fall into the family Pseudomonadaceae Winslow *et al.* 1917, and the tribe Pseudomonadeae Kluyver and van Niel 1936. Within this tribe, all the remainder except strain 16 fall into the genus *Pseudomonas* Migula 1894. Strain 16, because of its yellow, water-insoluble pigment and its proteolytic properties, is better placed in the genus *Xanthomonas* Dowson 1939. Strain 13 cannot be placed in the family Pseudomonadaceae as at present

defined, because it possesses peritrichial flagella; it appears to fit best into the family Achromobacteriaceae Breed 1945, and the genus *Achromobacter* Bergey *et al.* 1923.

Study of Green's description of *B. arsenoxydans* suggests that, if it were available for study today, it too would be placed in the genus *Achromobacter*; Green had great difficulty in determining the type of flagellation, as we did with strain 13, but he finally decided that it was peritrichous. Strain 13 resembles Green's lost microorganism in many other ways (Table 5), but its identity with *B. arsenoxydans* Green 1918 can never be proved by direct comparison.

Bergey's Manual does not refer to any arsenite-oxidizing bacteria. Indeed, it is unlikely that any of the species listed there has ever been examined for capacity to oxidize arsenite, except by Green and Kestell (1918); they found that, although strains of bacteria now referred to as *Alcaligenes faecalis, Pseudomonas aeruginosa, Ps. fluorescens,* and *Ps. putida* could be accustomed to grow in the presence of 0·1M or even 0·15M arsenite, oxidation of arsenite never occurred. Even if arsenite oxidation is disregarded, none of our 15 strains can be fully identified with any of the 148 species of *Pseudomonas,* the 47 species of *Xanthomonas,* or the 12 species of *Achromobacter,* described in the 6th Edition. Type I resembles to some extent *Ps. ambigua, Ps. sinuosa,* and *Ps. cruciviae*; but, *inter alia,* it has unique undescribed colonial characteristics; type II resembles in some respects *Ps. putida,* but does not grow at 37°C, and does not produce an odour of trimethylamine; type III does not resemble any listed species of *Achromobacter,* or the closely related *Alcaligenes,* but we believe it to be closely related to, and possibly identical with *B. arsenoxydans* Green 1918; type IV does not resemble any listed species of *Xanthomonas*; and although type V resembles in many ways *Ps. sinuosa* (Wright) Chester 1901, the description given is not complete enough to justify identification.

It is proposed to regard these provisional types, possibly with the exception of type III, as new species of the appropriate genera, and to allot them the specific names "*arsenoxydans*" hyphenated to Latin cardinal numbers, because the ordinals have already been used as specific epithets with other genera (e.g. *Vibrio prima, Clostridium tertium*). The resulting compound epithets are relatively short, and are to that extent preferable to descriptive Latin epithets hyphenated to the already long "*arsenoxydans*"; furthermore, if it were later considered necessary to reduce the specific epithets to a single word, with "arsenoxydans" as a varietal epithet, the cardinal numbers could be retained as the specific epithets, and regarded as indeclinable particles in apposition with the generic epithets: if bacterial nomenclature is to be restricted to the Classical languages, some grammatical liberties may have to be tolerated. The proposed nomenclature is therefore as follows:

        Type I   : *Ps. arsenoxydans-unus* sp. nov.
        Type II   : *Ps. arsenoxydans-duo* sp. nov.
        Type III : *Achr. arsenoxydans-tres* sp. nov.
        Type IV : *Xanth. arsenoxydans-quattuor* sp. nov.
        Type V : *Ps. arsenoxydans-quinque* sp. nov.

(d) Some Consequences of Arsenite Oxidation During Growth

(i) *Concomitant Decrease of pH.*—Because arsenic acid dissociates much more than does arsenious acid, the pH falls considerably as oxidation proceeds, even in a moderately well-buffered medium like KA3. This leads to an inverse relationship between the degree of oxidation and the pH, which is illustrated, with *Ps. arsenoxydans-duo* as an example, in Figure 3; in this instance the pH decreased 1·3 pH units, whereas in Koser medium without arsenite the change in pH was insignificant. Other species behaved similarly, although differences occurred in the extent of pH decrease, probably dependent upon the capacity of the particular strain to give rise to alkali from citrate.
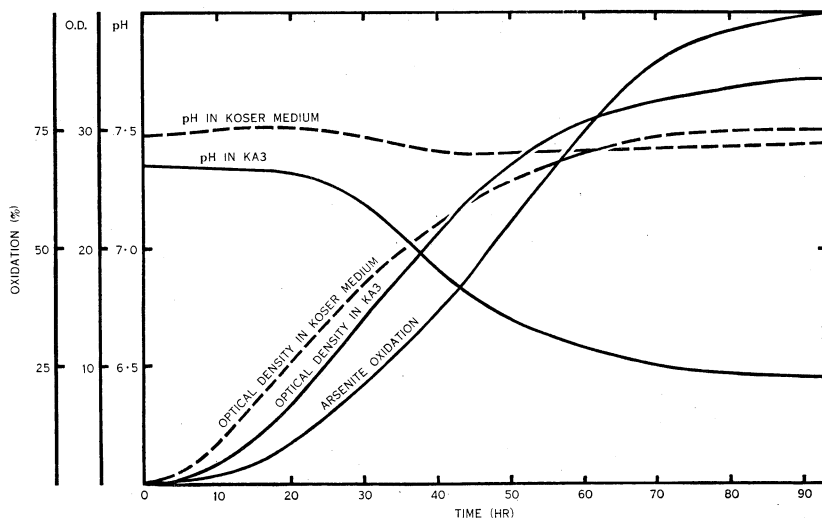
Fig. 3.—Inverse relationship between the percentage oxidation and the pH of *Ps. arsenoxydans-duo*, strain 5, grown in KA3 at 25°C.

(ii) *pH Optimum for Growth in KA3.*—*Ps. arsenoxydans-quinque* was sown into KA3 adjusted to the following pH values: 4·2, 5·1, 6·1, 7·2, 7·7, 9·4, 9·8, and 10·7. After 143 hr, growth and oxidation had reached virtual completion at all pH values between 6·1 and 9·4, but failed entirely at pH 9·8 and 10·7; at pH 5·1 oxidation had reached 26·9 per cent., but at pH 4·1 it had reached only 4·6 per cent. (Fig. 4).

This experiment revealed a wide pH tolerance for growth in an arsenical medium. The very sharp difference between the behaviour at pH 9·8, at which growth failed, and at pH 9·3, at which it was almost maximum, is to be explained by the fall in pH which accompanies oxidation; in this instance, the pH had fallen in 143 hr to 6·8, and it therefore appears that if the pH is not too alkaline to permit growth to start, the concomitant oxidation of arsenite progressively lowers the pH to more favourable levels for growth. When the initial pH is on the acid side of the optimum, the opposite effect must come into play. It happens that in arsenical dipping fluids the initial pH is high—of the order of 9·5—but not beyond the limit at which growth can begin.

(iii) *Progress of Oxidation in a "Model Dip."*—This is further illustrated in the following experiment. Commercial dip concentrate was diluted with tap water to prepare the standard dipping fluid, containing 0·2 per cent. As_2O_3, corresponding to approximately 0·02M arsenite, with pH = 9·55. In this was incorporated a filtered boiled decoction of bovine faeces, corresponding to 4·5 g dried faeces per l. It was placed in an earthenware jar, the depth of fluid being 16·5 cm, and 10 ml of a culture of *Ps. arsenoxydans-quinque* were added. The covered jar was left at room temperature (20°-10°C). The progress of oxidation, and the pH, are shown in Figure 5.
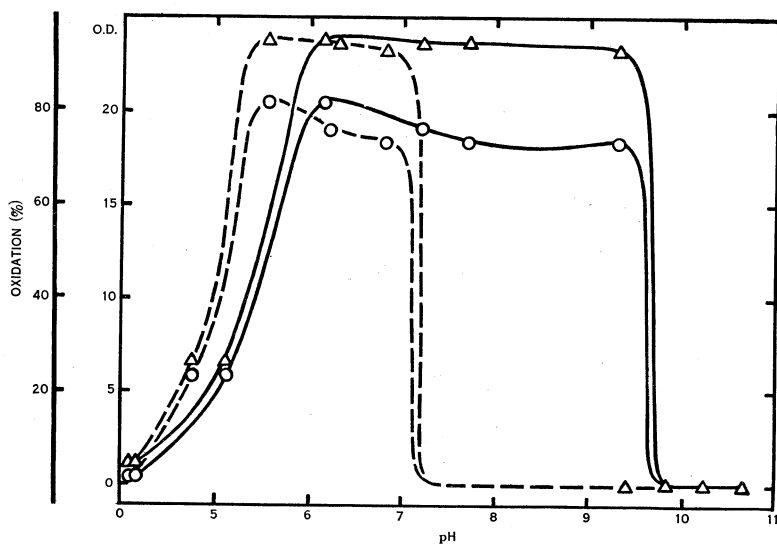
Fig. 4.—Influence of initial pH of KA3 upon growth and percentage oxidation by *Ps. arsenoxydans-quinque,* strain 17, at 25°C, after 143 hr. In the curves with continuous lines, the values are related to the original pH's; in the curves with broken lines, they are related to final pH's after 143 hr.

It is seen that oxidation began quite soon, and proceeded rapidly to reach a maximum level of more than 80 per cent. after about 55 days. During this period the pH fell inversely, and reached less than pH 4, doubtless because the buffering by faecal decoction was very much weaker than in KA3; probably the low pH prevented further growth and oxidation.

Records of samples of spontaneously oxidized dipping fluids received from the field suggest that in practice the pH does not often fall below pH 6 (Hitchcock, personal communication); this is partly because oxidation usually does not proceed so far under field conditions, where the activity of arsenate-reducing bacteria may also operate simultaneously (Green 1918b), and partly because accumulation of phosphate from excreta gradually improves the buffering properties.

(iv) *Influence of Arsenite Concentration.*—*Ps. arsenoxydans-quinque* was sown into Koser medium at pH 7·6, containing 0·02, 0·04, 0·06, 0·08, and 0·10M

arsenite. After 5 days at 25°C, growth had occurred equally well in the presence of 0·02 and 0·04M arsenite, but it was slightly inhibited at 0·06M, considerably inhibited at 0·08M, and almost completely inhibited at 1·0M. This is illustrated in Figure 6, which shows that, up to 0·06M arsenite, there was a positive correlation between growth, progress of oxidation, and pH; beyond 0·06M, the inhibitory effect of arsenite became apparent.

As incubation proceeded, growth improved even at the higher concentrations of arsenite, and by the twelfth day it was quite strong even in the presence of 0·10M arsenite.
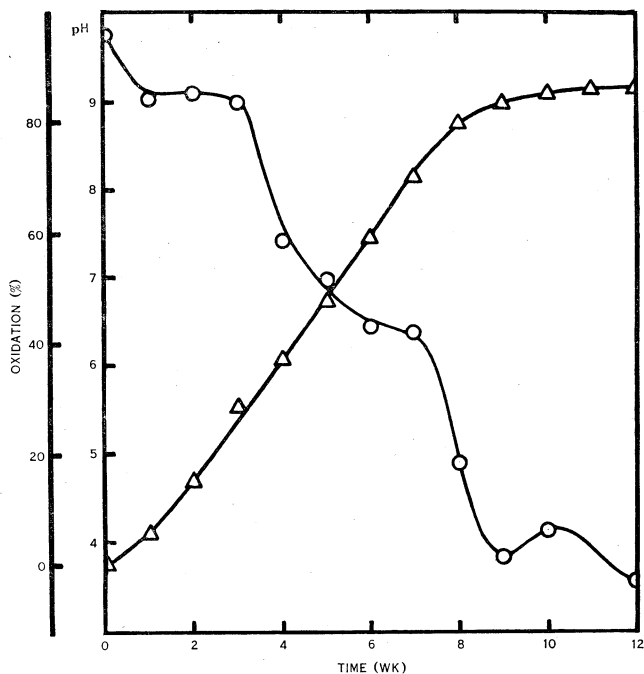
Fig. 5.—Progress of oxidation, and concomitant decrease of pH, in commercial dipping fluid enriched with faecal extract, sown with *Ps. arsenoxydans-quinque*, strain 17, and incubated at room temperature.

IV. Discussion

The development of enzyme systems for the oxidation of arsenite to the less toxic arsenate suggests itself as one mechanism for protecting vulnerable thiol groups from inactivation by arsenite. However, that this property is not essential to high tolerance towards arsenite is clear from the findings of Green and Kestell (1918) that certain arsenite-resisting fluorescent and non-fluorescent pseudomonads isolated from cattle-dipping fluids could be trained to tolerate 0·1M arsenite or more, without showing any evidence of arsenite oxidation. Nevertheless, it seems possible that, with the group of arsenite-oxidizing bacteria described in this paper, capacity to oxidize arsenite may favour survival in an

arsenical dip; we have observed that when cells of *Ps. arsenoxydans-unus* were held under anaerobic conditions in KA3, so that growth and oxidation were inhibited, the viable count began to fall, whereas, on admitting air, growth and arsenite oxidation followed a normal course. Further experiments of this sort, particularly with the facultatively anaerobic *P. arsenoxydans-duo*, are desirable to settle this point. A definite biological advantage of arsenite oxidation to these bacteria is the reduction of pH to values nearer the optimum for their growth, as the pH of freshly prepared dipping fluids is near the high limit of their pH tolerance. Calculation from the heats of formation of the acids in dilute solution indicates that oxidation of arsenite to arsenate could provide about 77 kcal per mole, which is comparable with the energy of anaerobic fermentation of glucose, for example; however, there was no evidence in the limited experiments that these organisms are able to utilize the energy of arsenite oxidation for growth purposes.
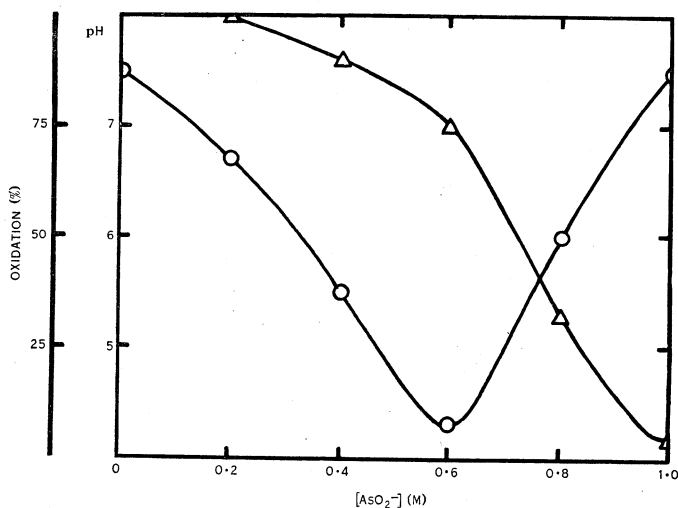
Fig. 6.—Influence of concentration of arsenite upon growth, percentage oxidation, and decrease of pH by *Ps. arsenoxydans-quinque*, strain 17, after 5 days at 25°C. The inhibition by $[AsO_2^-] > 0.06M$, apparent after 5 days was eventually overcome, and quite good growth even in 0.10M arsenite had occurred after 12 days.

The ultimate origin of arsenite-oxidizing strains, whether from soil, water, or faeces, cannot be stated with certainty. In experiments not reported here, attempts were made to accustom a dozen assorted strains of fluorescent pseudomonads, of South Australian origin, which had been isolated from soil, water, and pathological conditions; in no instance could they be trained to tolerate more than 0.01M arsenite in Koser medium, and none was able to oxidize it. Furthermore, we were unable to demonstrate arsenite-oxidizing bacteria in the faeces of horses and cows at Melbourne, when small amounts were sown into KA3 medium. However, when 40 g fresh bovine faeces collected from the

cement floor of stalls was added to 20 l. of dipping fluid made with tap water from commercial concentrate, and left at room temperature, oxidation was detected within a week; it eventually reached 83 per cent., and an arsenite-oxidizing pseudomonad, different from those described from Queensland material, was isolated. As the animal which was the source of the faeces was of local origin, and as the nearest arsenical cattle dips are over 600 miles away, it is unlikely that the pseudomonad isolated had had previous experience of arsenite; it could have been present in the faeces, on the concrete floor, or in the tap water, but it presumably had the potentiality to develop not only arsenite tolerance but an arsenite-oxidizing enzyme system. Green and Kestell (1918) found that arsenite-tolerant bacteria, without oxidizing power, were common in cattle faeces, but did not occur in soil; the genus *Bacillus,* and the "colon-typhoid group" had a low tolerance, but pseudomonads and *Alcaligenes faecalis* could be trained to withstand high concentrations.

Some discussion is called for on the classification of our strains. Fourteen of the strains are undoubtedly pseudomonads; 13 quite clearly belong to the genus *Pseudomonas,* but one (strain 16) is so different in colonial characteristics, pigment formation, and proteolytic properties that no objection can be raised against regarding it as a separate species, and a different genus. We consider it best placed in the genus *Xanthomonas,* and not in the genus *Flavobacterium,* because it has polar flagella. We have classified strain 13 in the genus *Achromobacter* because it is peritrichially flagellated, is inert towards carbohydrates, alcohols, etc., and does not produce pigment; but a case could be made for regarding it as an *Alcaligenes* with poor alkalinizing properties; indeed, Green (1918a) was inclined to classify his closely similar bacillus in this way, but was dissuaded because of the absence of alkali production in milk, and he was uncertain of the flagellation. At the present stage of taxonomy, the type of flagellation is accorded very great weight. Admittedly, the recent demonstration by Leifson and Hugh (1953) that the flagellar arrangement of *Aeromonas,* of *Xanthomonas,* of *Flavobacterium,* and of a *Vibrio* could undergo variation of several kinds, and that the formation of chains or filaments by polar flagellates could give the appearance of peritrichous flagellation, has thrown doubt on the validity of this criterion. The main matter on which some difference of opinion may exist is the differentiation of the three species allotted to the genus *Pseudomonas.* Type II, which is fluorescent and relatively cryophilic, can be clearly separated. Types I and V are rather similar in most cultural characteristics except colony shape on solid media. As pseudomonads are prone to develop mutants, this feature alone might not be sufficient for specific rank, but the very profound differences in dissimilative ability, and in the capacity to utilize carbon sources, justify their separation in our view.

Finally, some comment is appropriate on our finding that several species of bacteria are capable of oxidizing arsenite. On reflection, this appears no more surprising than that diverse species, with otherwise distinct characteristics, should be able to oxidize sulphur or hydrogen. It is possible that if Green had extended his investigation beyond the dip at Onderstepoort he might have found other oxidizing species.

V. Acknowledgments

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Explanation of Plates 1 and 2

Plate 1

Figs. 1-5.—*Ps. arsenoxydans-unus*: Fig. 1.—Colonies on McConkey agar (MCA) 10 days, × 2·5. Fig. 2.—24-hr Koser, electron micrograph, × 5600. Fig. 3.—Colonies on MCA, 10 days, transmitted light, × 2·5. Fig. 4.—Petri dish confluent growth on

MCA, 14 days, showing colonies flowing slowly down sides of dish, × 0·5. Fig. 5.—Smear, stained dil. basic fuchsin, PA, 2 days, × 2000.

Figs. 6-10.—*Ps. arsenoxydans-duo*: Fig. 6.—Colonies on PA, 4 days, × 10. Fig. 7.—Colonies on PA, 14 days, × 10. Fig. 8.—Smear, stained dil. basic fuchsin, PA, 2 days, × 2000. Fig. 9.—Smear, stained dil. basic fuchsin, Koser, 24 hr, × 2000. Fig. 10.—Smear, Leifson's flagella strain, Witte peptone veal infusion (WPVI), 2 days, × 2000.

PLATE 2

Figs. 1-4.—*Achr. arsenoxydans-tres*: Fig. 1.—Colonies on PA, 4 days, × 10. Fig. 2.—Smear, stained dil. basic fuchsin, PA, 2 days, × 2000. Fig. 3.—Smear, stained dil. basic fuchsin, KA3, 2 days, × 2000. Fig. 4.—Smear, Leifson's flagella stain, WPVI, 2 days, × 2000.

Figs. 5-9.—*Xanth. arsenoxydans-quattuor*: Fig. 5.—Colony on PA, 3 days, × 10. Fig. 6.—Confluent growth on PA, 3 days, × 10. Figs. 7 and 9.—Smear, Leifson's flagella stain, WPVI, 2 days, × 2000. Fig. 8.—Smear, stained dil. basic fuchsin, PA, 2 days, × 2000.

Figs. 10-13.—*Ps. arsenoxydans-quinque*: Fig. 10.—Colonies on PA, 3 days, × 10. Fig. 11.—Smear, stained dil. basic fuchsin, WPVI, 2 days, × 2000. Fig. 12.—Smear, stained dil. basic fuchsin, PA, 2 days, × 2000. Fig. 13.—Smear, Leifson's flagella stain, WPVI, 2 days, × 2000.

APPENDIX I

MONOGRAPHS OF THE PROPOSED NEW SPECIES[*]

The techniques used for certain of the tests were as follows:

(i) *Nitrate Reduction.*—The medium of Spray (1936) with the diphenylamine test for nitrate, the α-naphthylamine-sulphonic acid test for nitrite, and gas bubbles as evidence of reduction to N_2, NO_2, or both.

(ii) *Indole Production.*—Bacto-Tryptone peptone water, with the vanillin reagent.

(iii) *H_2S Production.*—Bacto lead acetate agar.

(iv) *Arsenite Oxidation.*—KA3 medium.

1.   *Pseudomonas arsenoxydans-unus* sp. nov.

Rods: 0·9-1·0 by 1·6 by 2·3 μ (measured from electron micrographs of cells grown in Koser's citrate medium); 0·4-0·6 by 1-2 μ (measured from photomicrographs of stained smears of cells grown on potato extract peptone agar). Tendency to form radiating clumps or rosettes (Plate 1, Fig. 5). Motile; single polar flagellum up to 7 μ long, with 2½ undulations (Plate 1, Fig. 2). Gram-positive. Spores not formed.

Pigment: None.

Gelatin: Not liquefied.

[*] Dried subcultures of the type strains have been submitted to the National Collection of Type Cultures, London.

Agar: Circular, domed, glistening, creamy colonies which may later develop greyish flecks or streaks; very soft and semi-fluid. Readily coalesce when contiguous (Plate 1, Fig. 1). By transmitted light, translucent with denser light brownish centres (Plate 1, Fig. 3). Colonies may eventually reach 2 cm in diameter; they readily flow under the influence of gravity, and may drip to the lower lid of an inverted petri-dish culture (Plate 1, Fig. 4).

When grown in presence of arabinose, xylose, adonitol, or erythritol, variants may develop which form small, whitish, opaque, circular, entire, raised colonies with a crateriform depression.

Broth: Turbid growth with formation of slight pellicle; later a tenacious mucinous deposit.

Litmus milk: Slowly becomes slightly alkaline, with decolorization in the depth.

Inspissated serum: Not liquefied.

Potato slope: Colourless, confluent growth.

Nitrate: Broken down to N_2.

Catalase: Positive.

Indole: Not formed.

Hippurate: Split.

Hydrogen sulphide: Small amount formed.

Oxygen requirements: Strongly aerobic. Does not grow anaerobically in meat infusion peptone broth. Very slight growth anaerobically on potato infusion agar or on blood agar. Grows in the depths of nitrate agar, or along the needle track in gelatine stabs, but only on the surface of glucose agar shakes.

Fermentations: Type of attack is oxidative. Gas not produced.

(a) In peptone water, acid as follows: arabinose, xylose, rhamnose, glucose, fructose, erythritol, and adonitol. Not with mannose, galactose, saccharose, maltose, lactose, trehalose, raffinose, starch, inulin, dextrin, glycogen, methanol, ethanol, glycerol, mannitol, dulcitol, sorbitol, salicin, aesculin, amygdalin, or inositol.

(b) With cells harvested from potato extract peptone agar, acid as follows: arabinose and xylose (weak); glucose (strong), fructose (weak). Not with any of other substances listed above.

Carbon sources utilized: All those listed above, except lactose, raffinose, starch, glycogen, salicin. In addition, utilizes following acids: formic, acetic, lactic, oxalic, mucic, tartaric, ascorbic, glycollic, succinic, fumaric, malic, aconitic, pyruvic, mandelic, adenylic, p-hydroxybenzoic, p-hydroxyphenylacetic, p-amino-benzoic, pimelic, phthalic, β-glycerophosphoric, uric; but not butyric, benzoic, o-aminobenzoic, salicylic, phenylpropionic, or α-naphthylacetic acid. Also utilizes phenol, aniline, pyridine, α-picoline, s-collidine, diethyleneglycol, cyclohexanol, methyl-cyclohexanone, glucose-1-phosphate, and fructose-1, 6-diphosphate; but not p-cresol.

Temperature requirements: No appreciable growth in 4 wk at 3°C; grows at 37°C but not at 44°C. Optimum near 25°C.

Arsenite oxidation: In Koser's citrate medium containing 0·02M arsenite, complete oxidation in 11 days at 25°C; less active at 37°C.

Source: Ten arsenical cattle-dipping fluids from southern Queensland. "Strain 1" is regarded as the type strain.

2. *Pseudomonas arsenoxydans-duo* sp. nov.

Rods: Rather sharply divided into two sizes of cells (Plate 1, Fig. 8): smaller 0·5 by 1 μ, larger 1 by 6 μ (cells grown on potato extract peptone agar). Cells grown in Koser medium not so thick (0·7 μ) (Plate 1, Fig. 9). Motile; flagella 5-6 μ long with 2½ undulations; single or in tufts up to four (Plate 1, Fig. 10). Gram-negative. Spores not formed.

Pigment: Greenish yellow fluorescent pigment.

Gelatin: Not liquefied. Slightly arborescent growth along the stab.

Agar: Large creamy-white, circular, entire, domed colonies (Plate 1, Fig. 6); later develop flattened margin (Plate 1, Fig. 7).

Broth: Heavy turbid growth without pellicle; later a heavy deposit which readily resuspends. Greenish yellow fluorescence.

Litmus milk: Slowly becomes slightly alkaline, with decolorization in the depths.

Inspissated serum: Not digested.

Potato slope: Confluent growth, very slight brown pigmentation.

Nitrate: No gas; reduction to nitrite, and later to NH_3.

Catalase: Positive.

Indole: Not formed.

Hippurate: Split.

Hydrogen sulphide: Small amounts formed.

Oxygen requirements: Grows only slightly under anaerobic conditions on surface of potato extract, peptone agar, blood agar, and McConkey agar, but grows well anaerobically in nutrient broth, in Koser medium, and in KA3.

Fermentations: Type of attack is oxidative. Gas not produced.

(*a*) In peptone water, acid as follows: xylose, arabinose, glucose, and fructose. *Not* with rhamnose, mannose, galactose, with any di-, tri-, or polysaccharides, with any mono-, di-, tri-, tetra-, penta-, or hexahydric alcohols, with glucosides, or with inositol.

(*b*) With cells harvested from potato extract peptone agar, acid as follows: arabinose, xylose, *but not* rhamnose; glucose, mannose, galactose, *but not* fructose; raffinose and polysaccharides not attacked; of the disaccharides, only maltose is attacked; of the alcohols, only ethanol and dulcitol are attacked; aesculin and amygdalin are attacked, *but not* salicin; inositol *not* attacked.

Carbon sources utilized: Arabinose, xylose, glucose, fructose, galactose, maltose, inulin, dextrin: methanol, ethanol, glycerol; amygdalin; glucose-1-phosphate; fructose-1, 6-diphosphate. Acids utilized: formic, acetic, lactic, oxalic, mucic, tartaric, ascorbic, glycollic, succinic, fumaric, malic, aconitic, pyruvic, mandelic, adenylic, butyric, *p*-hydroxyphenylacetic, benzoic, *p*-hydroxybenzoic, pimelic, phthalic, β-glycerophosphoric, phenylpropionic, uric, and α-naphthylacetic; *but not* *p*-aminobenzoic, *o*-aminobenzoic, or salicylic acid.

BACTERIAL OXIDATION OF ARSENITE.   I
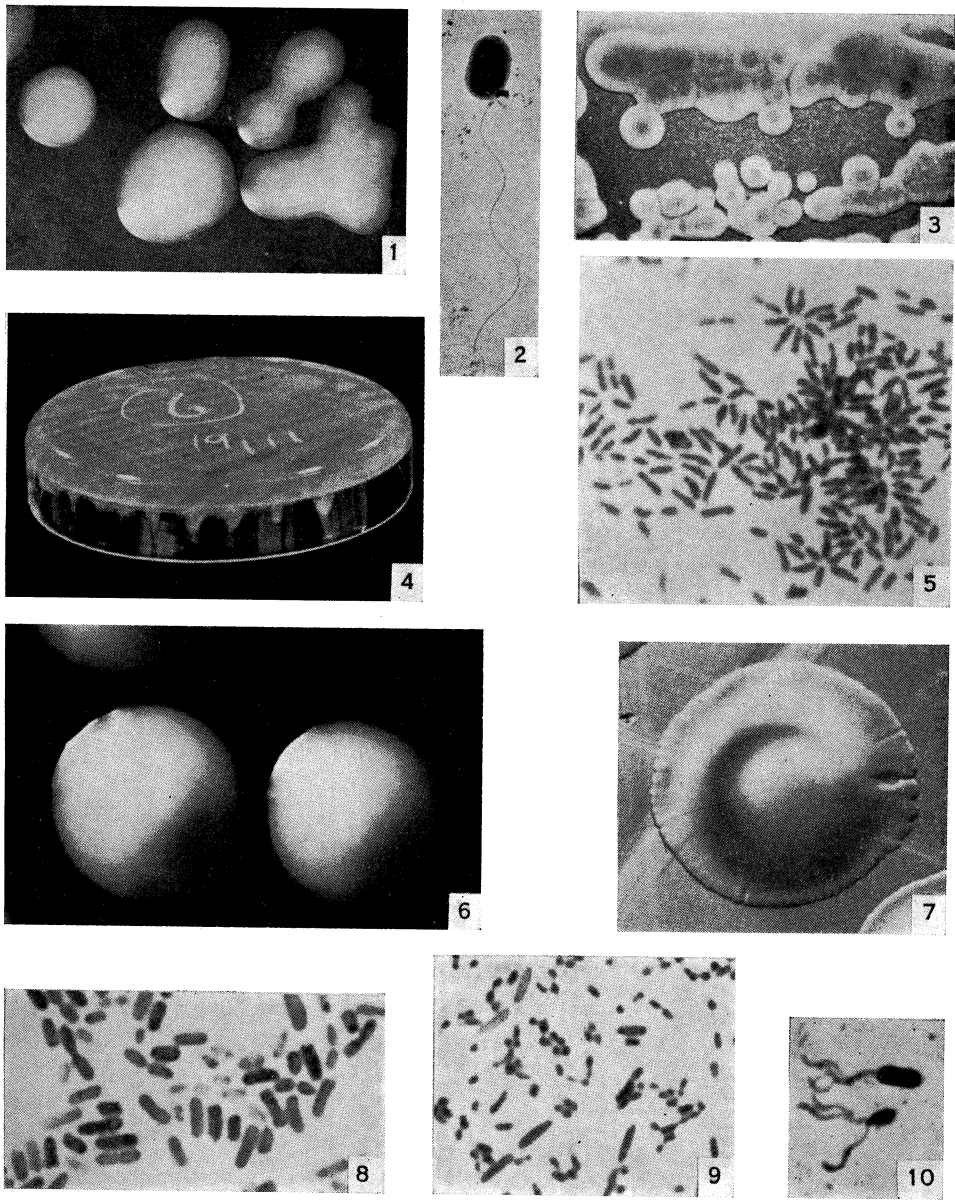
BACTERIAL OXIDATION OF ARSENITE. I
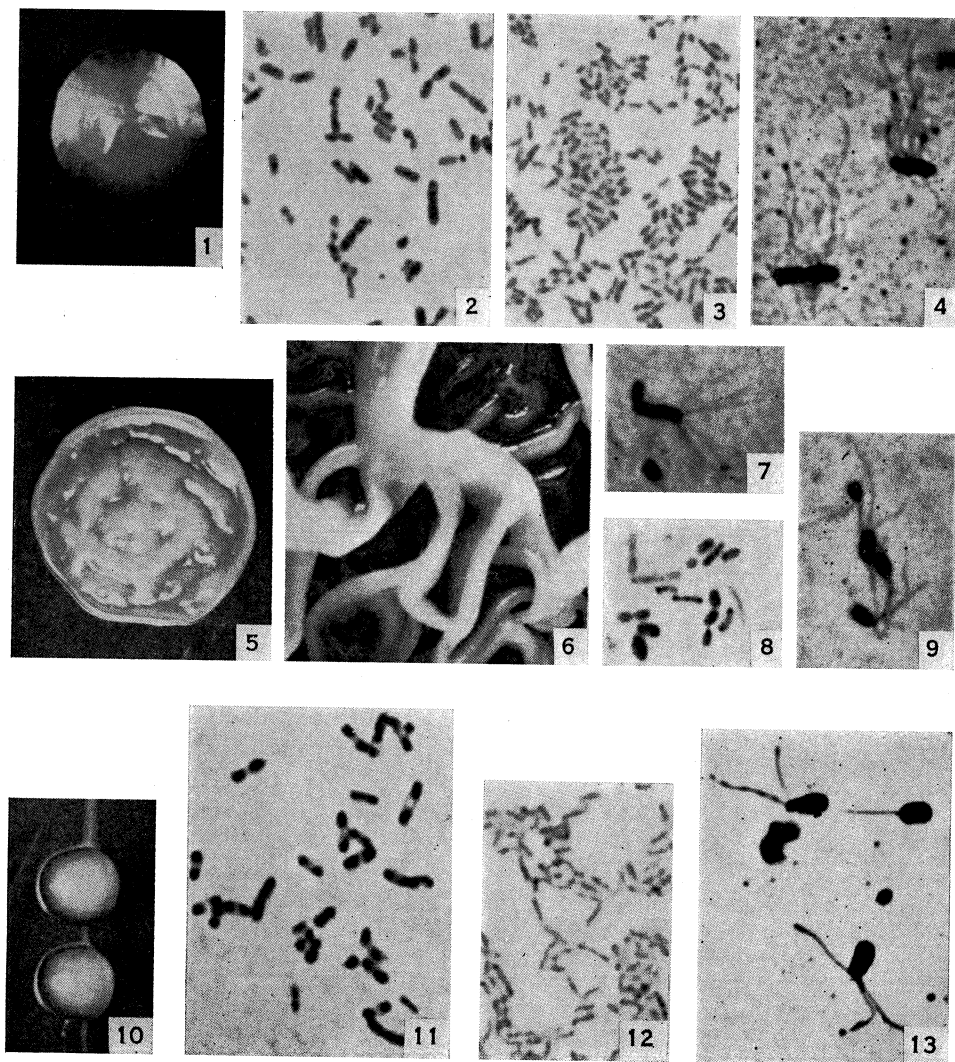
Also utilizes phenol, aniline, p-cresol, pyridine, α-picoline, s-collidine (one strain), diethyleneglycol, methyl-*cyclo*hexanone; *but not cyclo*hexanol.

Temperature requirements: Grows well on surface of potato peptone agar and in infusion peptone broth at 3°C; also at 25°C; but not at 37°C or higher. Optimum probably near 25°C.

Arsenite oxidation: In KA3 complete oxidation of 0·02M arsenite in 7 days at 25°C; does not grow at 37°C.

Source: Two arsenical cattle-dipping fluids from near south coast of Queensland. "Strain 5" is regarded as the type strain.

3. *Achromobacter arsenoxydans-tres* sp. nov.

Rods: 0·5 by 0·6 μ to 0·7 by 3 μ (cells grown on potato extract peptone agar) (Plate 2, Fig. 2); 0·35 by 0·5 μ to 0·45 by 2 μ (cells grown in KA3 medium) (Plate 2, Fig. 3). Motile; flagella 5-6 μ long, with 2½ undulations; 8-14 flagella, peritrichial (Plate 2, Fig. 4). Gram-negative. Spores not formed.

Pigment: Not formed.

Gelatin: Not liquefied.

Agar: Circular, entire, domed creamy colonies (Plate 2, Fig. 5); later a flat periphery develops.

Broth: Heavy turbid growth without pellicle; later a heavy deposit which readily resuspends.

Litmus milk: Slowly becomes slightly alkaline, with decolorization in the depths.

Inspissated serum: Not liquefied.

Potato slope: Colourless, confluent growth.

Nitrate: Gas not formed; reduction to nitrite, and later to NH_3.

Catalase: Positive.

Indole: Not formed.

Hippurate: Split.

Hydrogen sulphide: Small amount formed:

Oxygen requirements: Strongly aerobic; under anaerobic conditions, very slight growth on blood agar, but no growth on potato peptone agar, McConkey agar, in nutrient broth, in Koser medium, or in KA3. Grows in depths of nitrate media, but only on surface of glucose agar shakes.

Fermentations: Acid is not produced from pentoses, hexoses, saccharides, alcohols (mono-, di-, tri-, tetra-, or hexahydric), glucosides, or inositol, either by cultures in peptone water or by thick suspensions of cells harvested from potato extract peptone agar.

Carbon sources utilized: Of the same large range of substances, only methanol, ethanol, inulin, and amygdalin are able to support growth. Fructose-1, 6-diphosphate, *but not* glucose-1-phosphate, is also utilized. On the other hand, it can utilize a large variety of organic acids, namely formic, acetic, lactic, oxalic, tartaric, glycollic, succinic, fumaric, malic, aconitic, pyruvic, adenylic, butyric, p-hydroxyphenylacetic, p-aminobenzoic, pimelic, phthalic, and β-glycerophosphoric acid; *but not* mucic, o-aminobenzoic, ascorbic, salicylic, phenylpropionic, uric, or α-naphthylacetic acids. It utilizes aniline, pyridine, α-picoline, s-collidine,

diethyleneglycol, *cyclo*hexanol, and methyl-*cyclo*hexanone, *but not* phenol or *p*-cresol.

Temperature requirements: It does not grow appreciably at 3°C, but grows at 25°C and 37°C. The optimum is around 25°C.

Arsenite oxidation: In KA3, complete oxidation of 0·02M arsenite in 20 days at 25°C; slightly more active at 37°C.

Source: An arsenical cattle-dipping fluid from Gladstone, Qld. "Strain 13" is the type strain.

4. *Xanthomonas arsenoxydans-quattuor* sp. nov.

Rods: 0·3 by 1 μ to 1 by 3·5 μ (cells grown on potato extract peptone agar) (Plate 2, Fig. 8). In infusion peptone broth, forms chains and long, sinuous filaments. Motile; up to six polar flagella 5-6 μ long, with 2½ undulations (Plate 2, Figs. 7 and 9). Gram-negative. Spores not formed.

Pigment: Bright yellow or brownish yellow; water-insoluble.

Gelatin: Liquefied.

Agar: Pale yellow colonies which become tough and very wrinkled on continued incubation (Plate 2, Fig. 5); confluent growth develops great irregular folds (Plate 2, Fig. 6).

Broth: Turbid growth, slight pellicle which may sink; mucinous deposit later.

Litmus milk: Slowly becomes slightly alkaline, with decolorization in the depths.

Inspissated serum: Liquefied.

Potato slope: Thick, firm, tough, gelatinous, glistening, strongly wrinkled, yellowish brown.

Nitrate: Reduced to N_2.

Catalase: Positive.

Indole: Not formed.

Hippurate: Not split.

Hydrogen sulphide: Small amount formed.

Oxygen requirements: Strongly aerobic; under anaerobic conditions, no growth in any medium except in depths of nitrate agar, or along needle track in gelatine stabs; grows only on the surface of glucose agar shakes.

Fermentations: Type of attack oxidative. Gas not produced. With cells harvested from potato extract peptone agar, acid as follows: arabinose and xylose (weak); glucose, mannose and galactose (strong), fructose (weak); aesculin (weak).

Carbon sources utilized: Xylose, glucose, fructose, galactose, maltose, starch, dextrin, glycogen, methanol, ethanol, glycerol, mannitol, and amygdalin; *but not* arabinose, rhamnose, mannose, lactose, saccharose, trehalose, inulin, erythritol, adonitol, dulcitol, sorbitol, salicin, aesculin, or inositol. In addition, utilizes following acids: formic, acetic, lactic, oxalic, tartaric, ascorbic, glycollic, succinic, fumaric, malic, aconitic, pyruvic, adenylic, *p*-hydroxyphenylacetic, phthalic, β-glycerophosphoric, uric, α-naphthylacetic; *but not* mucic, butyric, benzoic, *p*-hydroxybenzoic, *p*-aminobenzoic, *o*-aminobenzoic, or salicylic acid. Also

utilizes phenol, aniline, pyridine, α-picoline, diethyleneglycol, methyl-*cyclo*-hexanone, glucose-1-phosphate and fructose-1,6-diphosphate; *but not* p-cresol, s-collidine, or *cyclo*hexanol.

Temperature requirements: No appreciable growth at 4°C; grows at 25°C, 37°C, and even 44°C. Optimum near 25°C.

Arsenite oxidation: In KA3, complete oxidation of 0·02M arsenite in 12 days at 25°C, but cultures are unable to oxidize arsenite at 37°C or 44°C.

Source: An arsenical cattle-dipping fluid from Stuart's Creek, Qld. "Strain 16" is the type strain.

5. *Pseudomonos arsenoxydans-quinque* sp. nov.

This species (strain 17) was chosen for study of enzymic mechanism of arsenite oxidation (Turner 1949; Turner and Legge 1954; Legge and Turner 1954; Legge 1954).

Rods: 0·45-0·50 by 1-2·3 μ (on potato extract agar) (Plate 2, Fig. 11); 0·5-0·8 by 1·5-3·5 μ (in meat infusion peptone broth) (Plate 2, Fig. 12). Cells stain bipolarly. Motile; one to three polar flagella 5-6 μ long, with 2½ undulations (Plate 2, Fig. 13). Gram-negative. Spores not formed.

Pigment: Virtually none; slight amounts of yellowish pigment associated with the deposit of cells in old cultures in Koser or KA3 media.

Gelatin: Slight softening after long incubation, but no liquefaction.

Agar: Small circular domed creamy whitish colonies with entire edge (Plate 2, Fig. 10).

Broth: Turbid growth, relatively slow to settle, thick mucinous deposit.

Litmus milk: Slowly becomes slightly alkaline, with decolorization in the depths.

Inspissated serum: Not liquefied.

Potato slope: Colourless confluent growth.

Nitrate: Reduced to N_2.

Catalase: Positive.

Indole: Not formed.

Hippurate: Not split.

Hydrogen sulphide: Small amount formed.

Oxygen requirements: Strongly aerobic; under anaerobic conditions, no growth in any medium. Grows in depths of nitrate agar, or along the needle track in gelatin stabs, but only on the surface of glucose agar shakes.

Fermentations: With cells from potato extract peptone agar, acid is not produced from any pentoses, hexoses, di-, tri-, and polysaccharides, alcohols, glucosides, or inositol.

Carbon sources utilized: Xylose, maltose, raffinose, inulin, dextrin, methanol, ethanol, glycerol, mannitol, amygdalin, glucose-1-phosphate, and fructose-1, 6-diphosphate; *but not* arabinose, rhamnose, any hexose, sucrose, lactose, starch, glycogen, erythritol, adonitol, dulcitol, sorbitol, inositol, salicin, or aesculin. In addition, utilizes following acids: formic, acetic, lactic, oxalic, mucic, tartaric, succinic, fumaric, malic, aconitic, pyruvic, adenylic, p-hydroxyphenylacetic, p-aminobenzoic, pimelic, phthalic, β-glycerophosphoric, uric, α-naphthylacetic;

but not ascorbic, butyric, benzoic, *o*-aminobenzoic, or *p*-aminobenzoic acid. Also utilizes phenol, aniline, pyridine, *α*-picoline, *s*-collidine, diethyleneglycol, methyl-*cyclo*hexanone, glucose-1-phosphate, and fructose-1, 6-diphosphate; *but not p*-cresol or *cyclo*hexanol.

Temperature requirements: Does not grow at 3°C or at 44°C, but grows at 25°C and 37°C; optimum near 25°C.

Arsenite oxidation: In KA3, complete oxidation of 0·03M arsenite in 6 days when grown at 25°C, but cultures are unable to oxidize arsenite in KA3 when grown at 37°C. Cells grown in KA3 at 25°C can oxidize arsenite at 37°C (Turner 1949).

Source: An arsenical cattle-dipping fluid from Richmond, Qld. "Strain 17" is the type strain.