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# Arthrobacter atrocyaneus, n. sp., and its Blue Pigment

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With 4 Figures in the text

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In the Fall of 1954, a dark blue bacterial colony was observed as a contaminant on a plate of Sabouraud's glucose agar which had been prepared for a class at the University of Pennsylvania in Philadelphia. The unusual pigmentation attracted the interest of one of the present writers; when preliminary examination suggested that the pigment formed by the Philadelphia bacterium was similar to that described by the other writer in *Corynebacterium insidiosum* (STARR 1955; 1958), a collaboration ensued (KUHN and STARR 1956). Our group is engaged in studies of several blue-colored bacteria and their pigments; the present report is restricted to a description of the blue Philadelphia bacterium, its identification as a new species of *Arthrobacter*, and the relationship of the pigment to indigoidine — a substance previously described in *Pseudomonas indigofera* by ELAZARI-VOLCANI (1939) and in *Corynebacterium insidiosum* by STARR (1955, 1958).

## I. Description of Arthrobacter atrocyaneus, n.sp.

### *Morphology*

The most outstanding feature of the blue organism, aside from its spectacular color, is its exceeding pleomorphism. A microscopic preparation from a mature culture shows cocci of two size ranges, rods of several lengths, and irregularly shaped elements.

By continuous observation with a phase microscope, of a culture developing from a single cell planted in a nutrient agar film, it can be seen that there is a characteristic cyclic development in shape and size. This is parallelled by changes in Gram-stain reaction, in possession of flagella and, thus, in motility. Fig. 1 is a photomicrographic record of the morphological alteration with time: the three adjacent coccoid cells grew into rods within a few hours, becoming relatively long. The division of these rods is accompanied by a typical snapping post-fission movement which, together with superficially similar arrangements due to development of rods or adjacent coccoid elements, is responsible for the typical V-shaped formations (STARR and KUHN 1960). These rods develop,

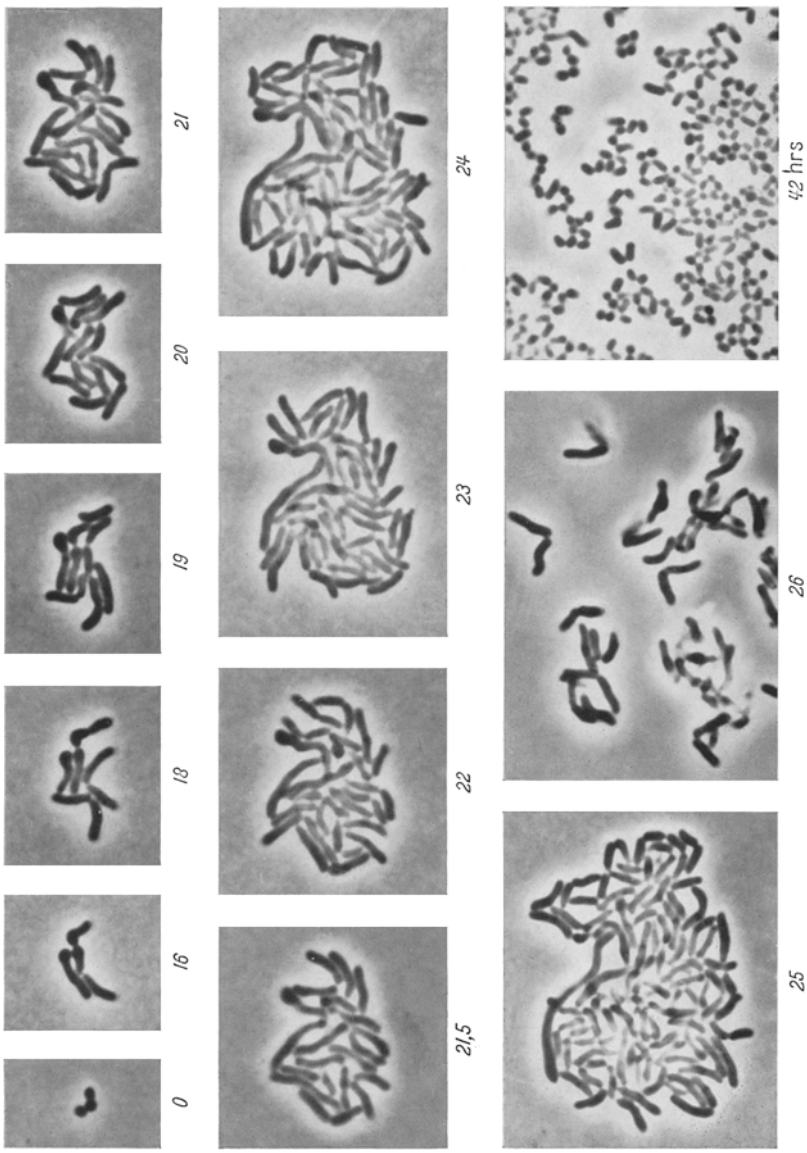


Fig. 1. Continuous phase-microscopic observation of the development of *Arthrobacter atrocyaneus* on a block of nutrient agar suspended in a slide-chamber; 22—23° C

finally, into short rods and coccoid elements; thus completing the cycle. The nature of the culture medium has a profound effect upon shape and size of the cells, in part by affecting the timing of the cycle.

The Gram-stain reaction of the blue bacterium varies with the cyclic alteration in shape and size. The coccoid cells are Gram-positive; the

rods may be Gram-negative, Gram-positive, or Gram-negative with Gram-positive polar granules.

The rod-shaped cells of most young cultures are motile and possess 1 to 3 flagella, often of considerable length, and arranged irregularly (Fig. 2) in the fashion termed "degenerate peritrichous" by CONN and WOLFE (1938). The cocci are generally non-motile and atrichous.

### *Cultural and Physiological Characteristics*

The determinative methods used for the characterization of this blue bacterium are described in the "Manual of Microbiological Methods"

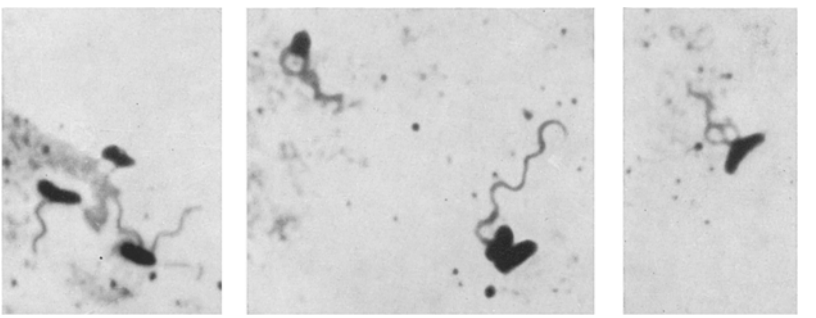

Fig. 2. Flagella stain (Gray's method) of young rod-shaped cells of *Arthrobacter atrocyaneus*. Photo: G. COSENS

(Soc. Amer. Bact., 1957) and in other contributions from this laboratory. Through the kind cooperation of various individuals (notably Drs. A. G. LOCHHEAD, L. E. SACKS, H. J. CONN, T. GIBSON and J. ØRSKOV), it was possible to compare this organism directly with authentic strains of practically every named species of *Arthrobacter* and possible relatives (except for *A. oxydans* which we isolated from soil using SGUROS', 1955, nicotine enrichment procedure).

In the conventional terms of these usual determinative tests, the blue bacterium can be described as follows:

Gelatin stab: Growth, but no blue pigment, best at surface; no liquefaction over a period of two months.

Nutrient agar colonies: Circular, three to four mm. in diameter, convex, entire, smooth, grey-white.

Nutrient agar slant: Growth filiform, raised.

Yeast extract-glucose-calcium carbonate (YDC) agar colonies: Circular, up to 5 mm. in diameter, convex, entire, smooth, blue to black with copper-like metallic luster.

Peptone agar colonies: Circular, up to 5 mm. in diameter, convex, entire, smooth, pale orange.

Sabouraud's (peptone plus glucose or maltose) agar colonies: Same appearance as on YDC agar.

Asparagine agar slant: No growth.

Sguros' nicotine agar: No growth.

Nutrient broth: Uniformly turbid; not viscous.

Potato: Pinkish brown growth.

Milk: No reaction except that a very slow reduction of the litmus is seen after 3 weeks.

Indole not produced in Bacto tryptone broth as tested with Kovac's reagent.

Hydrogen sulfide not produced in Bacto tryptone lead acetate agar, thiosulfate nutrient broth and cysteine nutrient broth.

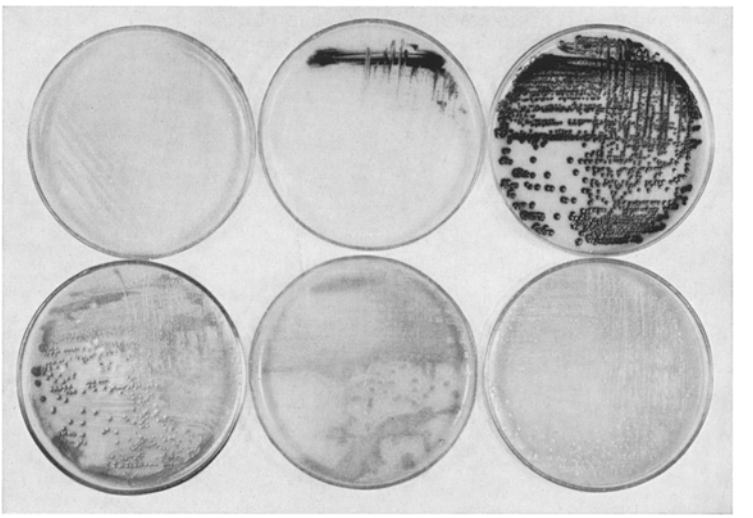

Fig. 3. Effect of temperature upon growth and pigmentation of *Arthrobacter atrocyaneus* on yeast-glucose-$CaCO_3$ (YDC) agar. Left to right, top row 5°C, 19°C, 28°C; bottom row 36°C, 39°C, 41°C (7 days old). No growth at 5°C; increasingly better growth up to 37°C; 41°C less growth than at 37°C; 45°C no growth. Pigmentation decreases with increasing temperature

Sugar media: Produces little (usually transient) or no acidity and no gas from various carbohydrates under conditions of the routine "fermentation" tests. However, addition of carbohydrates to peptone media permits blue pigment formation.

Cellulose not attacked.

Starch hydrolyzed; slightly orange-brown, mucoid colonies on starch agar.

Acetylmethylcarbinol not produced; methyl red test negative.

Nitrite produced from nitrate.

Urease not produced.

Unable to use nitrate or ammonium salts as sole nitrogen source, nor is citrate used as sole carbon source. Grows on various mixtures of amino acids; indifferent to the common B-vitamins; probably does not require ferrichrome or the terregens factor, but no systematic study has been made of its response to these growth factors.

Catalase-positive.

Aerobic.

Temperature relations: Optimum temperature for growth 37°C, at which temperature no blue pigment is produced; grows well at 24°C with abundant blue

pigmentation; at 14°C there is essentially no growth. Grows moderately well at 39°C and 41°C, but not at 45°C (Fig. 3).

Serology: Through the kindness of Dr. H. KATZNELSON, it can be stated that this organism is serologically "distinct from all the other named *Arthrobacter* species; although the antiserum does cross-react slightly with *A. tumescens* at a dilution of 1:20".

*Taxonomy*

The systematic position of this blue microbe is clearly in the genus *Arthrobacter* as redefined by CONN and DIMMICK (1947). This genus, which has been studied recently by LOCHHEAD (1957, 1958), SUNDMAN (1958), and SACKS (1954), is characterized by a cyclic alteration in morphology precisely as described herein.

The blue organism has been compared directly with authentic cultures of the named *Arthrobacter* species; it differs from all species of that genus listed in the VII Edition of "Bergey's Manual of Determinative Bacteriology" (LOCHHEAD 1957) and from the two new species recently described by LOCHHEAD (1958). The organism is actively motile in young cultures; of the presently recognized *Arthrobacter* spp., only the rods of *A. citreus* and *A. simplex* are reported to be feebly or occasionally motile. The organism does not liquefy gelatin; according to the literature (LOCHHEAD 1958) and our own experience, most *Arthrobacter* species possess this capacity. The temperature optimum for growth is comparatively high, much like that of *A. simplex* and *A. flavescens*.

The only blue-pigmented *Arthrobacter* species in the literature is *A. oxydans* (SGUROS 1955). SGUROS' organism forms, only on nicotine agar, a diffusible blue pigment which turns red to yellow-brown with age. The organism described here does not grow on nicotine agar; produces its non-diffusible blue pigment under quite different conditions as described below; and further differs from *A. oxydans* in being motile, not being able to grow on asparagine agar, not liquefying gelatin and in its optimum growth temperature.

Under these circumstances, we feel that there is ample justification for the claim that this blue bacterium is indeed a new species, for which we suggest the name *Arthrobacter atrocyaneus*, n. sp., meaning dark-blue.

In the determinative key prepared by LOCHHEAD (1957, 1958), *Arthrobacter atrocyaneus* would be grouped with the nutritionally more exacting species (*A. citreus*, *A. tumescens*, *A. terregens*, *A. flavescens*, and *A. duodecadis*) from which it is readily distinguished on the basis of blue pigmentation, lack of need for the terregens growth factor, diastatic action, inability to liquefy gelatin, and higher optimum growth temperature.

## II. The Blue Pigment of Arthrobacter atrocyaneus

The pigment of *A. atrocyaneus* has a brilliant blue color in dilute suspensions or solutions; it is produced so copiously on a suitable agar

medium that the colonies look almost black, with a beautiful metallic luster. Many cultural conditions affect pigmentation:

1. *A. atrocyaneus* produces the blue pigment only when sugar is present in a peptone medium; although heavy growth occurs without sugar, no blue pigment is formed (Fig. 4). Glucose, galactose, xylose, maltose and lactose have been found to bring about blue pigment formation;

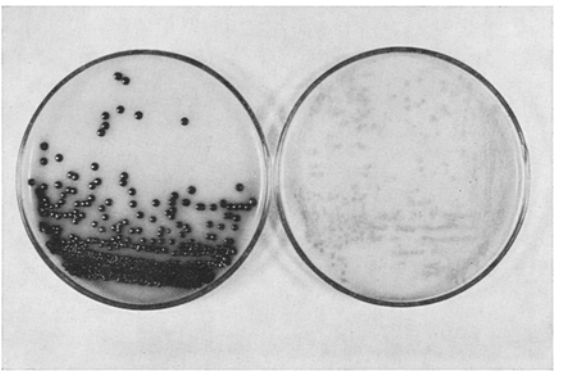

Fig. 4. Effect of sugar upon the blue pigmentation of *Arthrobacter atrocyaneus*. Left, yeast-glucose-$CaCO_3$ (YDC) agar; right, the same medium without glucose

the sugar alcohol sorbitol also serves this function. On starch or cellulose agar the organism forms orange-brown colonies, while on agar containing solely peptone or peptone and cellulose slightly orange colonies are found.

2. Temperature also plays a significant role in blue pigment formation. Although *A. atrocyaneus* grows best at 37°C, it does not produce any pigment at this temperature (Fig. 3). Pigment production in proportion to growth becomes greater with lower temperatures. At 18°C this proportion is high; however, in order to obtain large quantities of pigment, it is best to let the organism grow at room temperature of about 24°C. It is also interesting to note that colonies which are colorless due to incubation at 37°C turn blue within half an hour after being placed at room temperature.

3. The $p_H$ optimum for pigment formation is about 6.0.

4. Oxygen is essential for blue pigment formation. The organism evidently produces a reduced form of the pigment which is oxidized to its blue state through contact with molecular oxygen. This oxidation-reduction effect can be observed in a static liquid culture, in which the blue pigment appears upon shaking with air.

As in *Corynebacterium insidiosum* (*cf.* Fig. 1 of STARR 1958), the pigment from sugar-agar cultures accumulates extracellularly in granules which are usually several times as large as the bacterial cells. This

difference in mass facilitates the separation of the pigment from the cells, which can be accomplished by centrifugation at low speeds, followed by decanting the layer of cells, resuspending the pigment in water, recentrifuging and repeating the process several times. *A. atrocyaneus* does not exhibit the "crowding effect" as does *C. insidiosum* (*cf.* Fig. 4 of STARR 1958). This makes it possible to obtain large quantities of pigment from the confluent growth of *A. atrocyaneus* on the surface of agar in large trays. The pigment can then be harvested after 5 days' growth at room temperature.

The crude blue pigment of *A. atrocyaneus* possesses properties suggestive of the pigment indigoidine as that material is described from *Pseudomonas indigofera* by ELAZARI-VOLCANI (1939) and from *Corynebacterium insidiosum* by STARR (1955, 1958). The exact chemical nature of the blue pigment is at present under investigation.

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