PROPOSAL OF A NEW SPECIES PSEUDOMONAS KINGII¹

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ABSTRACT. The cultural, biochemical, and serological characteristics of an undescribed Pseudomonas species is presented. From the results obtained, it appears that the isolates studied constitute a new species. Pseudomonas kingii n.sp. is therefore proposed, and the type organism for the group, isolate A977 (ATCC 25609) is designated as the type strain.

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INTRODUCTION

The microorganism described in this paper was first isolated from a urine in New Jersey in 1951. Between August 11, 1951 and 1965, more than 135 isolates of this microorganism were submitted from 20 states and the District of Columbia to the National Communicable Disease Center, Atlanta, Georgia for identification. These microorganisms were isolated from a variety of clinical sources such as urine, blood, bronchial washings, eye, stool, umbilicus, anterior nares, throat, pyelonephritis, knee joint, vagina, vaginal secretions, hands, anesthesia bottle, sinus drainage, thyroid scar, kidney, myocardial abscess, incision wound, amniotic fluid, spleen at autopsy, hand dip, pleural fluid, lesion on head, thoracentesis, spinal fluid, sputum, and perhaps other sources.

These isolates were designated as Eugonic Oxidizers Group Number One (EO-1) by the late Miss Elizabeth King of the National Communicable Disease Center. The term 'eugonic oxidizers' refers to the ability of the organism to grow abundantly on most laboratory media and the oxidative utilization of carbohydrate substrate by the organism. The group number relates to a common characteristic among the isolates that separate them from other eugonic oxidizers in their biologic activity; namely, their overall biochemical similarity. The isolates were studied on the basis of morphological, cultural, biochemical, and serological characteristics, and animal pathogenicity in mice and guinea pigs.

¹ This investigation was performed as part of the Laboratory Directors' Training Program of the University of North Carolina, School of Public Health, in cooperation with the Laboratory Division, National Communicable Disease Center, Atlanta, Georgia. This program is supported by Training Grant No. 5T1 GM567, from the National Institute of General Medical Science, National Institutes of Health, United Public Health Service.

RESULTS

The morphological studies showed pleomorphic, Gram-negative bacilli with rounded ends, 0.5-0.8 μ in width and 1.1-2.5 μ in length.

The organisms were usually motile with one to several wavy polar flagella; lateral flagella were also present on some isolates.

Various biochemical tests showed that the EO-1 isolates are a relatively homogenous group of bacteria in their utilization of substrates. The isolates attacked a number of carbohydrates by an oxidative metabolism. Acid without gas was produced from glucose, xylose, lactose, maltose, cellobiose, and galactose by all of the isolates studied (135). Mannitol, sucrose, arabinose, inositol, levulose, mannose, trehalose, and glycerol were oxidized by most of the isolates (range 93.5-99.3%). Dextrin, erythritol, glycogen, inulin, melezitose, melibiose, rhamnose, and starch were not attacked. Oxidase was produced by all except three of the 138 isolates tested. A modification of the Kovacs oxidase test (1956) was used for the determination, using 0.5% of tetramethyl-p-phenylenediamine dihydrochloride. Of the 139 isolates tested, 88 oxidized gluconate to 2-keto-gluconic acid. Malonate was utilized by all 139 isolates tested and nearly all of them gave a positive reaction in the malonate broth within the first 4 days. On the other hand, sodium alginate was not utilized by any of the 139 isolates tested.

Urease was produced by 113 of 138 isolates tested (81.9%). Fat, in the form of corn oil (Mazola brand), was hydrolyzed by all except one of the 139 isolates tested (99.3%). Gelatin was hydrolyzed by 75% of 139 isolates tested. There were 128 of 138 isolates able to hydrolyze aesculin (92.1%). Ammonia was produced from peptone by 122 out of 138 isolates tested (88.4%). None of the 138 isolates of EO-1 tested was considered to have produced H_2S by either of the methods used (TSI slants with a lead acetate paper strip suspended over the medium). Indol was not produced by any of the 138 isolates of EO-1 studied.

The methyl red reaction was negative for all except four of the 139 isolates of EO-1 tested (2.9%). The four reactions were considered to be equivocal. The V-P reaction was negative for all of the 138 isolates. Nitrates were reduced to nitrites without formation of gas by 52 of the 139 isolates of EO-1 examined (37.4%). All of the 139 isolates of EO-1 tested gave a negative test for the presence of phenylpyruvic acid upon the addition of the ferric chloride reagent to the slant surface.

A yellow pigment was produced by 92 of the 139 isolates tested (66.2%), when they were grown on TSI agar slants with or without the phenyl red indicator. The pigment seems to be an iron dependent pigment produced only on iron-containing media. When spot tested with concentrated H_2SO_4 , the pigment failed to give the blue color supposedly characteristic of carotenoid substances (Steiger, 1941).

All 139 isolates of EO-1 studied grew on MacConkey agar slants and the majority grew on cetrimide agar slants (73.7%). Of the 139 isolates, 62 grew on SS agar slants, although only 5 of the 62 isolates (3.6%) were definitely able to grow on SS agar slants without any signs of inhibition. Only 57 isolates grew in the butt of the slant. This might indicate the tendency of the inoculum to accumulate at the base of the slant, thereby giving the organisms a foothold for multiplication. The temperature optimum for the organism is 37°C.

The colonial morphology of the EO-1 isolates was observed on blood agar plates, containing 5% defibrinated rabbit blood and incubated for 22-24 hours before reading. The colonial surface of all except 7 of the 134 isolates examined was smooth. These 7 exhibited a slightly rough surface. The colonies were opaque and glistening, and some colonies had a mottled appearance. The edge was generally entire and the form circular. The elevation was generally convex and the consistency butyrous. The size of all except 3 isolates was under 1 mm (punctiform). The two nonpunctiform isolates exhibited colonial size up to 2 mm. Most of the isolates lysed red cells without discoloration of the hemoglobin. This type of lysis was most marked where the growth was dense on the plate. The edge of the zone of lysis was not well defined as is the case with beta hemolysis.

The main distinguishing features of this group of bacteria may be given as follows: These pleomorphic, Gram-negative bacilli are obligate aerobes, which are usually motile with one to several wavy polar flagella (lateral flagella may be present), and which exhibit oxidative metabolism of carbohydrates, hydrolysis of fat, utilization of citrate, and the presence of catalase.

A serological study was based on tests with 19 different rabbit antisera prepared by injection of heated, washed, and formalinized antigens. By a tube agglutination test in four different dilutions (1:20, 1:40, 1:80, and 1:160) of the 19 respective sera, 137 isolates were screened; although a considerable amount of cross-reactivity occurred.

Among the isolates, the evidence obtained from the screening and final serum titrations indicates that there are several serological groups and subgroups among the 137 isolates. These groups have been designated on the basis of their reactions as Groups I, Ia, Ib, II, and III. These designations are tentative only, since serum absorptions were not carried out.

Virulence studies indicated that the EO-1 isolates are not pathogenic for mice or guinea pigs, except in extremely large dosages. The presence of an endotoxin substance was not demonstrated in the supernate of centrifuged broth cultures of an EO-1 isolate over a 7-day period. Rather, the lethal effects were shown to be due to the live organisms only.

On the basis of the evidence obtained in this study, the name Pseudomonas kingii n. sp. is proposed for these previously undescribed organisms.

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