Kurthia sibirica, A New Bacterial Species of the *Kurthia* Genus

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Six strains of aerobic, gram-positive, and non-spore forming bacteria of the genus *Kurthia* were isolated from the Magadan (or Susuman) mammoth found in the permafrost of Eastern Siberia. These strains are a phenotypically homogenous group different from the two known *Kurthia* species (*K. zopfii* and *K. gibsonii*) in that they require a great number of vitamins, do not grow in a 7% NaCl environment, and exhibit low DNA-DNA hybridization that does not exceed 45%. Furthermore, they differ from *K. zopfii* in their synthesis of yellow pigment, phosphatase activity, and lack of coccoid forms. They differ from *K. gibsonii* in their absence of growth in temperatures above 40°C.

The bacteria are referred to as *Kurthia sibirica* sp. nov. Strain type 13-2 has been deposited in the All-Union Collection of Microorganisms under reference BKM B-1549.

In the 8th edition of Bergey's manual [11] and in the approved lists of bacterial names [10] the genus *Kurthia* is represented by only one species, *K. zopfii.*

The first bacteria of the genus was isolated from the intestine of a chicken and described by German bacteriologist Kurth [15, 16]. After conducting careful microscopic observation of developing cultures of the bacteria, Kurth found that the shape of cells in young cultures was rods with long filaments, linked together. In older cultures, the shape was coccoid.

Later, the bacteria were observed by many scientists in meat and dairy products as well as in the feces of domesticated animals [12, 13], at the same time, both unpigmented and yellow-pigmented strains were isolated. After a comparative study of their properties, it was found that, unlike unpigmented strains, the yellow-pigmented strains grow at 45° [12, 17], do not require pantothenate [18], and their cells do not shorten into a coccoid shape as they age [3]. This was recently proposed [17] as a new species, *K. gibsonii*.

From samples of the stomach and intestinal contents of the Magadan mammoth, we isolated *Kurthia* strains that differ from the two known species by a number of pheno- and genotypical traits.

In this report, we propose a new species of *Kurthia* and provide a description using both newly obtained data and data previously published [2, 3, 7, 8].

Materials and Methods of Research

The object of the study was as follows: Strain type NCIB 9878^T of *K. zopfii* was acquired from the National Collection of Industrial Bacteria (Aberdeen, Scotland) and seven strains isolated in 1977 from the Magadan mammoth [2]. Six of these belong to the newly described *K. sibirica* (9, 12, 13-2^T, 40, 41, and 77) and one strain (38) of *K. gibsonii*. The identity and characteristics of the *K. gibsonii* strain were confirmed by data of earlier publications [17].

Most of the methods for studying the morphological, cultural, physio-biochemical, and chemotaxonomic properties of *Kurthia* have been described in earlier studies [2, 3, 8].

The type of flagella was determined with the help of electron microscopy of cell preparations, contrasted with phosphotungstic acid. The bacteria were grown on meat-peptone agar (MPA) at 25° for 18 hours.

Pigment formation was recorded visually, observing the growth of bacteria on various agar media: MPA, fish both with 0.005% diphenylamine and without, buffered phosphate salt medium [5] with 1% peptone and 0.3% yeast extract; the cultures were grown at various temperatures (5, 10, 15, 25, 30, and 37°). An approximate identification of pigments was carried out using spectrophotometry of ethanol extracts of the bacterial cells, grown for 3 days on fish agar (which showed the intense pigmentation). For the preparation of the fish agar, 5% dry fish preparation was dissolved in sea water.

To determine the requirements of individual vitamins, a phosphate buffered saline medium [5] was used. Into this medium was added 0.5% sodium acetate, 0.1% acid hydrolysate casein (Difco), 10 mg/L tryptophan, 10 mg/L guanine and the following vitamins listed (in mg/L): biotene – 0.005, thiamine HCl – 1, pantothenate Ca – 1, nicotinic acid – 0.05, pyridoxine HCl – 1, pyridoxamine – 1, pyridoxal HCl, pyridoxal 5-phospahte – 1, *n*-aminobenzoic acid – 0.005, folic acid – 0.05, cyanocobalamin – 0.5, *i*-inositol – 1.

The lack of bacterial growth in an environment after removal of one of the vitamins was assessed as a need for that vitamin in order to grow.

The composition of fatty acids was determined by gas-liquid chromatography; the acid pyrolysis was carried out with tetramethylammonium hydroxide [1]. For this analysis, the bacteria were grown on MPA at 25° for 24 hours.

Results and Discussion

Six strains (9, 12, 13-2^T, 40, 41, 77) of the bacteria under discussion, representing a homogenous group with great phenotypic similarities to known *Kurthia* types [2, 17] were isolated from the Magadan mammoth.

The cells of these bacteria (pictures a, b) have the form of rods with rounded ends. Their length varies considerably from the filaments and rods of the young cultures being 5-10 μm to the short rods of older cultures between 2-5 μm. The cells are motile by means of flagella (picture v), they are non-spore forming and gram positive, though can be variably colored.

The colonies (picture g) on meat-peptone agar are rhizoid and a stroke across the gelatin results in a “bird feather”-like appearance.

Bacteria are chemoorganotrophs. Growth is preferred in nutrient-rich environments containing peptone. Cells exhibit oxidative metabolism. Acid is formed from a few carbohydrates and alcohols, but not glucose. Starch is not hydrolyzed, gelatin not liquefied, nitrates not reduced, urease, lecithinase, and oxidase activity is not shown, but they are catalase positive. Bacteria exhibit hemolysis, and are sensitive to: penicillin (2 units), streptomycin (2 units), erythromycin (2 μg), novobiocin (5 μg), neomycin (5 μg), oleandomycin (5 μg), polymyxin B (300 μg), and when 0.1% triphenyltetrazolium chloride is present.

Aerobics: Bacteria grow in pH ranges 5.5 to 9.5 in temperatures of 5-37°.

Hydrolysates of cell walls contain lysine, glycine, alanine, leucine, valine, asparagine, and glutamic acid [2]. Basic fatty acids are branched and saturated among which 12-methyltetradecanoic (anti-iso-$C_{15:0}$) and 13-methyltetradecanoic (iso-$C_{15:0}$) are dominant. DNA GC content is around 37 mole% [2, 8].

Along with the previously mentioned similarities with the known *Kurthia* species, the group under discussion also have a number of distinctive features, many of which can be used to differentiate the various strains and species (see table). So, all six strains do not grow in a 7% NaCl environment, they need a vitamin rich environment and, apparently, the addition of yeast extract to an already rich environment (such as meat-peptone agar) significantly stimulates growth.

The optimal temperature for growth is around 20-25°, which is a few degrees below that of the other *Kurthia* [2]. Fresh isolated can exhibit growth as low as 0°. Unlike *K. zopfii*, these bacteria synthesize yellow pigment, exhibit phosphatase activity, and the ability to form acid from fructose and glycerol. In older cultures, cells do not shorten into a coccoid form.

At the same time, there are characteristics that bring them close to *K. gibsonii*, notably the yellow pigments that appear to belong to the same class. Other similarities include: carotenoids, which since they are not released into the environment, are not synthesized in a 0.005% diphenylamine environment, and their ethanol extracts have peaks in the range of 400-480 nm [4, 6]. However, in contrast to *K. gibsonii*, these strains do not grow in temperatures higher than 40° and their synthesis of pigment varies greatly depending on the cultivation.

How widely spread *K. sibirica* is in nature is not yet clear. Previously [2, 7] when analyzing a variety of natural substrates, the two known *Kurthia* species were found in meat and dairy products of varying freshness, the intestinal contents and feces of domesticated animals, on flies, in the stomach and intestinal contents of the Magadan mammoth, and the soft tissues of the Yuribeysky mammoth. But *K. sibirica* was only found in samples from the Magadan mammoth.

However, stating that these bacteria belonged to the native mammoth microflora (presuming we can admit the possibility of the bacteria being preserved throughout the millennia) is doubtful because the samples were not extracted immediately after the recovery of the mammoth form the permafrost. Instead the samples were extracted a few days later in the town of Magadan, where the mammoth was delivered in a frozen state [9].

Based on the observed phenotypic features of the group under discussion, as well as the observed low degree of DNA-DNA hybridization as compared to *K. zopfii* (around 20%) and *K. gibsonii* (around 40%), we consider it appropriate to classify these bacteria as their own species – *Kurthia sibirica.* Below is its description:

Kurthia sibirica sp. nov. si. bi ŕi. ca. M. L. fem. adj. sibirica (of Siberia), where the Magadan mammoth was found, from which the bacteria were isolated. Cells are regular rods with rounded ends, 0.6-1 μm in diameter and varying in length from 5-10 μm in young cultures to 2-5 μm in older cultures, but older cultures are not coccoid.

Colonies have a cream or yellow pigmentation depending on growth conditions. Nutritional requirements are complex, with good growth on peptone-containing media along with yeast extract. Biotin, thiamine pantothenate, nicotinic acid, pyridoxal 5-phosphate are required. Growth occurs in the range of 5-37°, with no growth above 40°. Freshly isolated strains can usually grow between 1-0°. Optimal temperature is between 20-25°.

Acid is produced from fructose and glycerol, but not from ribose or ethanol. Bacteria do not grow in media containing 7% NaCl. They are phosphatase positive. DNA GC content is 36.9 mole%. The typical strain is 13-2 (referenced as BKM B-1549 in the All-Union Collection).

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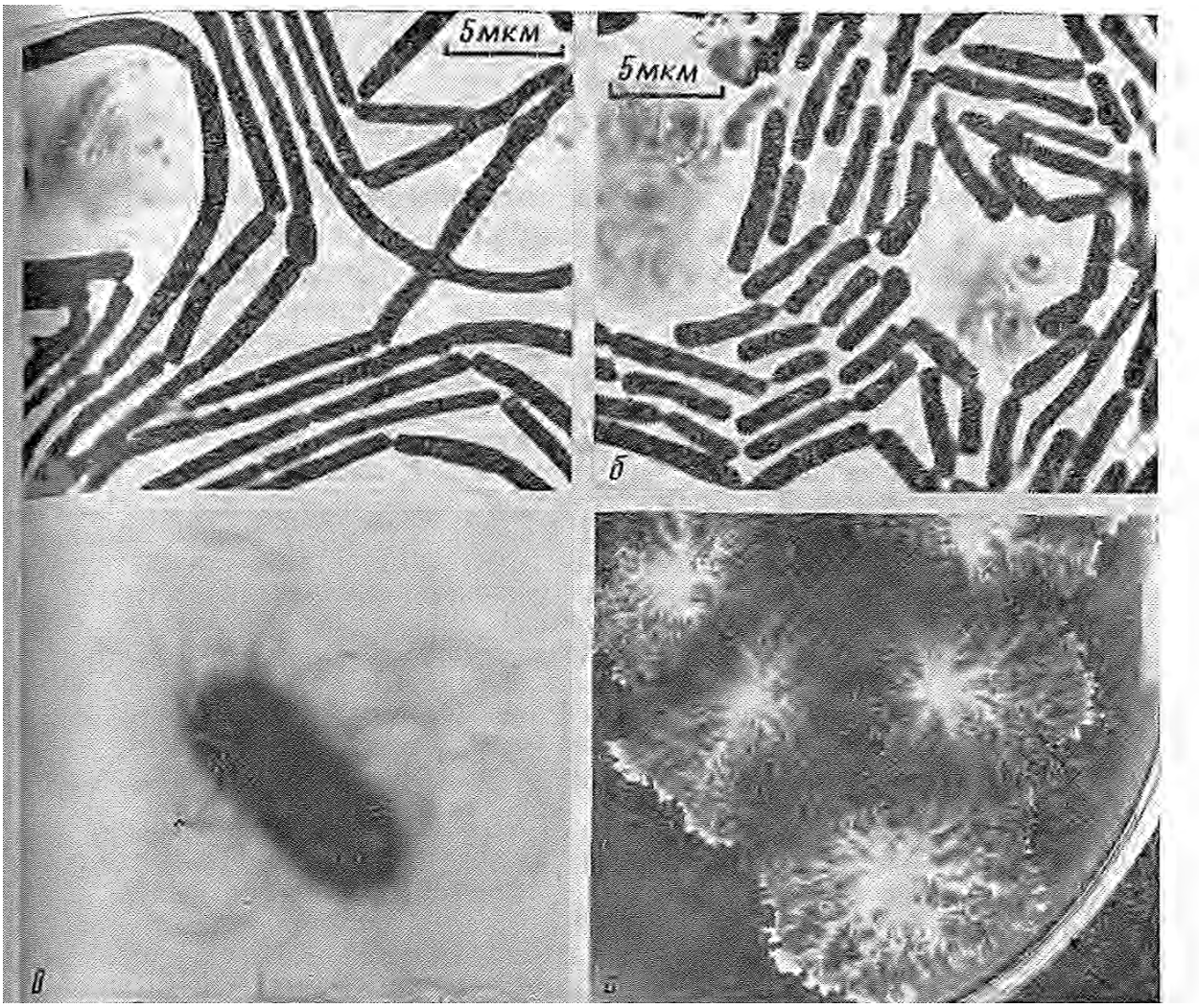

Morphology of cells and colonies of strain 13-2^{T}. View of cells growing on MPA through a micro-camera: *a* – after 18 hours, *b* – after 3 days, phase contrast, X2000, *v* – negative of a cell with flagella X12000, *g* – view of a colony on MPA cultured for 6 days, 4/5 scale.