NOTE

Runella zeae* sp. nov., a novel Gram-negative bacterium from the stems of surface-sterilized *Zea mays

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A Gram-negative bacterium, designated NS12^T, was previously isolated from duplicate treatments of surface-sterilized *Zea mays* stems. The plants were grown in synthetic soil under greenhouse conditions and watered with fertilizer containing no nitrogen. Strain NS12^T was not isolated from plants watered with the standard level or 20% (w/v) of the standard level of nitrogen. Cells were bent rods that formed chains of irregular shapes in R2A broth. Unlike its closest described relative *Runella slithyformis*, strain NS12^T fermented glucose and sucrose. The G+C content was 49 mol%. Phylogenetic analysis of the 16S rRNA gene showed that the strain was a member of the domain *Bacteria* and is most closely related to *R. slithyformis*, a member of the *Flexibacter* group within the *Cytophaga–Flexibacter–Bacteroides* phylum. Phenotypic and genotypic analyses indicated that strain NS12^T could not be assigned to any recognized species; therefore a new species designated *Runella zeae* sp. nov. is proposed, for which NS12^T (= ATCC BAA-293^T = LMG 21438^T) is the type strain.

Keywords: *Runella zeae* sp. nov., *Zea mays*, endophyte

The new taxon *Dyadobacter fermentans* was isolated from maize (*Zea mays*) cultivated without nitrogen fertilizer in the greenhouse (Chelius & Triplett, 2000). Chelius & Triplett (2000) examined the species richness of bacteria inhabiting the stem tissue of maize grown under different nitrogen levels. *D. fermentans* and a related co-isolate, NS12^T, resided in the same stem tissue of two plants receiving no nitrogen fertilizer. This unique strain (NS12^T) has similarity to *Runella slithyformis*, a member of the *Cytophaga–Flexibacter–Bacteroides* group. *Runella* is a monospecific genus containing *R. slithyformis*, a taxon with an aquatic lifestyle (Larkin & Williams, 1978). Here, evidence is presented based on a polyphasic taxonomic study that NS12^T is a distinct species for which the name *Runella zeae* sp. nov. is proposed.

Phenotypic and genotypic methods and results including nutritional and fatty acid analyses, antibiotic

sensitivity, plant tests, DNA G+C content, PCR amplification and sequencing, and phylogenetic inference have been previously reported (Chelius & Triplett, 2000). Briefly, NS12^T and *R. slithyformis* could not be unequivocally distinguished based solely on fatty acid profile or 16S rDNA sequence similarity. *R. slithyformis* and NS12^T had different responses to many phenotypic tests. For example, NS12^T could utilize more carbon sources for growth, ferment glucose and sucrose, and would not grow at 4 °C. Cell morphology and arrangement were also quite different between the two strains. When grown on R2A broth, NS12^T would produce long filaments with swollen ends that would fall out of solution, whereas *R. slithyformis* cells were circular with swollen ends that would not form filaments. In the present study, DNA–DNA hybridization analysis was performed to resolve the relationship between these two strains.

DNA–DNA hybridization of NS12^T and *R. slithyformis* strain 4 (ATCC 29530) was carried out as described by De Ley *et al.* (1970) with the modification described by Huss *et al.* (1983) and Escara & Hutton (1980) using a Gilford System model 2600 spectrometer equipped with a Gilford model 2527-R thermo-programmer and plotter. Renaturation rates were

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computed with the TRANSFER.BAS program by Jahnke (1992). DNA was isolated by chromatography on hydroxyapatite by the procedure of Cashion *et al.* (1977). Experiments were performed at 66 °C in 2 × SSC. The level of DNA–DNA homology between NS12^T and *R. slithyformis* was 19 %. Both strains are not related at the species level, when the threshold value of 70 % for the definition of species according to the recommendation of the ad hoc committee is considered (Wayne *et al.*, 1987).

NS12^T co-exists with maize and cannot grow at a low temperature indicative of an aquatic lifestyle maintained by other members of related bacteria. The plant hypersensitivity response (HR) test was done to identify a potential antagonistic relationship between strain NS12^T and *Zea mays* (Chelius & Triplett, 2000). There was no detectable HR in tobacco. However, this does not confirm the nature of the relationship between NS12^T and maize as a harmless one. To observe a potential growth response of maize to NS12^T, a bacterial inoculum was applied to maize seeds. There was no difference in dry weights among uninoculated controls and plants inoculated with NS12^T, over all three levels of N fertilizer tested. Thus NS12^T appears to be neither beneficial nor pathogenic to maize since inoculated plants appeared healthy and the yield of above-ground plant biomass was unaffected.

Phenotypic and phylogenetic analyses provided ambiguous evidence that NS12^T was a unique strain. Cluster analysis of fatty acid composition between *R. slithyformis* and NS12^T placed these within the same species and 16S rDNA sequence similarity between the two strains (94 %) was too distant to determine the degree of relatedness (Chelius & Triplett, 2000). NS12^T is phenotypically unique in that it: (i) has a cell morphology consisting of straight to slightly bent rods instead of a spiral-like form found with *R. slithyformis* and some members of the *Flexibacter* group; (ii) ferments glucose and sucrose; (iii) utilizes more carbon sources than *R. slithyformis*; (iv) grows on peptone water; and (v) does not tolerate a growth temperature of 4 °C (Chelius & Triplett, 2000). This evidence taken collectively with results of DNA–DNA hybridization analysis confirms that NS12^T represents a hitherto unknown *Runella* species. Consistent with the phenotypic characteristics of the *Runella* genus, NS12^T is aerobic, chemo-organotrophic, does not hydrolyse gelatin, and forms pale-pink colonies.

Description of *Runella zeae* sp. nov.

Runella zeae (ze'ae. N.L. gen. n. *Zae* of *Zea*, named because the organism was isolated from maize, *Zea mays*).

Most of the phenotypic data presented below were first published in Chelius & Triplett (2000). Cells are Gram-negative, straight to slightly bent rods that form chains of irregular shapes. Colonies are round, smooth and salmon in colour when grown on R2A at 28 °C. This

strain ferments, and produces acid from, glucose and sucrose. In contrast with *Runella slithyformis*, no acid produced from ribose. Weakly catalase-positive and oxidase-positive. Temperature range for growth is 15–37 °C and highest NaCl concentration tolerated is less than 1.5 %. This strain will grow on peptone water and weakly on Ayer's agar. It will not grow on litmus milk. This strain will grow on the following sole sources of carbon: acetate, amidon, fumarate, D-lyxose, malate, malonate, sucrose, tartrate, erythritol, D- and L-arabinose, β-methylxyloside, galactose, D-glucose, D-fructose, mannose, rhamnose, methyl α-D-mannoside, methyl α-D-glucoside, N-acetylglucosamine, amygdaline, arbutine, aesculin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, inulin, melezitose, D-raffinose, xylitol, β-gentiobiose, D-turanose, D-tagatose and D- and L-fucose. This strain grew weakly on dulcitol, inositol, 5-ketogluconate, sorbitol and sorbose. This strain did not grow on D- and L-arabitol, glycerol, mannitol, methanol or formate. This strain cannot hydrolyse agar, cellulose or starch. In contrast to *R. slithyformis*, this strain will not grow on D-arabitol, L-arabitol, glycerol or mannitol. Unlike *R. slithyformis*, this strain will grow on glycogen. Nitrate is not reduced to nitrite. This strain is resistant to ampicillin (25 µg ml⁻¹), kanamycin (50 µg ml⁻¹) and spectinomycin (10 µg ml⁻¹) but sensitive to rifampicin (25 µg ml⁻¹) and tetracycline (10 µg ml⁻¹). Unlike *R. slithyformis*, this strain is also sensitive to chloramphenicol (25 µg ml⁻¹), streptomycin (10 µg ml⁻¹) and trimethoprim (25 µg ml⁻¹). The level of DNA–DNA homology between NS12^T and *R. slithyformis* was 19 %. The principal fatty acids are 36.9 % 15:0iso 2-OH/16:1ω7c, 17.8 % 16:1ω5c and 16.1 % 15:0iso. The G + C content is 49 mol %. This strain was isolated from surface-sterilized *Zea mays* (cv. Mo17) stem and has been deposited in the American Type Culture Collection as ATCC BAA-293^T and the BCCM/LMG Bacteria Collection as LMG 21438^T.

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