***Dyadobacter fermentans* gen. nov., sp. nov., a novel Gram-negative bacterium isolated from surface-sterilized *Zea mays* stems**

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A Gram-negative bacterium, designated NS114^T, was 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 NS114^T could not be isolated from plants watered with the standard level or 20% (w/v) of the standard level of nitrogen. Cells occurred as pairs in young cultures that attached to form angled arrangements in R2A broth and occasionally formed rounded, horseshoe arrangements in YM broth. Cell variation resulted in flocculent chains of coccoid cells in old cultures. Strain NS114^T fermented glucose and sucrose. The G+C content was 48 mol%. Phylogenetic analysis of the 16S rRNA gene showed that the strain was a member of the domain *Bacteria* and branched from a point equidistant from an aquatic organism, *Runella slithyformis* and a marine isolate, '*Microscilla furvescens*'. Phenotypic and genotypic analyses indicated that strain NS114^T could not be assigned to any recognized genus; therefore a new genus and species, *Dyadobacter fermentans* gen. nov., sp. nov., is proposed, for which NS114^T is the type strain.

Keywords: *Dyadobacter fermentans* gen. nov., sp. nov., *Zea mays*, endophyte

INTRODUCTION

In an attempt to characterize the bacterial community that exists endophytically with *Zea mays*, we isolated a non-diazotrophic strain (NS114^T) that grew on a nitrogen-limited medium. This strain was found with duplicate plants that had been watered with a fertilizer lacking nitrogen and was not isolated from the same cultivar of maize that received the standard nitrogen level or 20% (w/v) of the standard nitrogen level. Species related to strain NS114^T include the aquatic species *Runella slithyformis* and a marine species *Microscilla* spp., in addition to soil-inhabiting *Cytophaga* spp. Although strain NS114^T was isolated from plants that were nutritionally deficient in nitrogen, the plants appeared healthy. Hypersensitivity response assays suggest that the interaction between

strain NS114^T and maize is a neutral one. Additionally, strain NS114^T did not attack cellulose or starch, suggesting that this strain does not have a saprophytic lifestyle.

In this report we describe the phenotypic and genotypic properties of strain NS114^T and employ molecular phylogenetic analyses to place strain NS114^T within the *Flexibacter* group of the *Cytophaga*–*Flexibacter*–*Bacteroides* phylum.

METHODS

Bacterial strains and culture conditions. Strain NS114^T was isolated from two surface-sterilized *Zea mays* stems, genotype Mo17. Maize seeds were sterilized for 5 min with 1.05% (w/v) sodium hypochlorite, planted in an autoclaved, synthetic sand/vermiculite soil and watered with a nitrogen-free nutrient solution. This experiment also included plants treated with fertilizer containing 2.5 and 12.5 p.p.m. nitrogen. After 6 weeks growth, the plants were harvested and stems were surface-sterilized for 5 min in 1.31% sodium hypochlorite followed by five washes with sterile water. Stems were crushed with a mortar and pestle and the fluid

Abbreviation: HR, hypersensitivity reaction.

The GenBank accession numbers for the 16S rDNA sequences of strain NS114^T and *Runella* sp. strain NS12 are AF137029 and AF137381, respectively.

fraction plated on R2A (Difco) and incubated at 28 °C. A sterility check was performed by touching the plant stem onto an agar plate and incubating at 28 °C. All strains were grown at 28 °C on R2A or a broth medium containing the same components as R2A without agar unless otherwise specified. All tests were replicated two or more times and performed with positive and negative control strains. *R. slithyformis* ATCC 29530 was used as a reference strain.

Carbon assimilation test. API 50CH was used according to manufacturer's instructions. Additional carbon assimilation tests (formate, methanol and sucrose) were done using a basal medium containing 0.1 % peptone, 0.1 % yeast extract, 0.3 % agar, 0.001 % bromothymol blue and 1 % of the substrate to be tested. Growth and alkali production on organic acids were tested on the same basal medium with the following substrates: fumarate, malate, malonate, tartrate and acetate. Samples were scored for growth and acid or base production after 3 weeks incubation.

Conventional tests. HL medium (Hugh & Leifson, 1953) was used for the oxidation/fermentation test. Catalase and cytochrome *c* oxidase tests were done with cells scraped from R2A and treated with 3 % (w/v) hydrogen peroxide or tetramethyl-*p*-phenylenediamine (Difco), respectively. The nitrate reduction test was done with a semi-solid nitrate broth (Smibert & Krieg, 1981). The motility test was done with motility medium (Difco) and scored after up to 3 weeks growth. AO medium was used to assess gliding motility (Anacker & Ordal, 1959). Starch agar (Difco) was flooded with iodine to test for amylase activity after 3 weeks growth. Proteinase activity was scored as positive when 3-week-old cultures liquefied a nutrient gelatin medium (Smibert & Krieg, 1981) that had been immersed in an ice-water bath. Growth over a range of temperatures was tested with strains inoculated on R2A.

Growth on various media. Growth on a cellulose medium (Drijber & McGill, 1992) was used to determine cellulase activity. Strains were inoculated into the following broth media to determine growth potential: LB (Difco); YM broth (Beringer, 1974); peptone water (0.2 % peptone, 0.1 % NaCl, pH 7.2); litmus milk (BBL); R2A supplemented with 0, 1.5 and 3 % (w/v) sodium chloride; Ayers agar (Ayers *et al.*, 1919); and AcD semi-solid agar (Burris, 1994) without yeast extract.

Antibiotic sensitivity. Antibiotic sensitivity was tested on R2A supplemented with 25 µg ml⁻¹ each of ampicillin, chloramphenicol, trimethoprim or rifampicin, 10 µg ml⁻¹ each of tetracycline, streptomycin or spectinomycin, or 50 µg kanamycin ml⁻¹.

Flexirubin and fatty acid analysis. Flexirubin-like pigments were tested by measuring the absorbance spectrum of an ethanol and alkaline-ethanol extract of lysed cells (Weeks, 1981). Fatty acid analysis was performed using standard methods and compared to the database of fatty acids in the MIDI Sherlock Microbial Identification System (Microbial ID). G + C content was determined by using HPLC (Mesbah *et al.*, 1989).

Microscopy. Strains were grown in R2A broth or YM at 30 °C on a rotary shaker at 200 r.p.m. and visualized under phase-contrast microscopy after 24 and 48 h growth. The same cultures were prepared for SEM using the following method. Cells were fixed in 4 % (w/v) glutaraldehyde, 0.5 % *p*-formaldehyde and 0.25 M phosphate buffer (pH 6.4) for 10 min, washed with 50 mM phosphate buffer (pH 6.8) twice

and serially treated with ethanol (15, 30, 50, 70, 95, 100 %, v/v) for 10 min at each concentration. Cells were examined using a Hitachi S570 scanning electron microscope at 10 kV.

Nitrogenase assay. The nitrogenase assay (Schöllhorn & Burris, 1967) was performed on 3-d-old cultures growing in AcD with and without yeast extract. Acetylene (3 ml) was introduced into tubes (30 ml) containing 10 ml medium and incubated for 1–24 h at 28 °C. Ethylene was measured by GC at 90 °C with a Porapak N column. *Klebsiella pneumoniae* was used as a positive control.

Plant tests. The hypersensitivity reaction (HR) in tobacco (*Nicotiana tabacum*) was tested as described by Fahy & Hayward (1983). Growth response of *Zea mays* to inoculation with strain NS114^T was tested by applying 1 ml 10⁶ bacteria in water to surface-sterilized seeds. Eight plants per treatment were grown in a synthetic, autoclaved sand/vermiculite mixture and watered with fertilizer containing 0, 2.5 or 12.5 p.p.m. nitrogen. After 6 weeks growth plant stems were harvested, dried and weighed.

PCR amplification and sequencing. 16S rDNA was PCR-amplified using primers (*Escherichia coli* numbering) 27f and 1492r (Lane, 1991). Purified DNA was used as template in a 50 µl PCR reaction containing 1 × PCR Buffer (Promega), 5 mM MgCl₂, 0.5 µg BSA, 200 µM dNTPs and 0.5 U *Taq* polymerase. Thermal cycling conditions were 3 min denaturation at 95 °C followed by 30 cycles at 94 °C for 20 s, 54 °C for 20 s and 72 °C for 1 min, followed by a final extension at 72 °C for 7 min. The PCR product was purified using a Qiagen PCR purification kit. The ABI cycle sequencing kit was used by the University of Wisconsin Biotechnology Center to bidirectionally sequence all strains using primers (*E. coli* numbering) 27f, 536f, 1115f, 343r, 907r, 1165r and 1492r (Lane, 1991). *Rumella* sp. strain NS12 was co-isolated with strain NS114^T and its 16S rDNA sequenced using the same methods as described above. *nifH* was PCR-amplified using the conditions described by Ueda *et al.* (1995). Sequencing of the PCR product was performed as described above but with the same *nifH* primers (19f and 407r) used for PCR amplification.

Phylogenetic analysis. The 16S rDNA sequences were assembled using Sequencher and the primary sequence of SSU was verified by manual construction of the secondary structure. Sequences were aligned against the following reference strains: *Arthrobacter globiformis* DSM 20124, *Bacteroides fragilis* ATCC 43183^T, *Chryseobacterium indologenes* ATCC 29897, *Cytophaga fermentans* ATCC 19072, *Cytophaga hutchinsonii* ATCC 33406, *Cytophaga lytica* ATCC 23178, *Flavobacterium ferrugineum* ATCC 13524, *Flavobacterium johnsoniae* DSM 425, *Flectobacillus major* ATCC 29496, *Flexibacter canadensis* ATCC 29591, *Flexibacter tractuosus* ATCC 23168, *Geotoga subterranea* strain CC-1, '*Microscilla furvescens*' ATCC 23129, *Myroides odoratus* ATCC 4651, *Pedobacter heparinus* ATCC 13125, *Persicobacter diffluens* ATCC 23140, *Planctomyces limnophilus* IFAM 1008, *Rumella slithyformis* ATCC 29530, *Sphingobacterium multivorum* strain OM-A8, *Spirosoma linguale* ATCC 23276, *Thermonema rossianum* strain AG3-1 and *Weeksella virosa* ATCC 43766. Phylogenetic relationships were inferred using SEQBOOT, DNADIST, FITCH and DNAPARS programs available in PHYLIP (Felsenstein, 1989). Unambiguously aligned positions (1064 nt) were used in the analysis. Distances were determined using the Kimura two-parameter method (Kimura, 1980) and distance data were clustered using the Fitch–Margoliash method (Fitch & Margoliash, 1967). Pseudo-replications were done with

bootstrapping using 100 resamplings. Phylogenetic trees were generated using TREEVIEW (Page, 1996).

RESULTS AND DISCUSSION

Runella sp. NS12

A sequence similarity analysis was done using the BLAST computer program available through NCBI. Strain NS12 had the highest sequence identity (94%) to *R. slithyformis* and 87% sequence identity to strain NS114^T over a length of 1393 nt. Phylogenetic analysis placed strain NS12 within the *Flexibacter* group as a close relative to *R. slithyformis* (Fig. 1). The genotypic data suggest that strain NS12 and *R. slithyformis* are different species and the phenotypic data are in strong support of this assumption (Tables 1–3). However, because DNA–DNA hybridization analysis has not been done, a species designation has not been assigned.

Enrichment and isolation

Strain NS114^T was isolated from healthy maize stem tissue that had been surface-sterilized in sodium hypochlorite. *Runella* sp. strain NS12 was co-isolated from the same stems as strain NS114^T. Both of these isolates were found in duplicate plants watered with fertilizer containing no nitrogen. Neither of these isolates was detected in plants receiving the intermediate- and standard-level nitrogen fertilizer treatments. Because all three watering solutions (0, 2.5 and 12.5 p.p.m. nitrogen) were prepared from the same stock solutions, it is unlikely that the watering solution was a source of contamination. Without microscopic

evidence that strain NS114^T is living within plant tissue and did not merely survive the sterilization treatment, we cannot unequivocally conclude that this strain lives endophytically. However, the cumulative data suggest that strain NS114^T does associate with maize grown under limited nitrogen nutrition.

Genotypic features

The G + C content of strain NS114^T is 48 mol%. The DNA base composition of related bacteria varies widely (Table 1) with *R. slithyformis* having the most similar DNA base composition of 49 mol% (Larkin & Williams, 1978).

A sequence similarity analysis was done using the BLAST computer program available through NCBI. Strain NS114^T had the highest sequence identity (87.6%) to *R. slithyformis* over a length of 1393 nt. The phylogenetic placement of strain NS114^T consistently fell along the *R. slithyformis* branch within the *Flexibacter* group, when both distance and parsimony analyses were used (Fig. 1). This placement was supported in 100% of bootstrap resamplings. Distance analysis placed strain NS114^T approximately equidistant from both *R. slithyformis* and '*M. furvescens*'; however, it is phenotypically very different from both species (Table 1). This is reflected in the type of habitat all three strains occupy; *R. slithyformis* is aquatic, '*M. furvescens*' is marine and strain NS114^T associates with maize. 16S rDNA signature sequences that define the flavobacteria and relatives (Woese *et al.*, 1990) were identified in the secondary structure of strain NS114^T.

Fig. 1. Phylogenetic tree based on 16S rDNA sequence comparisons of strains NS114^T, NS12 and bacteria within the *Cytophaga*–*Flexibacter*–*Bacteroides* group. Bootstrap values at 50% or higher are shown at the branch points. Scale bar indicates 10 inferred nt substitutions per 100 nt. GenBank accession numbers are indicated in parentheses.

Table 1. Some genotypic, phenotypic and nutritional characteristics that distinguish strains NS114^T and NS12 from related bacteria

Data are from this study, Raj (1977) and Reichenbach (1989). +, Positive; –, negative; NA, data not available from the sources used; SL, slight.

Characteristic	<i>Runella slithyformis</i>	<i>Flectobacillus major</i>	<i>Cytophaga hutchinsonii</i>	<i>Flexibacter tractuosus</i>	' <i>Microscilla furvescens</i> '	Strain NS114 ^T	Strain NS12
Colony colour	Salmon	Pink	Bright yellow	Orange	Orange	Yellow	Salmon
Motility	None	None	Gliding	Gliding	Gliding	None	None
Cell Morphology:							
Spiral-like	+	+	–	–	–	–	–
Filaments	+	+	+	+	+	+	+
Cell variations	–	+	–	–	–	+	+
G + C content of type strain (mol %)	49	40	39	37	44	48	49
Catalase	SL +	SL +	–	NA	NA	+	SL +
Oxidase	+	+	+	NA	NA	+	+
Flexirubin reaction	–	–	+	NA	–	+	–
Maximum growth temp. (°C)	37	NA	NA	30–45	NA	37	37
Highest NaCl tolerated (%)	<1.5	NA	NA	0–>1.5	>1.5	1.5	<1.5
Growth on peptone	–	NA	–	NA	NA	+	+
Nitrate reduced	–	–	NA	–/+	–	–	–
Fermentative metabolism	–	–	NA	–	NA	+	+
Degradation of:							
Gelatin	–	SL +	NA	±	+	–	–
Starch	SL +	+	–	±	+	–	–
Cellulose	–	–	+	–	–	–	–
Agar	–	–	–	–	+	–	–
Habitat	Fresh water	Fresh water	Soil	Marine	Marine	Plant	Plant

Cell morphology and growth characteristics

In an actively dividing 24 h culture on R2A broth, two to four polarly attached cells of strain NS114^T grew in V-shaped or angled arrangements of rods averaging 2 µm in length (Fig. 2a). As the cells aged, they formed long filaments made up of individual coccoid cells approximately 0.75 µm in length (Fig. 2b). Although these filaments demonstrated curved morphology, they formed as a result of nutritional deprivation in old cultures. This contrasts with the cell morphology observed in a 24 h culture of *R. slithyformis* on R2A broth which grew as rings and spirals at the onset of growth when they were actively dividing (Fig. 2c). *R. slithyformis* was originally reported to rarely form long spirals (Larkin & Williams, 1978); however, in the work reported here, this strain often formed this arrangement in R2A broth. After approximately 24 h growth, filaments formed by strain NS114^T would fall out of solution and settle to the bottom of the culture vessel. In a 24 h culture on YM broth, polarly attached cells of strain NS114^T averaging 1 µm in length formed rounded semi-circles (Fig. 2d) and an occasional horse-shoe structure (Fig. 2e). When growing on R2A agar, strain NS114^T formed a yellow-gold colony that

became dry and flaky with age. The cells produced copious slime when grown on a nitrogen-limited agar medium (AcD).

Phenotypic features

Strain NS114^T is an aerobic, heterotrophic micro-organism that is non-motile, ferments glucose and sucrose, and can grow on various carbon sources (Tables 1 and 2). This is in striking contrast to *R. slithyformis* and some other members of this group that do not demonstrate a fermentative metabolism and/or utilize many carbon sources for growth. Strain NS114^T is oxidase- and catalase-positive, does not require growth factors as evidenced by luxuriant growth on a simple, defined medium (Ayers medium), and cannot use nitrate as electron acceptor. The temperature range for growth is 15–37 °C, as opposed to *R. slithyformis* having a broader growth range down to 4 °C. Strain NS114^T will grow in the presence of 1.5% (w/v) NaCl, but not at 3.0% NaCl. It will grow on R2A supplemented with several antibiotics. Strain NS114^T grows well on peptone water, YM broth, AcD containing at least 10 p.p.m. nitrogen and Litmus milk.

Fig. 2. Phase-contrast (a–d) and scanning electron (e) micrographs of strain NS114^T (a, b, d, e) and *R. slithyformis* (c). Cells were grown for 24 h (a, c) or 48 h (b) on R2A broth; strain NS114^T grew as polarly attached rods in bent arrangements and *R. slithyformis* formed long spirals of filamentous growth. (d) Growth of strain NS114^T on YM broth after 24 h. (e) Electron micrograph shows attached cells that form horseshoe arrangements in YM broth. Bar, 1 µm.

Pigment and fatty acid features

Strain NS114^T produces a flexirubin-like pigment. A cellular ethanol extract has an absorbance that broadens upon addition of sodium hydroxide (Fig. 3), characteristic of flexirubin-like pigments (Weeks, 1981). The fatty acid profile of strain NS114^T is dominated by branched species with substantial quantities of 2- and 3-OH fatty acids (Table 3). This general pattern is similar between *R. slithyformis* and strain NS114^T. However, the two strains differ in the total number of fatty acids and relative abundance of shared species (Table 3). Strain NS114^T could not be assigned to a known genus when compared with a database of fatty acid profiles in the MIDI Sherlock Microbial Identification System (Microbial ID).

Strain NS114^T–*Zea mays* association

The plant HR test was done to identify a potential antagonistic relationship between strain NS114^T and *Zea mays*. There was no detectable HR in tobacco. However, this does not confirm the nature of the relationship between strain NS114^T and maize as a harmless one. To observe a potential growth response of maize to strain NS114^T, a bacterial inoculum was applied to maize seeds. There was no significant

difference in dry weight among uninoculated controls and plants inoculated with strain NS114^T over all three levels of nitrogen fertilizer tested. Thus, strain NS114^T appears to be neither beneficial or pathogenic to maize since inoculated plants appeared healthy and the yield of above-ground plant biomass was unaffected.

Previous work has shown that the addition of small amounts of nitrogen present in the form of yeast extract to a nitrogen-free medium can enhance the diversity of nitrogen-fixing bacteria isolated from the rhizosphere (Watanabe & Barraquio, 1979). Although strain NS114^T demonstrated turbid growth on AcD containing yeast, it did not reduce acetylene in this medium. When strain NS114^T was grown in AcD lacking nitrogen, no growth was observed. Furthermore, PCR amplification of *nifH* was negative for this strain. It appears that strain NS114^T has some capacity to survive in nitrogen-limited growth conditions and under these conditions might compete with the plant for this resource.

Description of *Dyadobacter* gen. nov.

Dyadobacter (Dy.a.do.bac'ter. G. n. *dyas* two in number, pair; L. masc. n. *bacter* from the Gr. n. *baktron* rod or staff; M.L. n. *Dyadobacter* rod or staff occurring in pairs).

Table 2. Comparison of strains NS114^T and NS12 with *R. slithyformis*

+, Positive; — negative; w, weak reaction; O, oxidation; F, fermentation; ND, not determined.

Characteristic	<i>R. slithyformis</i>	NS114 ^T	NS12
Growth on:			
Peptone water	—	+	+
Ayer's agar	—	+	w
Litmus milk	—	Acid and reduction	—
1.5 % NaCl	—		—
Acid produced from:			
Glucose	—	+	+
Ribose	+	+	—
Sucrose	w	+	+
Sole carbon sources used for growth:*			
D-Arabitol	w	+	—
L-Arabitol	—	w	—
Dulcitol	w	w	w
Inositol	w	+	w
Glycerol	—	w	—
Mannitol	w	+	—
Sorbitol	w	w	w
Methanol, formate	—	—	—
Amidon	—	+	+
Glycogen	+	—	+
D-Lyxose	—	+	+
Sorbose	w	+	w
Acetate	—	+	+
Fumarate	—	+	+
5-Ketogluconate	—	+	w
Malate	—	+	+
Malonate	—	+	+
Tartrate	—	+	+
Oxidation/fermentation:			
Glucose	O	F	F
Sucrose	O	F	F
Antibiotic resistance ($\mu\text{g ml}^{-1}$):			
Ampicillin (25)	+	+	+
Chloramphenicol (25)	—	+	—
Kanamycin (50)	+	+	+
Rifampicin (25)	—	—	—
Spectinomycin (10)	+	+	+
Streptomycin (10)	—	+	—
Tetracycline (10)	—	—	—
Trimethoprim (25)	—	+	—
Growth at:			
4 °C	+	—	—
15–37 °C	+	+	+
Plant tests:			
HR on tobacco	ND	—	—
Growth response in maize	ND	—	—

* All strains used the following as a sole carbon source in the API 50 CH test: erythritol, D- and L-arabinose, D- and L-xylose, 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, saccharose, trehalose, inulin, melezitose, D-raffinose, xylitol, β -gentiobiose, D-turanose, D-tagatose, and D- and L-fucose. None could use the following: adonitol, gluconate and 2-ketogluconate.

Fig. 3. Absorbance spectrum of an ethanol (■) and an alkaline-ethanol (●) extract of strain NS114^T.

Table 3. Fatty acid composition (%) of strains NS114^T, NS12 and *R. slithyformis* grown at 28 °C on R2A

Data for two fatty acids that were present at levels less than 1% in all strains are not included.

Fatty acid	<i>R. slithyformis</i>	NS114 ^T	NS12
Unknown (ECL 13:57)	1.83	1.32	2.10
15:1 iso G	2.36	0.00	2.03
15:0 iso	19.8	16.8	16.1
15:0 anteiso	4.34	0.00	4.00
15:0	1.47	1.37	3.15
15:0 iso 2-OH/16:1 ω7c	32.1	43.5	36.9
16:1 ω5c	16.5	17.5	17.8
16:0	7.13	4.76	3.98
15:0 iso 3-OH	4.13	2.74	4.48
16:0 3-OH	2.00	4.66	1.87
17:0 iso 3-OH	7.00	7.38	6.62

Gram-negative rods in straight to curved arrangements, occurring in pairs in young culture and forming chains of coccoid cells in old cultures. Cells are 0.75–2 µm in length. Non-motile, oxidase- and catalase-positive. Cells produce a non-diffusible, yellow, flexirubin-like pigment. Aerobic and chemo-organotrophic, capable of fermenting glucose and sucrose. Does not hydrolyse cellulose or starch. G+C content is 48 mol%. Isolated from surface-sterilized *Zea mays* stems. Phylogenetic position is the *Flexibacter* group of the *Cytophaga–Flexibacter–Bacteroides* phylum. Closest related genera are *Runella* and *Microscilla*. Type species is *Dyadobacter fermentans*.

Description of *Dyadobacter fermentans* sp. nov.

Dyadobacter fermentans (fer.men'tans. L. part. *fermentans* causing fermentation).

Morphological and chemotaxonomic characteristics are the same as those described for the genus. This strain can grow on acetate, D- and L-arabitol, amidon, fumarate, 5-ketogluconate, glucose, glycerol, D-lyxose, malate, malonate, sucrose, tartrate, erythritol, D- and L-arabinose, D- and L-xylose, β-methylxyloside, galactose, D-glucose, D-fructose, mannose, L-sorbose, rhamnose, dulcitol, inositol, mannitol, sorbitol, methyl α-D-mannoside, methyl α-D-glucoside, N-acetylglucosamine, amygdaline, arbutine, aesculin, salicin, cellobiose, maltose, lactose, melibiose, saccharose, trehalose, inulin, melezitose, D-raffinose, xylitol, β-gentiobiose, D-turanose, D-tagatose, and D- and L-fucose. It can ferment glucose and sucrose and produce acid from ribose. This strain cannot hydrolyse agar, cellulose, gelatin or starch. Temperature range for growth is 15–37 °C. The principal fatty acids are 43.5% 15:0 iso 2-OH/16:1 ω7c, 17.5% 16:1 ω5c and 16.8% 15:0 iso. The type strain, NS114^T, was isolated from surface-sterilized *Zea mays* (cv. Mo17) stem and has been deposited in the American Type Culture Collection as ATCC 700827^T.

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