**Verrucosispora sonchi** sp. nov., a novel endophytic actinobacterium isolated from the leaves of common sowthistle (**Sonchus oleraceus** L.)

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A novel actinobacterium, designated strain NEAU-QY3ᵀ, was isolated from the leaves of **Sonchus oleraceus** L. and examined using a polyphasic taxonomic approach. The organism formed single spores with smooth surface on substrate mycelia. Phylogenetic analysis based on the 16S rRNA gene sequence indicated that the strain had a close association with the genus **Verrucosispora** and shared the highest sequence similarity with **Verrucosispora qiuiae** RTII47ᵀ (99.17 %), an association that was supported by a bootstrap value of 94 % in the neighbour-joining tree and also recovered with the maximum-likelihood algorithm. The strain also showed high 16S rRNA gene sequence similarities to **Xiangella phaseoli** NEAU-J6ᵀ (98.78 %), **Jishengella endophytica** 202201ᵀ (98.51 %), **Micromonospora eburnea** LK-2-10ᵀ (98.28 %), **Verrucosispora lutea** YIM 013ᵀ (98.23 %) and **Salinispora pacifica** CNR-114ᵀ (98.23 %). Furthermore, phylogenetic analysis based on the gyrB gene sequences supported the conclusion that strain NEAU-QY3ᵀ should be assigned to the genus **Verrucosispora**. However, the DNA–DNA hybridization relatedness values between strain NEAU-QY3ᵀ and **V. qiuiae** RTII47ᵀ and **V. lutea** YIM 013ᵀ were below 70 %. With reference to phenotypic characteristics, phylogenetic data and DNA–DNA hybridization results, strain NEAU-QY3ᵀ was readily distinguished from its most closely related strains and classified as a new species, for which the name **Verrucosispora sonchi** sp. nov. is proposed. The type strain is NEAU-QY3ᵀ (=CGMCC 4.7312ᵀ=DSM 101530ᵀ).

The genus **Verrucosispora** was originally described by Rheims *et al.* (1998) as a member of the family **Micromonosporaceae** and emended subsequently by Xi *et al.* (2012). Members of the genus **Verrucosispora** are described as aerobic, Gram stain positive, non-acid-fast actinobacterium that forms a well-developed substrate mycelium but lacks aerial mycelium or sporangia (Rheims *et al.*, 1998; Goodfellow *et al.*, 2013). Smooth, warty or hairy-surfaced spores are borne singly, in pairs or in clusters on the substrate mycelium (Liao *et al.*, 2009). The genus is characterized chemotaxonomically by the presence of *meso*-diaminopimelic acids in the cell wall, mannosae as the diagnostic whole-cell sugar, MK-9(H₄) as the predominant menaquinone, diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylinositol mannosae as major polar lipids [phospholipid type II after Lechevalier *et al.* (1977)] (Rheims *et al.*, 1998; Liao *et al.*, 2009; Xi *et al.*, 2012) and iso-C₁₅:₀ and iso-C₁₆:₀ as the predominant cellular fatty acids. At the time of writing, the genus comprised eight species with validly published names (http://www.bacterio.net/verrucosispora.html). In recent years, the genus has drawn much attention, as it has
proved to be a source of potential antitumour compounds such as abyssomicin, gifhornelenolones and proximicins (Bister et al., 2004; Keller et al., 2007; Riedlinger et al., 2004; Schneider et al., 2008; Shirai et al., 2010). *Sonchus oleraceus* L., an annual or biennial herb of the family *Compositae*, grows in badlands throughout north and south China. It has various medicinal functions, such as preventing anaemia, treating diarrhoea and inflammatory diseases, helping wound healing, anti-tumour and anti-infection (Zhao et al., 2009; Liu & Zhao, 2010; Li et al., 2012; Huyan et al., 2016). During the course of investigating endophytic actinobacteria from medicinal plants, strain NEAU-QY3T was isolated from the leaves of *S. oleraceus* L. In this study, we performed polyphasic taxonomy on this strain and proposed that it represents a new species of the genus *Verrucosispora*, for which the name *Verrucosispora sonchi* sp. nov. is proposed.

Strain NEAU-QY3T was isolated from the leaves of *S. oleraceus* L., which was collected from Fenghuang Mountain in Wuchang, Heilongjiang province, north China (42° 21’ N 127° 99’ E). Two leaves were processed as described by Ma et al. (2015). After 21 days of aerobic incubation at 28°C, colonies were transferred and purified on oatmeal agar [International Streptomyces Project (ISP) 3 medium] (Shirling & Gottlieb, 1966) and maintained as glycerol suspensions (20%, v/v) at −80°C.

Cultural characteristics were determined after 14 days at 28°C using ISP 2–7 and nutrient agar (NA) (*Shirling & Gottlieb, 1966; Waksman, 1961, 1967*). The ISCC-NBS colour charts were used to determine the designations of colony colours (Kelly, 1964). Morphological characteristics were observed by light microscopy (Nikon Eclipse E200) and electron microscopy (Hitachi S-3400N) using cultures grown on ISP 3 agar at 28°C for 6 weeks. Motility was assessed by light microscopic (Nikon Eclipse E200) observation of cells suspended in phosphate buffer (pH 7.0, 1 mM). The utilization of sole carbon and nitrogen sources, decomposition of cellulose, hydrolysis of starch and aesculin, reduction of nitrate, peptonization of milk, liquefaction of gelatin and production of H2S were examined as described previously by Gordon et al. (1974) and Yokota et al. (1993). Production of catalase, esterase and urease was tested as described by Smibert & Krieg (1994). Growth at different temperatures (4, 10, 15, 20, 28, 32, 35, 40, 42 and 45°C) was determined on ISP 3 agar after incubation for 14 days. Tolerance of pH range (pH 4, 5, 6, 7, 8, 9, 10 and 11), using buffer system described by Xu et al. (2005), and NaCl tolerance (0, 1, 2, 3, 4, 5, 6 and 7%, w/v) for growth were determined after 14 days of growth in ISP 2 medium in shake flasks (250 r.p.m.) at 28°C.

Strain NEAU-QY3T was an aerobic, Gram-stain-positive actinobacterium that formed extensively branched substrate mycelia and lacked aerial mycelia. Single, non-motile, spherical spores (0.6–0.9 µm in diameter) were observed; the spore surface was smooth (Fig. S1, available in the online Supplementary Material). The morphological characteristics were affiliated with the genus *Verrucosispora*. Strain NEAU-QY3T grew well on ISP 3 and NA but poorly on ISP 4, ISP 5, ISP 6 and ISP 7, and no growth was observed on ISP 2 agar. However, strain NEAU-QY3T grew well on ISP 2 broth. No soluble pigments were produced on any of the media tested. Colonies on ISP 3 were moderate orange yellow and presented vivid yellow on NA (Fig. S2). Strain NEAU-QY3T grew well between pH 7 and 9, with an optimum pH of 7 and 8. The temperature range for growth was determined to be 10°C to 40°C, with the optimum temperature at 28°C. Growth was observed in the presence of 0 to 1% NaCl (w/v). Detailed physiological and biochemical properties are presented in the species description.

Biomass for chemical studies was prepared by growing the strain in ISP 2 broth on a rotary shaker at 250 r.p.m. for 14 days at 28°C. Cells were harvested by centrifugation, washed twice with distilled water, re-centrifuged and freeze-dried. The isomer of diaminopimelic acid in the cell wall hydrolysates was derivatized according to McKerrow et al. (2000) and analysed by the HPLC method described by Yu et al. (2013). The whole-cell sugars were analysed according to the procedures developed by Lechevalier & Lechevalier (1980). The N-acyl group of muramic acid in peptidoglycan was determined by the method of Uchida et al. (1999). The phospholipids in cells were examined by two-dimensional TLC and identified using the method of Minnikin et al. (1984). Menaquinones were extracted from freeze-dried biomass and purified according to Collins (1985). Extracts were analysed by an HPLC-UV method according to procedures described by Wu et al. (1989). The presence of mycolic acids was checked by the acid methanolysis method as described previously by Minnikin et al. (1980). Fatty acid methyl esters were extracted from the biomass as described by Gao et al. (2014) and analysed by GC-MS using the method of Xiang et al. (2011).

Strain NEAU-QY3T contained meso-diaminopimelic acid as diamino acid. The whole-cell hydrolysates were mannose and xylose. The same compositions were reported for *Verrucosispora quiae*, *Verrucosispora maris* and *Verrucosispora fiedleri* (Xi et al., 2012; Goodfellow et al., 2012, 2013). The N-acyl type of muramic acid was determined to be glycolyl. The phospholipid profile consisted of diphosphatidylglycerol, phosphatidyl-ethanolamine and phosphatidylinositol mannoside, which was consistent with the description of the genus *Verrucosispora* (Fig. S3). The menaquinones detected were MK-9(H4) (53.9%), MK-9(H6) (39.5%) and MK-9(H8) (6.6%). The fatty acids were identified as iso-C16:0 (51.1%), C17:0 3-OH (16.1%), C15:0 (9.0%), 10-methyl C17:0 (7.2%), C16:0 (7.0%), C18:0 (6.1%) and anteiso-C17:0 (3.5%). A high proportion (>10%) of unsaturated fatty acid C17:1ω9c as the major fatty acid was also reported by Goodfellow et al. (2013) for *V. fiedleri*. Therefore, the fatty acid composition of strain NEAU-QY3T is affiliated with the genus *Verrucosispora*. Mycolic acids were not detected.
Extraction of genomic DNA and PCR-mediated amplification of the 16S rRNA gene were carried out using a standard procedure (Lee et al., 2003; Loqman et al., 2009). The PCR product was purified and cloned into the vector pMD19-T (Takara) and sequenced by using an Applied Biosystems DNA sequencer (model 3730XL). Almost full-length 16S rRNA gene sequence of strain NEAU-QY3\(^T\) (1509 bp) was obtained and compared with multiple sequences obtained from the GenBank/EMBL/DDBJ databases using CLUSTAL X 1.83 software. 16S rRNA gene sequence similarities between strains were calculated based on pairwise alignment using the EzTaxon-e server (Kim et al., 2012). Strains that have a similarity with NEAU-QY3\(^T\) higher than 97% were chosen to reconstruct phylogenetic trees. Phylogenetic trees were reconstructed by the neighbour-joining (Saitou & Nei, 1987) and maximum-likelihood (Felsenstein, 1981) tree-making algorithms by using the software packages MEGA version 6.06 (Tamura et al., 2013). Confidence values of branch nodes were evaluated using the bootstrap resampling method with 1000 replications (Felsenstein, 1985). A distance matrix was generated using Kimura's two-parameter model (Kimura, 1980). All positions containing gaps and missing data were eliminated from the dataset (complete deletion option). Bootstrap values above 50% (based on 1000 replications) are shown at branch points. Bar, 0.01 substitutions per nucleotide position.

![Fig. 1](downloaded_from_images) Neighbour-joining tree based on nearly complete 16S rRNA (1362 bp, by omitting unaligned regions) gene sequence showing the relationship between strains NEAU-QY3\(^T\) and other related taxa. Asterisks denote branches that were also recovered using the maximum-likelihood method. Bootstrap values above 50% (based on 1000 replications) are shown at branch points. Bar, 0.01 substitutions per nucleotide position.

The nearly complete 16S rRNA gene sequence (1509 nt) of strain NEAU-QY3\(^T\) was based on EzTaxon-e analysis containing gaps and missing data were eliminated from the dataset (complete deletion option). PCR amplifications and sequencing of the gyrase B subunit (gyrB) gene were carried out using primers GYF1 and GYR3B (Garcia et al., 2010). Sequencing and phylogenetic analysis were performed as described above. The G+C content of the genomic DNA was determined by the thermal denaturation (T\(_m\)) method as described by Mandel & Marmur (1968), and *Escherichia coli* JM109 was used as the control. DNA–DNA relatedness tests between the novel strain and its closest phylogenetic relatives were carried out as described by De Ley et al. (1970) under consideration of the modifications described by Huss et al. (1983), using a model Cary 100 Bio UV/VIS spectrophotometer equipped with a Peltier-thermostatted 6×6 multiecell changer and a temperature controller with *in situ* temperature probe (Varian). The DNA samples used for hybridization were diluted to OD\(_{260}\) around 1.0 using 0.1× SSC (saline sodium citrate buffer) and then sheared using a FY92-II ultrasonic cell disruptor (ultrasonic time 3 s, interval time 4 s, 90 times). The DNA renaturation rates were determined in 2× SSC at 70 °C. The experiments were performed with three replications, and the DNA–DNA relatedness value is expressed as a mean value.
showing together with members of the family Micromono-
sporaceae (>97.0 % similarities). The highest sequence simi-
larities (>97.0%) were between strain NEAU-QY3\textsuperscript{T} and
members of the genera Dactylosporangium, Pilimelia, Actino-
planes, Asanoa, Polymorphospora, Phytohabitans, Plantacti-
nospora, Salinispora, Micromonospora, Jishengella, Xiangella
and Verrucosispora (97.0–99.2 %). The phylogenetic results
based on 16S rRNA gene and gyrB gene clearly demonstrated
that strain NEAU-QY3\textsuperscript{T} belonged to the genus Verrucosis-
pora (Figs 1 and S4), with the highest 16S rRNA gene and
gyrB gene sequences similarities to V. qiuiae RtIII47\textsuperscript{T} (99.2,
96.5 %) and Verrucosispora lutea YIM 013\textsuperscript{T} (98.2, 95.2 %) in
the genus Verrucosispora. However, further research on
strain NEAU-QY3\textsuperscript{T} and its closest relatives V. qiuiae
RtIII47\textsuperscript{T} and V. lutea YIM 013\textsuperscript{T} showed that the DNA relat-
edness between strain NEAU-QY3\textsuperscript{T} and the latter was
49.8±0.7 and 57.2±0.5 %, respectively. These values are
below the threshold value of 70% recommended by Wayne
et al. (1987) for assigning strains to the same species. The
DNA G+C content of the type strain is 71.5±0.3 mol%.

Besides the genotypic evidence above, strain NEAU-QY3\textsuperscript{T}
could also be distinguished from V. qiuiae RtIII47\textsuperscript{T} and
V. lutea YIM 013\textsuperscript{T} by several phenotypic characteristics (Table 1
and Fig. S2). Strain NEAU-QY3\textsuperscript{T} was observed to form single
spores, while V. lutea YIM 013\textsuperscript{T} could produce pairs and clus-
ter spores. The spore surface of strain NEAU-QY3\textsuperscript{T} was
smooth, which apparently distinguishes it from V. qiuiae
RtIII47\textsuperscript{T}. The novel strain could not utilize L-arabinose and D-
ribose as the sole carbon sources, in contrast to V. lutea YIM
013\textsuperscript{T} and V. qiuiae RtIII47\textsuperscript{T}, which could. The isolate could
grow with 1 % (w/v) NaCl but not with 2 %. In contrast, V.
lutea YIM 013\textsuperscript{T} and V. qiuiae RtIII47\textsuperscript{T} could grow above 7 %
NaCl. Meanwhile, strain NEAU-QY3\textsuperscript{T} could also be differen-
tiated from its closest related strains V. qiuiae RtIII47\textsuperscript{T} and V.
lutea YIM 013\textsuperscript{T} by different colonial characteristics on ISP 3
and NA media, degradation of Tween 40 and Tween 80,
hydrolysis of starch, reduction of nitrate and patterns of nitro-
gen utilization. In addition, strain NEAU-QY3\textsuperscript{T} could also be
differentiated from other type strains of closer genus that
shared high 16S RNA gene sequence similarities with the iso-
late by morphological, physiological and chemotaxonomic
characteristics (Table 2).

It is evident from the genotypic and phenotypic data that
strain NEAU-QY3\textsuperscript{T} represents a novel species of the genus
Verrucosispora, for which the name Verrucosispora sonchi sp.
nov. is proposed.

**Description of Verrucosispora sonchi sp. nov.**

Verrucosispora sonchi [son’chi. L. n. sonchus, name of a plant
and also a botanical generic name (Sonchus); L. gen. n. son-
chi of Sonchus, referring to the isolation of the type strain
from Sonchus oleraceus].

Aerobic, Gram-stain-positive, mesophilic and non-motile
actinobacterium that forms well-developed and branched
substrate hyphae. Aerial mycelium is absent. Non-motile, sin-
gle smooth spores are produced on substrate hyphae. Growth
occurs at pH 7 to 9 (optimum pH 7–8), 10 to 40 °C (optimum
28 °C) and in the presence of 0 to 1 % (w/v) NaCl. It is pos-
tive for degradation of Tween 40, hydrolysis of aesculin and
starch, coagulation and peptonization of milk, decomposition

<table>
<thead>
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<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
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</thead>
<tbody>
<tr>
<td>Aerial mycelium</td>
<td>Absent</td>
<td>Absent</td>
<td>Sparse</td>
</tr>
<tr>
<td>Spore shape</td>
<td>Smooth, single</td>
<td>Warty, single*</td>
<td>Smooth, single, pairs, clusters†</td>
</tr>
<tr>
<td>Colony colour on ISP 3</td>
<td>Moderate orange yellow</td>
<td>Deep orange yellow</td>
<td>Vivid yellow</td>
</tr>
<tr>
<td>ISP 3</td>
<td>NA</td>
<td>Strong orange</td>
<td>Strong yellow</td>
</tr>
<tr>
<td>Reduction of nitrate</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Utilization as sole carbon source</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>D-Arabinose</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Inositol</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>D-Mannitol</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<tr>
<td>L-Rhamnose</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>D-Ribose</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Utilization as sole nitrogen source</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>L-Alanine</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>L-Arginine</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>L-Glutaminic acid</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>L-Serine</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>DPG, PE, PIM, PL</td>
<td>DPG, PE, PIM, PL*</td>
<td>DPG, PE, PL, PIM, PL</td>
</tr>
</tbody>
</table>

DPG, diphasphatidylglycerol; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PIM, phosphatidylinositol mannoside; PL, unknown polar lipids.

*Data from Xi et al. (2012).
†Data from Liao et al. (2009).
Table 2. Differential phenotypic properties of strain NEAU-QY3T and its related strains of closer genera

| Characteristic                  | 1  | 2* | 3† | 4‡ | 5§ | 6|| |
|---------------------------------|----|----|----|----|----|----|
| Spore shape                     | Smooth, single | Smooth, single | Unevenly warty, single | Unevenly warty, single | Rough and nodular, single | Smooth, single, clusters |
| Colony colour on ISP 3          | Moderate orange yellow | Strong orange | Orange to black | Vivid orange to dull orange | 0–4 | ND |
| Growth at NaCl range (%)        | 0–1 | 0–4 | 0–3 | 0–2 | 0–4 | ND |
| Hydrolysis of starch            | +  | +  | –  | +  | +  | +  |
| Reduction of nitrate            | –  | –  | –  | –  | –  | +  |
| Phospholipids                   | DPG, PE, PIM | DPG, PE, PME, PG, PI, PM, PGL, PL | PE, PME, PC, PI, PIM | PE, DPG, PS, PIM | PE, DPG, PI, PIM | DPG, PE, PME, PG, PI, PIM |
| Whole-cell sugars               | Man, Xyl | Ara, Gal, Glc, Rha, Rib, Xyl | Man, Gal, Glc | Xyl, Man, Ara, Rib, Glc | Xyl, Ara | Gal, Ara, Xyl |
| Major menaquinones              | 9(H4a) | 9(H4a) | 9(H4a) | 9(H4a), 10(H4) | 9(H4) |

DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PME, phosphatidylmethylethanolamine; PG, phosphatidylglycerol; PC, phosphatidylcholine; PS, phosphatidylserine; PI, phosphatidylinositol; PIM, phosphatidylinositol mannoside; PL, unknown polar lipids; PGL, phosphoglycolipid; Ara, arabinose; Gal, galactose; Glc, glucose; Man, mannose; Rha, rhamnose; Rib, ribose; Xyl, xylose.

*Data from Supong et al. (2013).
†Data from Wang et al. (2013).
‡Data from Xie et al. (2011).
§Data from Thawai et al. (2005).
||Data from Ahmed et al. (2013).

of cellulose and liquefaction of gelatin and negative for production of H2S, catalase and urease, degradation of Tween 20 and Tween 80 and reduction of nitrate. D-Fructose, D-galactose, D-glucose, maltose, D-mannose, raffinose, D-sorbitol, sucrose and D-xylose are utilized as sole carbon source, but D-arabinose, dulcitol, inositol, lactose, D-mannitol, L-rhamnose and D-ribose are not. L-Asparagine, L-aspartic acid, creatine and L-glutamic acid are utilized as sole nitrogen source, but L-alanine, L-arginine, L-glutamine, glycine, L-proline, L-serine, L-threonine and L-tyrosine are not. The diagnostic amino acid of the cell wall is meso-diaminopimelic acid, and whole-cell hydrolysates contain mannose and xylose. The predominant menaquinones are MK-9(H2) and MK-9(H4). The phospholipid profile contains diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylinositol mannoside. The major fatty acids (>10 %) are iso-C16:0 and C17:ω7c. Mycolic acids are absent.

The type strain is NEAU-QY3T (≡CGMCC 4.7312T=DSM 101530T), isolated from the leaves of Sonchus oleraceus collected from Fenghuang Mountain in Wuchang, Heilongjiang province, north China. The DNA G+C content of the type strain is 71.5 mol%.

Acknowledgements

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