Bifidobacterium apri sp. nov., a thermophilic actinobacterium isolated from the digestive tract of wild pigs (Sus scrofa)

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Abstract

Fresh samples of intestinal contents of three wild pigs originating from the Central Bohemia region were examined for the presence of bifidobacterial strains. During the study, we isolated many fructose-6-phosphate phosphoketolase-positive, strictly anaerobic, irregular rod-shaped bacterial isolates. Three of them were preliminarily identified as representing a novel species of the genus Bifidobacterium because their 16S RNA gene sequence similarity with the closest relatives of thermophilic bifidobacteria (Bifidobacterium DSM 20432, Bifidobacterium thermophilum DSM 20210, Bifidobacterium therma cidophilum subsp. porcinum LMG 21689) was in the range of 97.9 – 98.4 %. All three bacterial isolates had identical 16S rRNA, dnaJ1, fusA, gyrB and rplB gene sequences. Isolate RP115 was chosen as a representative of the bacterial group and DNA G+C content (mol%) determination, biochemical tests and analyses of physiological and morphological characteristics, habitat and chemotaxonomic traits (peptidoglycan structure, cellular fatty acids and polar lipids profile) were performed. The DNA–DNA hybridization analyses of RP115 and species representing the group of thermophilic bifidobacteria revealed values in the range from 33 to 53 %. This fact, together with relatively low sequence similarities of particular phylogenetic markers among examined bacterial strains and the phenotyping and chemotaxonomic results obtained, indicated that the evaluated bacterial isolate should be classified as representing a separate taxon within the specific group of thermophilic bifidobacteria. The name Bifidobacterium apri (of boar) sp. nov. has been proposed for the representative strain RP115 (=CCM 8605=DSM 100238=LMG 28779).

Digestive tract of domesticated pigs represents, along with humans and primates [1–3], the largest reservoir of bifidobacterial diversity [4, 5]. Bifidobacterium choerinum, Bifidobacterium longum subsp. suis, Bifidobacterium longum subsp. suillum, Bifidobacterium psychraerophilum and Bifidobacterium thermophilum subsp. porcinum are exclusively found in domesticated pigs [6–9]. Other species, such as Bifidobacterium animalis, Bifidobacterium pseudolongum, Bifidobacterium minimum, Bifidobacterium thermophilum and Bifidobacterium boum are often present in the digestive tract of domesticated pigs [4, 10–13]. In addition, other representatives of the family Bifidobacteriaceae (genera Aeriscardovia and Pseudocardovia) occurring in the digestive tract of domestic and wild pigs have been recently described [11, 14, 15]. Some researchers even believe that many species of bifidobacteria inhabiting the intestinal tract of pigs have not been described yet [11, 16]. Despite the high diversity, the number of bifidobacteria in pigs appears to be lower compared with other bacterial groups and reaches about 106 g–1 of intestinal faecal samples [4, 17, 18]. However, it should be noted that these studies were based on culture-dependent methods using media that are not fully selective and that the real number could be higher. Unfortunately, desirable culture-independent studies aimed at determining the real number of bifidobacteria in different parts of pig’s digestive tract have not yet been performed. The probiotic effect of bifidobacteria in pigs was studied mainly in connection with immunoregulation [19, 20], improved feed conversion and reduced mortality [21].

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Keywords: Bifidobacterium; wild pigs; thermophilic bifidobacteria; MLSA.

Abbreviations: DDH, DNA–DNA hybridization; FAMEs, Fatty acid methyl esters; MLSA, multilocus sequence analysis.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA, dnaJ1, fusA, gyrB, rplB gene sequences and 16S rRNA-23S rRNA intergenic spacer region of strain RP115 are KT343145, KT343155, KT343133, KT343148, KT343130 and KT343137, respectively.

Six supplementary figures and two supplementary tables are available with the online Supplementary Material.
Eurasian wild pigs represent the ancestor of most domestic pig breeds. Hence, bifidobacterial population inhabiting their digestive tracts could be considered as an archetype from which bifidobacteria occurring in domesticated pigs have evolved. Our research is focused on isolation and characterization of a novel species of the genus *Bifidobacterium* related to the thermophilic group represented by species *B. thermophilum*, *B. thermacidophilum* subsp. *porcinum* and *B. boum* often found in domesticated pigs.

Three bacterial strains (RP115\(^T\), RP6 and RP210) originating from large and small intestines of three wild boar individuals shot in two different locations in Central Bohemia [Prague (Divoká Šárka) and Hlavenec] during the years 2013–2015 were isolated and preliminarily classified using 16S rRNA gene sequencing based on methods described in our previous work [14]. Reference type strains *B. boum* DSM 20432\(^T\), *B. thermacidophilum* subsp. *porcinum* LMG 21689\(^T\), *B. thermacidophilum* subsp. *thermacidophilum* DSM 15837\(^T\) and *B. thermophilum* DSM 20210\(^T\) representing the thermophilic and ‘*B. boum*’ phylogenetic group [22] were revealed as the closest relatives to the examined strains having the same 16S rRNA and other gene sequences. Similarity values from 16S rRNA comparative analysis using the EzTaxon database [23] were 98.4, 98.0, 98.2 and 97.9 %, respectively. The sequence of the selected strain RP115\(^T\), deposited in the NCBI database (GenBank accession number KT343145) using the BankIt application, was used for purpose of the analyses. This strain was isolated from the large intestine contents of a wild boar from the locality of Prague (Divoká Šárka). Only sequences with 100 % completeness (length 1399 nt of compared gene fragment) were taken into account. Recently, the value of 98.65 % 16S rRNA gene similarity has been proposed for species delineation [24]. Therefore, we have decided that bacterial strain RP115\(^T\) might represent a novel *Bifidobacterium* taxon. Owing to relatively high values of similarities, exceeding 97.0 %, the former cut-off value for species assignment [25], this had to be confirmed by DNA–DNA hybridisation and multilocus sequence analysis.

Although problems associated with the reproducibility of the DNA–DNA hybridization method (DDH) have been discussed [22, 26], it is still an accepted tool for distinguishing different bifidobacterial species [27]. High-molecular-weight genomic DNAs of RP115\(^T\), *B. boum* DSM 20432\(^T\), *B. thermacidophilum* subsp. *porcinum* DSM 17755\(^T\) and *B. thermophilum* DSM 20210\(^T\) for DDH experiments were obtained using methods based on the protocol of Gevers et al. [28]. Previously described protocols [29, 30] of the microplate method [31] were performed. The value of DNA G+C content determined for RP115\(^T\) (59.4 mol%, SD 0.08 from three independent measurements) using the modified HPLC method [15] was applied to calculate the hybridization temperature [29]. The hybridization temperature (47 °C) was calculated from the DNA G+C content with the formula of De Ley [32] and corrected for the presence of 50 % formamide in the hybridization mixture [33]. The DNA–DNA relatedness percentages were calculated as means based on four independent hybridizations. Reciprocal reactions were performed and also considered as independent experiments. DDH results are shown in Table 1. The range 33–53 % of DNA–DNA hybridization values was determined for RP115\(^T\) compared with the other three type strains, the lowest value corresponded to *B. boum* and the highest to *B. thermacidophilum* subsp. *porcinum*, respectively. Concerning the latter, almost the same value (52 %) was obtained for *B. thermophilum*. The threshold value of ≤70 % is generally accepted for separated prokaryote species [34]. Values achieved in the study were lower. Thus, the evaluated strain RP115\(^T\) can be proposed as representing a novel species of the genus *Bifidobacterium*. Notably, phylogenetic relationship to the level of strain belonging to the same species was found between the *B. thermacidophilum* subsp. *porcinum* and *B. thermophilum* type strains (DNA–DNA relatedness 88 %). A very similar result (82 %) has been obtained by von Ah et al. [35]. Therefore, it would be worth considering reclassifying these taxa based on a polyphasic approach [36] or comparative genomics [22]. In this context, we would like to point out discrepancies between genomic sequences of *B. thermophilum* JCM 1207\(^T\) (NCBI accession number NZJGZV00000000) and *B. thermophilum* DSM 20210\(^T\) (NZJDU80000000). Our example of comparative analysis based on *thrS* (encoding threonyl-tRNA synthase; NCBI accession numbers for the genomes of *B. thermophilum* JCM 1207\(^T\) and *B. thermophilum* DSM 20210\(^T\) are NZJGZV01000002 and NZJDU80000005, respectively) and *dnaK* (encoding chaperone protein DnaK; NCBI accession numbers NZJGZV01000006 and NZJDU80000014, respectively) gene sequences revealed in Geneious v7.1.7 software (Biomatters) after CLUSTALW alignment the pairwise identities of 93.7 and 96.0 %, respectively. This means that the two type strains differ, even though identical sequences would be expected. Based on comparative genomics, Lugli et al. [22] mentioned a very close relationship between *B. thermophilum* JCM 1207\(^T\) and *B. boum* LMG 10736\(^T\) rather than referring to the same taxon. However, verification and assignment of particular type strain for *B. thermophilum* seem to be necessary with regarding to our above findings.

Multilocus sequence analysis (MLSA) was performed to confirm the genetic difference between RP115\(^T\) and related taxa. Fragments of *dnaI*, *fusA*, *gyrB* and *rplB* genes were amplified using primers and PCR conditions, as described

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by Ventura et al. [37] and Delétoile et al. [38], respectively. In addition, the 16S–23S rRNA [internal transcribed spacer (ITS)] region was amplified [39] and sequenced for purposes of comparative analyses. Genomic DNA of RP115 was extracted using the PrepMan Ultra Sample Preparation Reagent (Applied Biosystems) according to manufacturer’s instructions and used for PCRs at 10–100 ng. The PCR amplifications, performed in a volume of 25 µl, consisted of 1× PPP Master Mix (Top-Bio) and 0.5 µM of each primer. Amplicons were checked using 1.5 % agarose (Top-Bio) electrophoresis (110V, 30 min) under a UV lamp and purified with a PCR purification kit (Qiagen). Sanger sequencing of RP115 based on samples derived from forward and reverse primers was performed in the Sequencher software (http://www.mbio.ncsu.edu/bioedit/page2.html) in the Republic). Final sequences were obtained in BioEdit v7.2.5 (Ibis Biosciences, Foster City, CA). Sequences of RP115 were deposited in NCBI database with the widely used Banklt application. Results of comparative sequence analyses conducted in the Geneious program are listed in Table S1 (available in the online Supplementary Material). Available genomic and NCBI sequences of certain genes and ITS regions of B. boum, B. thermacidophilum subsp. porcinum, B. thermacidophilum subsp. thermacidophilum and B. thermophilum type strains were used. Calculated ranges of dnaJ, fusA, gyrB, rplB and ITS pairwise similarities were 83.0–86.9, 96.9–98.5, 92.5–93.2, 93.6–94.8 and 82.1–90.3 %, respectively. In most cases, the highest values were observed to B. thermacidophilum subsp. porcinum and B. thermophilum which was consistent with DNA–DNA comparisons.

**Fig. 1.** Phylogenetic relationship of RP115 to representatives of the family Bifidobacteriaceae based on 16S rRNA gene sequences using the maximum-likelihood algorithm. Hypervariable positions were removed by the Gblocks application (final length of 1183 nt). Only sequences of type strains retrieved from complete genomes and the nucleotide search tool of the NCBI database was applied. The Tamura–Nei model was used for reconstruction. The tree was reconstructed as unrooted. Bootstrap percentages from 1000 datasets above 70 are given at branching points. Some representatives of the phylum Actinobacteria were also included in order to ensure accurate topology. Numbers in parentheses correspond to the NCBI accession numbers. Bar, 0.02 substitutions per nucleotide position.
also demonstrated by the tree reconstructed on the basis of concatenated amino acid sequences obtained from dnaJ, gyrB, rplB, and ftsA gene fragments (overall 542 amino acids). Maximum-likelihood statistical analysis using the Jones–Taylor–Thornton model and nearest neighbour interchange heuristic method was performed in MEGA 5.05 [41] for this reconstruction. The examined novel isolate is located on a separated branch within the cluster together with B. thermacidophilum subsp. porcinum and B. thermacidophilum subsp. thermacidophilum (Fig. S5). However, the distance from the taxonomic unit is illustrated by length of the phylogenetic branch.

Methodological procedures for phenotypic characterization including biochemical diagnostic kits API 50CH, RAPID ID32A and API ZYM (bioMérieux), evaluation of physiological, morphological properties and chemotaxonomic techniques [cellular fatty acid methyl esters (FAMEs), polar lipid profiling and peptidoglycan structure analysis] are described in our previous study [14]. Only temperature growth range was extended with temperatures of 49 and 50°C. Biochemical tests and determination of physiological features were performed for RP115T and the other two strains (RP6 and RP210) representing a proposed novel species, B. boum DSM 20432T, B. thermacidophilum subsp. porcinum DSM 17755T and B. thermophilum DSM 20210T in triplicates. Information relating to DNA G+C content, type of peptidoglycan structures and habitat were obtained from Bergey’s Manual of Systematic Bacteriology [42] and our previous publications [14, 43]. The resulting data are presented in Table 2. The DNA G+C content (59.4 mol%) determined for RP115T is similar to those quantified (60.0–61.0 mol%) in other representatives of the ‘B. boum’ thermophilic’ phylogenetic group and in accordance with classification of the genus Bifidobacterium [44]. Amino-acid composition of the peptidoglycan interbridge for RP115T [A4β l-Orn(l-Lys)–d-Ser–d-Glu, type A21.12 based on DSMZ database: www.peptidoglycan-types.info] is different compared with those of B. boum (A4α l-Lys–d-Ser–d-Glu, type A11.38) and B. thermophilum [A4β l-Orn(l-Lys)–d-Glu, type A21.6], respectively. The concrete peptidoglycan interbridge A4β l-Orn(l-Lys)–d-Ser–d-Glu revealed in the evaluated strain is rare in bifidobacteria. Nevertheless, it was recently determined in Bifidobacterium avesanii [3]. The apparent similarity between RP115T and B. thermophilum peptidoglycan structures corresponds with relatedness found on the basis of the results of DNA–DNA and multilocus sequence analyses. Similarly, results of biochemical tests confirmed previous findings. RP115T differs from B. thermophilum and B. thermacidophilum subsp. porcinum in hydrolysis of aesculin and fermentation of arbutin, methyl α-D-glucopyranoside and salicin and ability to synthesize glutamic acid decarboxylase, acid phosphatase, leucyl glycine and alanine arylamidases. However, strains representing the proposed novel species differ among themselves in ability to ferment methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, amygdalin, melezitose and trehalose (Table 2). The substrate utilization profile of B. boum is more distinct. We have demonstrated the ability of all representatives of the thermophilic group to grow at temperatures of 49–50°C. This is in contrast to results of Mitsuoka [45] and Zhu et al. [7], authors who described B. thermophilum and B. thermacidophilum subsp. porcinum. They mentioned 46.5°C as the highest temperature for growth. The ability to grow at 50°C was very recently observed also in Bifidobacterium ramosum, B. avesanii and Bifidobacterium aerophilum [3]. RP115T grows at 10°C, in contrast to the other species. Within the family Bifidobacteriaceae, the ability to grow at low temperatures (4–10°C) has been recently noticed in Bombiscardovia coagulans, Pseudocardovia suis, Bifidobacterium aquitrifir, Bifidobacterium bombi, Bifidobacterium bohemicum, Bifidobacterium commune, Bifidobacterium crudilactis and Bifidobacterium psycheorophilum [14, 44, 46–50].

Using FAMEs analysis it was determined that C16:1ω7c, C14:1ω6t and C13:0 3-oxo were the main fatty acids in cells of novel bifidobacterial strain. Similar results were obtained for B. thermophilum and B. thermacidophilum subsp. porcinum type strains (Table S2). However, higher proportions of C16:1ω7c and C13:0 3-oxo and conversely lower proportion of C18:2 ω9t were detected in RP115T compared with related taxa. The prevailing cellular fatty acids C16:0, C14:0 and C18:1 ω9c were recently determined in novel species of bifidobacteria isolated from faeces of common marmosets [51], Bifidobacterium moukalabense [52], isolated from the faeces of wild west lowland gorilla, and also in Bifidobacterium fuscace [53] and Bifidobacterium kashiwanohense [54], both originating from human faeces.

Cellular polar lipid analysis revealed eight different glycolipids, five unidentified phospholipids and two phosphoglycolipids in RP115T (Fig. S6). These three groups of polar lipids were observed in members of the genera Alloscardovia, Bombiscardovia, Pseudocardovia, Scardovia and Neo scardovia within the family Bifidobacteriaceae [14, 40, 44, 55, 56]. Glycolipids and specific phospholipids are present in cells of some bifidobacteria [57]. Although phosphatidylglycerol and diphosphatidylglycerol are considered to be the main polar lipids in bifidobacteria [58, 59] and have been detected in many scardovial species, they were not found in RP115T.

The detailed cellular morphology of RP115T is illustrated by scanning electron micrographs (Fig. 2). Cells growing in anaerobic TPY broth occur mainly singly and rarely in pairs. Cells are rod-shaped of variable sizes, occasionally slightly curved and club-shaped. Branching and constrictions occur rarely. Similar morphology has been documented for B. thermophilum and B. thermacidophilum subsp. porcinum in the description of the genus Bifidobacterium [42].

Comparative genotypic and phenotypic analyses indicate that bacterial RP115T is sufficiently different from other representatives of the thermophilic bifidobacteria to be considered as representing a novel taxon for which the name
Bifidobacterium apri sp. nov. is proposed. Given the fact that Eurasian wild pigs represent the ancestors of most domestic pig breeds, their intestinal microbiota could include the original phylogenetic bacterial species from which new ones have evolved over evolutionary time in domestic pigs or in some other livestock. The hypothesis can be supported by the fact that different species of farm animals often live side by side in a small space which can

Table 2. Phenotypic characteristics, DNA G+C contents (mol%) and habitats of phylogenetically related thermophilic bifidobacteria which differentiate the group

(Subspecies: 1, B. apri RP115† (physiological and biochemical properties also provided for RP6 and RP210 strains); 2, B. thermacidophilum subsp. porcinum DSM 17795†; 3, B. thermacidophilum subsp. thermacidophilum DSM 15837†; 4, B. thermophilum DSM 20210†; 5, B. boum DSM 20432†. All strains ferment D-galactose, D-glucose, D-fructose, maltose (not determined for subspecies 3), melibiose, sucrose, raffinose, starch, glycogen (weakly in species 4) and turanose (weakly in species 4). None produce acid from glycerol, erythritol, D-arabinose, L-xyllose, D-adonitol, methyl-β-D-xylopyranoside, D-mannose, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, N-acetyl-glucosamine, cellobiose, inulin, xylitol, gentiobiose, L-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, potassium gluconate (weakly in species 5), potassium 2-ketogluconate and potassium 5-ketogluconate. For the unfermented substrates, not determined for subspecies 3 except for D-mannose, L-sorbose, L-rhamnose, D-mannitol, D-sorbitol and inulin. All strains were positive for α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, arginine arylamidase, proline arylamidase, leucine arylamidase, tyrosine arylamidase, serine arylamidase, esterase (C4) (weakly positive reaction in subspecies 2), naphtol-AS-BI-phosphohydrolase and glycine arylamidase (the latter two weakly positive reaction in all tested strains). All were negative for urease, arginine dihydrolase, β-galactosidase-β-phosphate, α-arabinosidase, β-glucuronidase, N-acetyl-β-glucosaminidase, α-fucosidase, nitrate reduction, indole production, alkaline phosphatase, pyrogallnic acid arylamidase, glutaryl glutamic acid arylamidase, lipase (C14), cystine arylamidase, trypsin, α-chymotrypsin, α-mannosidase, gelatin hydrolysis, catalase and oxidase. Enzyme characteristics of subspecies 3 (except for catalase and oxidase) were not determined. +, Positive reaction; (+), weakly positive reaction; --, negative reaction. no, no data available.

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<td>Intestinal tract of domesticated pigs</td>
<td>Waste water of a bean-curd farm</td>
<td>Digestive tract of domesticated pigs, human and calves</td>
<td>Digestive tract of cattle, domesticated and wild pigs, horses, Asian elephant</td>
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**API 50 CHL:**
- L-Arabinose
- D-Ribose
- L-Xylose
- Methyl α-D-mannopyranoside
- Methyl α-D-glucopyranoside
- Amygdalin
- Arbutin
- Aesculin
- Salicin
- Melezitose
- Trehalose
- Lactose

**Rapid ID 32A + API ZYM:**
- Phenylalanine arylamidase
- Leucyl glycine arylamidase
- Glutamic acid decarboxylase
- Alanine arylamidase
- Histidine arylamidase
- Esterase lipase (C8)
- Valine arylamidase
- Acid phosphatase

*Data are from this study and for subspecies 3 from previous studies [7, 60].
†Negative in RP115†.
‡Negative in RP6.
§Weakly positive in all strains tested.

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lead to oral transmission of originally host-specific bacterial taxa. Typical examples of host-non-specific bifidobacteria inhabiting the digestive tract of various farm animals (pigs, cattle and poultry) are B. thermophilum, B. pseudolongum subsp. globosum and B. pseudolongum subsp. pseudolongum [6, 42, 45]. Owing to the habitat and results of phylogenetic analyses, we proposed that the novel species could be the ancestor of the phylogenetic group including thermophilic species of bifidobacteria. However, it is necessary to state here that this hypothesis must be confirmed by modern genomic and phylogenetic analyses.

DESCRIPTION OF BIFIDOBACTERIUM APRI SP. NOV.

Bifidobacterium apri (a’pri. L. gen. n. apri of a wild pig, referring to the fact that the organism was isolated from wild pigs).

Gram-staining-positive, non-motile, non-spore-forming, slightly irregular rods of variable (0.3–0.8 × 1.5–6.0 µm) sizes (Fig. 2). Growth occurs only under anaerobic conditions. Colonies on TPY agar after 72 h cultivation under anaerobic conditions are large (1.4–2.8 mm in diameter), glossy, creamy white, round, disc-shaped in profile, sharply defined, soft and very bulging above the surface of the agar. A wide range of growth temperatures was found (10–49 °C), optimum 37 °C. Cells can grow in the pH range 3.5–7.5, optimally at pH 6.5. Fructose-6-phosphate phosphoketolase-positive, catalase-, oxidase and indole negative. Able to ferment arbutin, aesculin and salicin and to produce glutamic acid decarboxylase, acid phosphatase, leucyl glycin and alanine arylamidases unlike the type strains of phylogenetically related species B. thermophilum, B. thermacidophilum subsp. porcinum and B. boum. Variable in ability to produce acids from methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, amygdalin, melezitose and trehalose. Other biochemical characteristics are listed in Table 2. FAMEs analysis revealed most common fatty acids to be C16:1, C14:1, C18:0 and C13:0 in that order. Peptidoglycan contains the amino acids D-alanine, L-alanine, D-glutamic acid, L-ornithine, L-lysine and D-serine in an approximate ratio of 2.7:1.0:1.6:1.2:0.4:0.1 belonging to the type A4B, variation A21.12. Polar lipids consist of a variety of glycolipids, phospholipids and phosphoglycolipids.

The type strain, RP115T (=CCM 8605T=DSM 100238T=LMG 28779T), was isolated from the large intestine contents of a wild boar from the locality Prague-Dívoká Šárka (Czech Republic). The DNA G+C content of the type strain is 59.4 mol%.

Figure 2. Cell morphology of B. apri RP115T illustrated by scanning electron microscopy.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

References


