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D-ribose utilisation differentiates porcine *Brachyspira pilosicoli* from other porcine *Brachyspira* species

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Abstract

D-ribose utilisation was studied in 60 *Brachyspira pilosicoli* strains and 35 strains of other *Brachyspira* species, the majority of which were of porcine origin. Utilisation of D-ribose was demonstrated indirectly by measuring the reduction in pH of densely inoculated tryptone–peptone broth supplemented with 7% foetal calf serum and 1% D-ribose. Among *B. pilosicoli* strains, the mean reduction in pH units was 1.72 (range 0.95–2.28) in broth with D-ribose and 0.27 (range 0.10–0.40) in sugar-free control broth. For *Brachyspira* strains other than *B. pilosicoli*, the corresponding reductions in pH units were 0.37 (range 0.12–0.49) and 0.37 (range 0.15–0.58). In conclusion, porcine *B. pilosicoli* can be differentiated from other porcine *Brachyspira* species by a test for D-ribose utilisation.

Keywords: *Brachyspira* species; *Brachyspira pilosicoli*; D-ribose; pH; Intestinal spirochaete

The weakly β-haemolytic intestinal spirochaete *Brachyspira pilosicoli* is an aetiological agent of porcine intestinal spirochaetosis [1] and causes diarrhoea among weaned pigs and fatteners. *B. pilosicoli* can also infect or colonise a variety of other animal species as well as humans [2–7]. All of the described species of *Brachyspira* are anaerobic but are sufficiently aerotolerant [1] to be handled in a normal atmosphere for a short period of time. *Brachyspira* bacteria grow well on solid, blood-supplemented agar media, but their growth in liquid broth requires continuous shaking or stirring of the broth [8]. A common scheme for biochemical classification of porcine *Brachyspira* species includes tests for indole production from tryptophane, hippurate hydrolysis, α-galactosidase activity, α-glucosidase activity and β-glucosidase activity [9]. *B. pilosicoli* can be distinguished by its positive hippurate hydrolysis reaction and lack of β-glucosidase activity. Some *B. pilosicoli* strains are, however, hippurate negative [10]. A species-specific polymerase chain reaction (PCR) [11,12] distinguishes *B. pilosicoli*, especially hippurate-negative biovariants, from other weakly β-haemolytic *Brachyspira* species. In low-throughput laboratories, specific PCRs might not be readily accessible; in such cases, additional phenotyping tests for confirmation of *B. pilosicoli* would be beneficial.

Trott et al. [1,13] showed that the *B. pilosicoli*-type strain, two porcine *B. pilosicoli* field strains and three human *B. pilosicoli* strains fermented D-ribose. Neither the type strains of porcine *B. hyodysenteriae, B. innocens, B. murdochii* and *B. intermedia* nor avian *B. alvinipulli* were found to utilise D-ribose [1,14,15]. In these studies the base media contained substrates supporting minimum growth, and utilisation of particular sugar was shown by enhanced growth yield after addition of the sugar to the media. No studies of D-ribose utilisation by a large number of *B. pilosicoli* isolates have been reported to date.

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Utilisation of a single sugar can be detected indirectly also by measuring the reduction in pH of the broth culture [16]. A sugar-free control is also used because the supplements added to meet the minimum growth requirements of *Brachyspira* bacteria can cause some reduction in pH. In this work, we studied D-ribose utilisation in 60 *B. pilosicoli* strains and 35 strains representing other *Brachyspira* species, the majority of which were of Finnish porcine origin. To do this, we applied a method that recorded pH reduction in the broth culture. The effect of the primary cell density on pH reduction was evaluated, and reproducibility of the test was examined before the final tests were carried out.

The strain information is provided in Table 1. Altogether 90 Finnish *Brachyspira* spp. field strains were isolated during 1997–2004. Of the 60 *B. pilosicoli* strains, 36 represented genotypes that had previously been determined by pulsed-field gel electrophoresis [10,17]. Seven of these were hippurate-negative biovariants [10]. Seven of the ten *B. hyodysenteriae* strains were included in the study because of their dissimilar lipo-oligosaccharide cell wall profiles, as earlier confirmed by SDS-PAGE [18]. The remaining strains were obtained from unrelated sources. In addition, five ATCC type or reference strains of genus *Brachyspira* were included. Except for one canine *B. pilosicoli* and two avian *B. intermedia* field isolates, all strains were of porcine origin. The species of field strains had been determined biochemically [9,10], and for *B. pilosicoli* strains, also by species-specific PCR targeting 16S rDNA [11] and/or 23S rDNA [12].

The strains were stored at −70 °C. After thawing, the strains were cultivated on prereduced fastidious anaerobe (FA) agar (LabM, Lancashire, UK) and incubated for 3–4 days at 43 °C in jars under anaerobic conditions (90% N, 10% CO_2, <1% O_2) achieved by anaer generator sachets (AnaeroGen™, Oxoid Ltd, Basingstoke, UK). The second subculture on two prereduced FA-agar plates was used for the D-ribose tests in broth cultures. The base broth (TS-broth, the control) contained 1% tryptone and 1% neopeptone (Bacto™, Becton Dickinson Europe, France) and 7% inactivated foetal calf serum in water. TSR-broth contained 1% D-ribose (MerckTM, Darmstadt, Germany) in TS-broth. The broths were portioned in aliquots of 4 mL in glass tubes of 8 mL. The tubes had flat bottoms and were sealed by loose caps that allowed diffusion of gases. The bacteria were harvested by cotton swabs and transferred to the broths by vigorously spinning the swabs. The cell density in the broth inocula was adjusted by using a CrystalSpek™ Nephelometer (Beckton Dickinson and Company, Sparks, Maryland, USA), which showed the turbidity of the culture in McFarland units. Prior to this, the correlation between the true cell density and McFarland units had been determined microscopically by using a Buerker chamber, separately for *B. pilosicoli* (which is distinctly smaller than the other porcine *Brachyspira* species) and for *B. intermedia* (with a size representative of the other porcine *Brachyspira* species).

To evaluate the reproducibility of the test and the optimal cell density in the broths, two *B. pilosicoli* field strains and the *B. pilosicoli*-type strain were tested three times in TSR-broth by using two-fold cell densities of 2.3×10^7, 4.5×10^7 and 9.0×10^7 cells/mL, representing McFarland units 2, 4 and 8, respectively. In the final tests, each culture was suspended simultaneously in TS-broth and TSR-broth. Cell densities of 6.4–10.9×10^7/mL (McFarland units 5.7–9.7) and 3.2–5.4×10^7/mL (McFarland units 5.4–9.7) were used for *B. pilosicoli* and non-*B. pilosicoli* strains, respectively. Next, 1.6 mL of the inoculated broth was transferred to another tube for measurement of baseline pH (Meterlab™, PHM210,

Table 1
Reduction in pH of serum-supplemented tryptone–peptone broth in presence or absence of D-ribose

Species	No. of strains	Reduction in pH units, mean ± SD	
		TS-broth[a]	TSR-broth[b]
B. pilosicoli[c]	60	0.27 ± 0.08	1.72 ± 0.36
B. intermedia[d]	9	0.39 ± 0.08	0.39 ± 0.07
B. hyodysenteriae[e]	10	0.31 ± 0.11	0.29 ± 0.10
B. murdochii[f]	3	0.32 ± 0.09	0.42 ± 0.15
B. innocens, α-glu negative[g]	8	0.38 ± 0.05	0.39 ± 0.07
B. innocens, α-glu positive[h]	5	0.47 ± 0.09	0.42 ± 0.05

[a]Tryptone–peptone broth with 7% foetal calf serum.
[b]Tryptone–peptone broth with 7% foetal calf serum and 1% D-ribose.
[c]Fifty-eight Finnish strains of porcine origin, and one of canine origin. One type strain, *B. pilosicoli* P43/6/78^T, ATCC 51139.
[d]Six Finnish strains of porcine origin, and two of avian origin. One type strain, *B. intermedia* PWS/A^T, ATCC 51140.
[e]Nine Finnish strains of porcine origin. One reference strain, *B. hyodysenteriae* B 204, ATCC 31212.
[f]Two Finnish strains of porcine origin. One reference strain, *B. murdochii* 155–20, ATCC 700173.
[g]Phenotype which is α-glucosidase negative [22]. Eight Finnish strains of porcine origin.
[h]Phenotype which is α-glucosidase positive [22]. Four Finnish strains of porcine origin. One type strain, *B. innocens* B256^T, ATCC 29796.