**SHORT ARTICLE**

**Lipo-oligosaccharide profiles of *Serpulina pilosicoli* strains and their serological cross-reactivities**

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The purpose of this study was to determine the presence of lipopolysaccharide-like material in the intestinal spirochaete *Serpulina pilosicoli* and the extent of antigenic cross-reactivity of this material in different strains of the species. Hot water-phenol, aqueous-phase extracts from five porcine and three human strains of *S. pilosicoli*, and from seven strains of four other *Serpulina* spp., were separated by SDS-PAGE and silver-stained profiles were obtained. All *S. pilosicoli* strains had a predominant band at c. 16 kDa. Some also had a partial ladder-like profile, consistent with the presence of semi-rough lipo-oligosaccharide (LOS); this was more obvious in Western immunoblot analysis. LOS from each *S. pilosicoli* strain was serologically distinct in immunoblot analysis and there was no cross-reactivity with other *Serpulina* spp. The serological diversity found amongst the LOS of *S. pilosicoli* strains may help to explain why individual people and animals can suffer repeated infections with different strains of the organism.

**Introduction**

*Serpulina pilosicoli* was named recently as the aetiological agent of porcine intestinal spirochaetosis [1]. It colonises the large intestine and may induce a colitis and diarrhoea [1, 2]. *S. pilosicoli* is also recognised as the agent of intestinal spirochaetosis in man [3, 4], birds [5] and dogs [6]. In all species, masses of the spirochaete may be found attached by one cell end to the mucosa, forming a ‘false brush border’ [1, 4]. Other interesting features of the infection include the occurrence of prolonged and repeated episodes of colonisation (on average occurring once yearly and lasting for 4 months in villagers in Papua New Guinea [7]), and the presence of multiple strains of the organism both in pig herds [8] and in closed human communities [3, 9]. Such persistence and repeated occurrence of infection suggest that the spirochaete may have mechanisms for avoiding the host immune response.

Very little is known about the composition of the outer envelope of *S. pilosicoli* although, by analogy with the related intestinal spirochaete *S. hyodysenteriae*, the agent of swine dysentery, it would be predicted to contain lipopolysaccharide (LPS)-like material [10]. In *S. hyodysenteriae*, this material has a rough or semi-rough character, consistent with it being lipo-oligosaccharide (LOS) [11, 12]; all strains possess one or more components of mol.wt 10–16 kDa, representing lipid A-core polysaccharide regions, with some strains apparently also having short O-antigen side chains [12]. This material is extremely important in *S. hyodysenteriae* because, in convalescent pigs, the protective immune response is largely directed against it [13]. LOS extracts also have been used widely as the basis for serological typing of *S. hyodysenteriae* strains [10, 14]. Eleven serogroups have been described, some of which contain multiple serovars [15].

The purpose of the present study was to investigate the occurrence of LPS-like material in *S. pilosicoli*, and to characterise and define the extent of antigenic relationships amongst extracts from different strains.

**Materials and methods**

**Bacterial strains and culture conditions**

Fifteen strains of intestinal spirochaetes from the collection held at the Reference Centre for Intestinal Spirochaetes at Murdoch University, Western Australia...
were used (Table 1). They comprised eight strains of *S. pilosicoli*, two strains each of *S. hyodysenteriae*, *S. intermedia* and *S. innocens*, and one strain of *S. murochii* [16]. Three human isolates of *S. pilosicoli* were from patients with diarrhoea or intestinal disease (WesB, HRM2 and HRM7); all the other spirochaetes were from pigs with diarrhoea (Table 1). The *S. pilosicoli* strains had all been subjected to analysis by multilocus enzyme electrophoresis [3], and their electrophoretic types (ETs) are presented in Table 1. The eight strains used were selected from four different parts of the dendrogram, but included one group of four strains (in ETs 30–33) and one of two strains (in ETs 88 and 89) which were closely related. This selection was intended to give a representation of strains from across the species, and to include closely related strains to determine their potential similarities.

The strains were cultured in trypticase soy broth supplemented with fetal bovine serum 2% and an ethanolic cholesterol 1% solution under anaerobic conditions until they reached mid-log phase (c. 10⁸ cells/ml) [17].

**Extraction of LPS-like material**

LPS-like material from all the strains was extracted by the hot water-phenol method, as modified for *S. hyodysenteriae* [10, 14]. In addition to these aqueous-phase extracts, LPS-like material was also extracted from the phenol phase of five *S. pilosicoli* strains (Table 2) by the method described for *Leptospira* spp. [18]. A hexose assay was used to estimate the concentrations of the LPS-like material extracted from both phases [19]. All samples were stored at –20°C until required.

**Preparation of sera for immunoblot analysis**

Spirochaete cells were obtained by centrifuging 500 ml of fresh mid-log phase cultures at 10000 g for 20 min at 4°C, washing twice in phosphate-buffered saline (PBS) and resuspending in formaldehyde 0.3% in PBS. This suspension was adjusted to an absorbance of 1.0 at 420 nm, and inactivated for 24 h with constant stirring at room temperature. The bacterin was stored at –20°C in small volumes.

Hyperimmune sera used for immunoblot analysis were obtained from rabbits of 2–3 kg body weight, as described previously for *S. hyodysenteriae* [14]. Each animal received 1 ml of bacterin, emulsified in 1 ml of Freund’s incomplete adjuvant, at two intramuscular sites twice at fortnightly intervals. Three weeks later, each rabbit was inoculated intravenously with 1 ml of bacterin without adjuvant at weekly intervals for 5 weeks. Blood was collected 1 week after the last injection and the serum was removed and stored at –20°C until required.

**SDS-PAGE and Western blot analysis**

Extracts from all the intestinal spirochaete strains were subjected to SDS-PAGE and silver staining, as described previously for *S. hyodysenteriae* [12]. Approximately 2 µg of hexose equivalent was added to each well. Separated extracts were also analysed by Western immunoblots with rabbit antiserum raised against each isolate, again with the reagents and

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**Table 1. Sources of spirochaetes used in examination of LOS profiles and antigenicity**

<table>
<thead>
<tr>
<th>Species</th>
<th>Strain no.</th>
<th>Origin</th>
<th>ET*</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. pilosicoli</em></td>
<td>P43/6/78†</td>
<td>Pig, UK</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>3295/90B</td>
<td>Pig, Victoria, Australia</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>HRM2</td>
<td>Man, Italy</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>HRM7</td>
<td>Man, Italy</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>155-4</td>
<td>Pig, Western Australia</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>WesB</td>
<td>Man, Western Australia</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>7082</td>
<td>Pig, New South Wales</td>
<td>88</td>
</tr>
<tr>
<td></td>
<td>181792</td>
<td>Pig, Queensland, Australia</td>
<td>89</td>
</tr>
<tr>
<td><em>S. hyodysenteriae</em></td>
<td>B78†</td>
<td>Pig, USA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>WA1</td>
<td>Pig, Western Australia</td>
<td></td>
</tr>
<tr>
<td><em>S. intermedia</em></td>
<td>PWS/A†</td>
<td>Pig, UK</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2818.5</td>
<td>Pig, Tasmania</td>
<td></td>
</tr>
<tr>
<td><em>S. innocens</em></td>
<td>B256†</td>
<td>Pig, USA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>155-5</td>
<td>Pig, Western Australia</td>
<td></td>
</tr>
<tr>
<td><em>S. murochii</em></td>
<td>155-21</td>
<td>Pig, Western Australia</td>
<td></td>
</tr>
</tbody>
</table>

*Electrophoretic types of *S. pilosicoli* in multilocus enzyme electrophoresis analysis, according to Lee and Hampson [3].
†Type strain.

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**Table 2. Comparison of hexose concentration of LOS from aqueous-phase and phenol-phase extracts of five strains of *S. pilosicoli***

<table>
<thead>
<tr>
<th>Strain no.</th>
<th>LOS extract (µg/ml)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>aqueous phase</td>
</tr>
<tr>
<td>P43/6/78</td>
<td>400</td>
</tr>
<tr>
<td>3295/90B</td>
<td>350</td>
</tr>
<tr>
<td>HRM2</td>
<td>1000</td>
</tr>
<tr>
<td>155-4</td>
<td>500</td>
</tr>
<tr>
<td>WesB</td>
<td>400</td>
</tr>
</tbody>
</table>

*Hexose concentration of LOS.
条件描述先前用于分析S. hyodysenteriae LOS [12]。

**Results**

**Characterisation of LOS by SDS-PAGE**

银染SDS-PAGE谱系的水相提取物的S. pilosicoli菌株谱系如图1所示。预染蛋白分子量标记物用于获得LOS带的大小估计。多重带在所有菌株的准备中被发现，这些带在所有菌株中，这些带的大小和数量之间有变化。所有菌株有一个或更多的主导带在约16 kDa。一般来说，主要成分是在低分子量(<30 kDa)，但较弱的带也在在高分子量区域(30–60 kDa)。个人带宽，且被染成灰色或棕色。此外，在S. pilosicoli菌株155–4（图1，第6条）以及其它S. pilosicoli菌株中，一个阶梯状的重复单元模式明显，即使在银染谱系中这些带不明显（图2）。

**Immunoblot analysis of S. pilosicoli**

抗血清针对每株S. pilosicoli菌株反应强烈只与LOS提取物从同源性菌株。这些血清不反应与主要c. 16-kDa成分，但反应与和高亮标记的重复性成分在每个菌株c. 20–30 kDa，即使在这些菌株中这些带不明显在银染谱中（图2）。这些血清针对与LOS准备从其它Serulina spp.测试，但这些抗血清针对未反应与LOS准备从其它菌属测试。

**Comparison of LPS-like material from S. pilosicoli strains extracted from the phenol phase and water phase**

S. pilosicoli LOS也在被检测在苯酚相提取物中，尽管在较低浓度下，与水相提取物相比（表2）。这种苯酚相材料具有相似的电泳图谱，该材料从水相提取物中（数据未显示）。

**Discussion**

LPS的革兰氏阴性菌是经典‘光滑’，带有重复O-抗原寡糖单位形成一个规则的阶梯状模式从高到低分子量(在银染聚丙烯酰胺凝胶[20])。重复的寡糖单位的数目通常从两个(半光滑)到40(光滑型LPS)。在S. hyodysenteriae,水相粗糙材料从螺旋体拥有一两个在16-kDa范围内，有另外几个但不规则的bands通常观察在较低(<30 kDa) mol. wt region [11, 12, 21]。这些主要带被认为代表脂质A核心材料，而其它低mol.wt的材料被假定代表LOS，而螺旋体已被认为具有半光滑型LOS profile [11, 12]。
Fig. 2. Immunoblot analysis of LOS from *S. pilosicoli* strains. Antiserum raised against *S. pilosicoli* strain 3295/90B was used for detection. Lane 1, mol. wt marker (kDa); 2, strain P43/6/78; 3, 3295/90B; 4, 7082; 5, 181792; 6, 155-4; 7, HRM2; 8, HRM7; 9, WesB (lanes 2–6, pig isolates and lanes 7–9, human isolates). Note the strong homologous reaction with LOS from strain 3295/90B (lane 3) and the appearance of a regular ladder-like pattern in the region c. 20–30 kDa.

As with *S. hyodysenteriae* [12], the profiles of the aqueous-phase extracts of the *S. pilosicoli* strains stained grey or brown with silver, consistent with the presence of specific sugars or fatty acid constituents of lipid A [22]. The *S. pilosicoli* strains also resembled *S. hyodysenteriae* in possessing one or more predominant molecules in the 16-kDa region. Again, by analogy, these probably represent the lipid A-core material. Unlike *S. hyodysenteriae*, the *S. pilosicoli* strains all showed a regular series of low mol. wt bands to a greater or lesser extent in their silver-stained profiles. This was most pronounced in strain 155-4, with fairly regular multiple bands extending down from the c. 70-kDa region, but was more sparse and in more irregular patterns for the other strains (Fig. 1). The low mol. wt of this material is more consistent with it being LOS than LPS. Repeat ladder-like patterns were more demonstrable by immunoblotting than by silver stain. For example, with homologous rabbit antisera, *S. pilosicoli* strains 3295/90B and HRM2 were seen to have ladder-like bands at 20–30 kDa, yet these were not obvious by silver staining (Fig. 2). Five bands were present in strain 3295 and seven in strain HRM2. The lipid A-core material did not stain with the antisera. These findings suggest that *S. pilosicoli* strains differ from *S. hyodysenteriae* in having a smoother type of LOS, with some strains such as 155-4 having more repeat oligosaccharide subunits than others. Clearly, these findings should be confirmed by detailed biochemical analysis of the LOS components.

Subtle differences between silver-stained and immunostained LOS profiles have been observed for *S. hyodysenteriae* strains [11]. The more obvious differences in profiles found here with the two techniques may reflect the differences between the LOS of the two species. As *S. pilosicoli* strains apparently have relatively smooth LOS profiles, the immune response against the spirochaete is likely to be directed more at these external oligosaccharides than at the internal core material. Consequently, the core material would not react with the antiserum. The LOS of the *S. pilosicoli* strains partitioned into both the aqueous and phenol phases of the hot water-phenol extraction. The solubility of LPS is dependent on the relative proportion of hydrophilic polysaccharides and hydrophobic lipid A [23]. Interestingly, there are examples of spirochaetes (e.g., *L. interrogans* serovar *hardjo*) which have LPS-like materials that are more soluble in the phenol phase than in the aqueous phase [24]. Again, further work is required to investigate the physicochemical basis for this partitioning in *S. pilosicoli*.

The LOS extracts from the eight strains of *S. pilosicoli* were all antigenically distinct from each other, as well as from the LOS of the other *Serpulina* spp. tested. *S. hyodysenteriae* strains can be divided into 11 LOS serogroups, although the majority of isolates examined have belonged to only two or three of these [15]. The main component involved in the serological reactivity described for *S. hyodysenteriae* is the lipid A-core material, but in the case of *S. pilosicoli*, hyperimmune serum does not appear to recognise this factor. The overall interpretation of the failure to find *S. pilosicoli* strains with antigenically similar LOS must be tempered by the fact that only eight strains were examined and that these were from two different species (pigs and man) and from different geographical localities. However, by multi-locus enzyme electrophoresis, strains P43/6/78T, 3295/90B, HRM2 and HRM7 apparently belonged to one clonal group, and strains 7082 and 181792...
were also very closely related, separated by a single allele [3]. As shown with *S. hyodysenteriae* [12], genetically related strains of *S. pilosicoli* do not necessarily have the same serological specificity.

*S. pilosicoli* strains are genetically diverse [8, 9], and the results of this study suggest that they are also antigenically diverse. This finding may help explain the tendency for infections to persist or recur in piggeries and human communities where there is extensive exposure to multiple strains [8, 9]; under these conditions, antigenically diverse strains will not necessarily stimulate a cross-protective immune response. Currently it is not clear to what extent the LOS antigens of *S. pilosicoli* strains are important in the development of protective immunity and further work is required to examine this.

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References