Identification of *Bilophila wadsworthia* by specific PCR which targets the taurine:pyruvate aminotransferase gene

Heike Laue¹, Theo H. M. Smits¹, Ulrike K. Schumacher², Marina C. Claros³, Ralf Hartemink⁴ & Alasdair M. Cook¹

¹Department of Biological Sciences, University of Konstanz, Konstanz, Germany; ²Institute for Medical Microbiology & Hygiene, Eberhard-Karls-University, Tübingen, Germany; ³Institute of Medical Microbiology and Infectious Epidemiology, University of Leipzig, Leipzig, Germany; and ⁴Department of Food Technology and Nutritional Sciences, Wageningen University and Research Centre, Wageningen, The Netherlands

**Abstract**

The bile-resistant, strictly anaerobic bacterium *Bilophila wadsworthia* is found in human faecal flora, in human infections and in environmental samples. A specific PCR primer set for the gene encoding the first metabolic enzyme in the degradative pathway for taurine in *B. wadsworthia*, taurine:pyruvate aminotransferase (*tpa*), was developed and tested. In addition, enrichment cultures were started from faecal samples of primates and felines and shown to contain *B. wadsworthia*. These were subcultured on agar media and then identified by PCR fingerprinting. PCR for *tpa* was successful in all positive enrichment cultures and showed no amplification signal in a variety of other bacterial species. Therefore, this PCR method could be a promising tool for rapid detection of *B. wadsworthia* in biological samples.

**Introduction**

The strictly anaerobic bacterium *Bilophila wadsworthia* has been recovered from a variety of intra-abdominal infections, especially appendicitis, and extraabdominal infections (Baron et al., 1992; Finegold et al., 1992; Rautio et al., 2000), as well as human and pig faeces (Baron et al., 1989; Schumacher et al., 1997; McOrist et al., 2001), anaerobic digestors of communal wastewater treatment plants (Laue et al., 1997) and pristine lake sediments (K. Denger and A.M. Cook, unpublished). *Bilophila wadsworthia* was named for its apparent requirement for 20% bile in the growth medium (Baron et al., 1989). Bile could be replaced by taurine (2-aminoethanesulphonate)-conjugated bile acids (Schumacher et al., 1996), and growth with taurine was elucidated as an anaerobic respiration (Laue et al., 1997). *Bilophila wadsworthia* conserves energy by sulphite reduction, with taurine as a source of sulphite, in combination with organic (e.g. formate) or inorganic electron donors (Laue et al., 2001). Taurine is usually the most abundant low-molecular-weight organic compound in mammals (Huxtable, 1992), and one of its many functions is conjugation with bile acids to form bile salts.

At least six enzymes are involved in the formation of the metabolic end products (ammonium, acetate and sulphide ions) during the degradation of taurine by *B. wadsworthia* (Laue et al., 1997, 1999). Taurine:pyruvate aminotransferase (*Tpa*) [EC 2.6.1.77] catalyzes transamination of taurine to sulphaocetaldehyde and alanine (Laue & Cook, 2000b). Alanine dehydrogenase [EC 1.4.1.4] regenerates pyruvate and releases the ammonium ion (Laue & Cook, 2000a). A sulphaocetaldehyde acetyltransferase [EC 2.3.3.15] is presumed to generate acetyl phosphate and release sulphite.
(Ruff et al., 2003). Dissimilatory sulphite reductase (DSR, encoded by dsrABC) [EC 1.8.99.3] generates sulphide from sulphite (Laue et al., 2001). A phosphate acetyltransferase [EC 2.3.1.8], to yield acetyl-CoA for biosynthesis, and an acetate kinase [EC 2.7.2.1] to yield ATP (and acetate) (Cook & Denger, 2002) have been detected at high levels (K. Denger & A.M. Cook, unpublished data). Just as the presence of taurine is widespread, its utilization by bacteria is also widespread, and many organisms initiate dissimilation via Tpa (Cook & Denger, 2002; Denger et al., 2004; Gorzynska et al., 2006). Further, anaerobic bacteria seem to initiate the assimilation of sulphur from taurine via Tpa (Chien et al., 1997; Masepohl et al., 2001).

There is very little gene-specific sequence information available to identify B. wadsworthia. The DSR shares more than 80% amino acid identity with other DSRS (Laue et al., 2001), and the alanine dehydrogenase shares more than 60% identity with other alanine dehydrogenases (Laue & Cook, 2000a). In contrast, Tpa usually has low similarity to other amino transferase genes with known function (Laue & Cook, 2001), and the alanine dehydrogenase shares more than 60% identity with other alanine dehydrogenases (Laue & Cook, 2000a). A phosphate acetyltransferase with known function (Laue & Cook, 2000b; Supplementary Fig. S1). Genome sequence data reveal that the Tpa from Rhodobacter capsulatus SB1003, involved in the assimilation of sulphonate sulphur (Masepohl et al., 2001), shares 58% identity with the Tpa of B. wadsworthia. The highest level of amino acid sequence identity (around 60%) is to hypothetical proteins from the (unfinished) genome sequences of three strains of R. sphaeroides (strains 2.4.1, ATCC 17025 and ATCC 17029). At least one of these does not have an active Tpa when the strain is growing on taurine as sole carbon or nitrogen source (T.H.M. Smits, K. Denger & A.M. Cook, unpublished data).

The tpa gene thus seems to be a suitable candidate for the specific identification of B. wadsworthia. To test this possibility, and to investigate the general abundance of both B. wadsworthia and its tpa gene, a specific PCR primer set for the tpa gene of B. wadsworthia was evaluated for its specificity. Enrichments with taurine started from faecal samples of several felines and primates contained B. wadsworthia, and their presence was confirmed by specific PCR for the tpa gene and dsrAB genes.

### Materials and methods

#### Growth media and enrichment cultures

The freshwater mineral salts medium of Widdel & Pfennig (1981) was used as the basis for the anoxic media. Enrichment cultures for, and pure cultures of B. wadsworthia were incubated in medium with 10 mM taurine and 60 mM sodium formate. All liquid cultures were incubated at 30 °C under an atmosphere of N₂ plus CO₂ (80:20) in serum bottles sealed with butyl rubber septa.

Faecal samples for enrichment cultures were collected from eight species of primates and five species of felines (Table 1) at several zoos in The Netherlands, and two samples of sheep faeces were collected near Konstanz. Faeces (10–25 g) were suspended in 90 mL buffered peptone water (Oxoid) supplemented with cysteine HCl (0.5 g L⁻¹) and resazurine, and homogenized in an anoxic chamber. The flask was filled with sterile glycerol (final concentration about 50%) and stored at −80 °C before use.

<table>
<thead>
<tr>
<th>Source of inoculum</th>
<th>Growth*</th>
<th>Sulphide¹</th>
<th>tpa¹</th>
<th>dsr²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Celebes Macaque</td>
<td>Macaca nigra</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Chimpanzee</td>
<td>Pan troglodytes</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Galago</td>
<td>Galago sp.</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Gorilla</td>
<td>Gorilla gorilla</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Lesser Mouse Lemur</td>
<td>Microcebus murinus</td>
<td>–</td>
<td>–</td>
<td>ND</td>
</tr>
<tr>
<td>Mangabeys</td>
<td>Cercocebus toquates</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Siamese</td>
<td>Hylobates syndactulus</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Orangutan</td>
<td>Pongo pygmaeus</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Bobcat</td>
<td>Lynx lynx</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Cheetah</td>
<td>Acinonyx jubatus</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Panther</td>
<td>Panthera pardus</td>
<td>++</td>
<td>++</td>
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<tr>
<td>Tiger</td>
<td>Panthera tigris</td>
<td>++</td>
<td>++</td>
<td>+</td>
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<tr>
<td>Lion</td>
<td>Panthera leo</td>
<td>–</td>
<td>–</td>
<td>ND</td>
</tr>
<tr>
<td>Sheep</td>
<td>Ovis aries</td>
<td>–</td>
<td>–</td>
<td>ND</td>
</tr>
</tbody>
</table>

* Growth was monitored as turbidity: +++, strong growth; ++, significant growth; +, no growth.

¹Sulphide formation: ++, large amounts; +, significant amounts; –, none detected.

²Amplification by tpa-specific PCR of a 1.0 kb DNA fragment.

Partial gene fragments of the dissimilatory sulphite reductase genes dsrAB were detected by the 1.9 kb amplification product obtained with the consensus PCR primers DSR1F and DSR4R (Wagner et al., 1998). ND, not determined.
For the initial enrichment cultures, about 10% (v/v) inoculum was used. Enrichment cultures were transferred to fresh medium after 5 days for the initial cultures, and after 2–4 days for later cultures. Pure cultures were isolated from the fourth transfer of the enrichment cultures by serial streaking the cultures on Bacteroides Bile Esculin (BBE) agar plates, which were supplemented with 1% taurine and incubated under anoxic conditions at 37 °C.

**Bacteria**

The following pure cultures of *B. wadsworthia* were used in addition to the new isolates: the type strain (ATCC 49260) (Baron et al., 1989), two environmental isolates, RZATAU (DSM 11045) and KNATAU (Laue et al., 1997), and 11 clinical isolates (Claros et al., 1999) recovered from blood (one), appendicitis (two), abdominal specimens (two), human faeces (one), the trachea (one), ulcus cruris (one), a vaginal specimen (one), peritonitis (one), and an ear abscess (one). A further 14 characterized pure cultures and their growth characteristics are cited in the supplementary data.

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**Results**

**Development of PCR assays for *B. wadsworthia***

Few genes encoding metabolic enzymes have been sequenced from *B. wadsworthia* RZATAU, and they are all involved in the anaerobic dissimilation of taurine (Laue & Cook, 2000a, b; Laue et al., 2001). We designed a primer set, which is highly specific for the *tpa* gene, and which should yield a 1003 bp PCR fragment. The primer set was tested on the *B. wadsworthia* environmental isolates RZATAU and KNATAU, on the type strain ATCC 49260 and on 11 clinical isolates of *B. wadsworthia*. All yielded the 1 kb fragment (not shown). This suggests that little genetic diversity is present among isolates of the genus *Bilophila*, which is currently represented by only one species (*B. wadsworthia*).

The specificity of the primer set was tested on a range of clinically relevant Gram-positive and Gram-negative organisms (not shown), and on a set of sulphonate-degrading bacteria including sulphate reducers of the related genus *Desulfovibrio* (Table S1). No amplification product for *B. wadsworthia* gene, and which should be regarded as specific for *B. wadsworthia*. The dissimilatory sulphite reductase genes *dsrAB* of *B. wadsworthia* RZATAU have been sequenced (Laue et al., 2001). A primer set that had been developed to detect a 1.9 kb fragment of the *dsrAB* genes common to all sulphate reducers (Wagner et al., 1998) was used with the same set of organisms mentioned above. In this case, all strains of *B. wadsworthia* (not shown) and the...
Desulfovibrio spp. (Table S1) gave a positive signal at 1.9 kb, whereas no other tested strain yielded the PCR fragment. This confirms that the primer set is unable to discriminate between sulphate reducers and the phylogenetically related Bilophila wadsworthia.

**Enrichment of B. wadsworthia from animal faeces**

Anoxic enrichment cultures for B. wadsworthia in taurine-plus-formate-minimal-salts medium were set up with faecal samples from primates, felines and an ungulate. All of these cultures, with the exceptions of inocula from the lesser mouse lemur, lion and sheep, grew in about 5 days, produced significant amounts of sulphide (Table 1) with simultaneous utilization of taurine (data not shown), and could be reproducibly subcultured. After about four subcultures to fresh liquid medium, 11 enrichments were considered stable and positive. These grew in 2–4 days and contained predominantly short, nonmotile rods, i.e. the morphotype of B. wadsworthia (Baron et al., 1989). Pure cultures were obtained from these enrichments after plating on taurine-supplemented BBE agar. Convex colonies with black centres and translucent margins were observed in each culture, which indicated the presence of B. wadsworthia (Baron et al., 1989). These identifications were confirmed by PCR fingerprinting using the primer T3B (Hunt Gerardo et al., 1997), yielding in all cases the characteristic band for B. wadsworthia.

**Application of tpa and dsrAB primers on the enrichment cultures**

DNA isolated from the 11 positive enrichment cultures (Table 1) was used as the template for the tpa-specific primer pair, and amplification of the 1 kb DNA fragment was observed in each case (Fig. 1). In addition, the presence of genes encoding DSR in all 11 enrichment cultures was indicated by the amplification of the 1.9 kb dsrAB PCR fragment (not shown). The presence of B. wadsworthia in the enrichment cultures was thus indicated by the positive PCR reactions for tpa and dsrAB and corresponded well with the results obtained by microscopy, by colony morphology on BBE agar plates, and by PCR fingerprinting (see above).

**Discussion**

Bilophila wadsworthia has been recovered from the faeces of 60% of all humans tested (Baron et al., 1992; Schumacher et al., 1997) which indicates that the human gastrointestinal tract is a natural habitat for this organism (Baron et al., 1989; Eckburg et al., 2005); it has also been detected in the intestinal tract of pig, chicken and eland (Baron et al., 1992; McOrist et al., 2001, 2003; Nelson et al., 2003). The data in Table 1 indicate that B. wadsworthia is also widespread but not omnipresent in the intestinal tract of primates and felines.

We were unable to isolate B. wadsworthia from the faeces of sheep, lion and lesser mouse lemur. The sample of lion faeces, however, was old, and B. wadsworthia tends to be lost from samples, which were exposed for longer time to oxic conditions (K. Denger & A.M. Cook, unpublished data). An alternative possibility is that, as in the case of humans, pigs and chickens (Baron et al., 1992; McOrist et al., 2001, 2003; Eckburg et al., 2005), not all individuals contain a detectable population of B. wadsworthia within their gastrointestinal tract: populations of B. wadsworthia in human faeces vary between $10^3$ and $10^8$ g$^{-1}$ (wet weight) of faeces, with a total of $10^1$ anaerobes g$^{-1}$ (wet weight) (Baron et al., 1992). The lower limit number might not allow successful isolation of B. wadsworthia from faecal samples.

In all vertebrates except for mammals, bile salts are the conjugates of taurine and cholesterol derivatives (Huxtable, 1992). Mammals, however, use glycine in addition to taurine, though some animals, including the carnivorous polar bear, are described as purely glycine-conjugators (Huxtable, 1992). As taurine-conjugated bile acids are a source of taurine for B. wadsworthia (Schumacher et al., 1996), this species presumably belongs to the normal flora of intestinal tracts, which contain taurine-conjugated bile acids.

Bilophila wadsworthia is one of the most important anaerobic pathogens especially in appendicitis and intra-abdominal infections (Schumacher et al., 1997). Culture-based detection and identification of B. wadsworthia on conventional agar media is time-consuming (4–7 days). Therefore, a more rapid and specific detection method would improve the laboratory diagnosis of this important pathogen. The PCR test involving the tpa primer set is highly specific for the B. wadsworthia tpa gene, with no false

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**Fig. 1.** Amplification by PCR of a fragment (1003 bp) of the tpa gene from representative enrichment cultures in taurine-and-formate-supplemented minimal-salts medium. The enrichment cultures are detailed in Table 1. Lane 1, 1 kb ladder; lane 2, inoculum from a gorilla; 3, inoculum from a chimpanzee; 4, inoculum from a mangabey; 5, inoculum from a panther; 6, inoculum from aCelebes macaque; 7, inoculum from a siamang; 8, inoculum from a bobcat; 9, inoculum from a cheetah; 10, template from a pure culture of Bilophila wadsworthia RZATAU; 11, control (water).
negatives in *B. wadsworthia* isolates from clinical and environmental sources and no false positives from closely related genera. Based on a recent database search at NCBI (February 2006), no bacterial sequences were obtained that would allow PCR with this primer set. The amino acid sequence most similar (60%) to the *B. wadsworthia* Tpa is a putative aminotransferase from the genome sequences of three strains of *R. sphaeroides* represented by strain 2.4.1: this gene product has negligible Tpa activity (T.H.M. Smits, K. Denger & A.M. Cook, unpublished data). Functional Tpa proteins (Masepohl et al., 2001; Denger et al., 2004; Gorzynska et al., 2006), whether involved in the assimilation of taurine sulphur, the assimilation of taurine nitrogen or the dissimilation of taurine carbon, do not cluster in phylogenetic trees, but are interspersed with proteins of different or unknown functions (Fig. S1).

The *dsrAB* primer set (Wagner et al., 1998) could be used in a multiplex PCR approach. The amplification of the *dsrAB* genes using the current primer set in combination with the *tpa* gene by PCR is convincing proof for the presence of *B. wadsworthia* because the corresponding enzymes are both involved in the taurine degradative pathway. As this gene has been sequenced for *B. wadsworthia* RZATAU (Laue et al., 2001), it would be possible to design a gene-specific primer set that only detects the *dsrAB* genes of *B. wadsworthia*.

We have shown the presence of *B. wadsworthia* *tpa* genes in clinical isolates and enrichments from a broad range of sources, demonstrating that the primer set is applicable to identify *B. wadsworthia*, whether from clinical or environmental sources. The primer set for *tpa* we developed, with the *dsrAB* primers, would allow rapid screening of clinical samples using PCR technology, including quantitative PCR using real-time PCR technology (Bustin, 2000, 2002; Mackay, 2004). Further studies are required to develop the applications for medical laboratories.

**Acknowledgements**

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**References**


Supplementary data

Supplementary data associated with this article can be found in the online version, at doi: 10.1016/j.femsle.2006.07.023.

**Fig. S1.** Dendrogram of orthologues of taurine:pyruvate aminotransferase (Tpa) within COG161.

**Table S1.** Some characteristics of pure cultures of sulfonate-degrading bacteria and the detection of the *tpa* gene and the *dsrAB* genes

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