Phenotypic characterization of Sodalis praecaptivus sp. nov., a close non-insect-associated member of the Sodalis-allied lineage of insect endosymbionts

Abhishek Chari,1 Kelly F. Oakeson,1 Shinichiro Enomoto,1 D. Grant Jackson,1 Mark A. Fisher2,3 and Colin Dale1

1Department of Biology, University of Utah, 257 South 1400 East, Salt Lake City, UT 84112, USA
2Associated Regional and University Pathologists (ARUP) Institute for Clinical and Experimental Pathology, 500 Chipeta Way, Salt Lake City, UT, USA
3Department of Pathology, University of Utah School of Medicine, 15 North Medical Drive East, Salt Lake City, UT 84132, USA

A Gram-stain-negative bacterium, isolated from a human wound was previously found to share an unprecedentedly close relationship with Sodalis glossinidius and other members of the Sodalis-allied clade of insect symbionts. This relationship was inferred from sequence analysis of the 16S rRNA gene and genomic comparisons and suggested the strain belonged to a novel species. Biochemical and genetic analyses supported this suggestion and demonstrated that the organism has a wide repertoire of metabolic properties, which is consistent with the presence of a relatively large gene inventory. Among members of the Sodalis-allied clade, this is the first representative that has sufficient metabolic capabilities to sustain growth in minimal media. On the basis of the results of this study, we propose that this organism be classified as a representative of a novel species, Sodalis praecaptivus sp. nov. (type strain HS T = DSM 27494 T = ATCC BAA-2554 T).

This study describes the physiological and biochemical characterization of a novel bacterium isolated from a human wound, previously described as ‘strain HS’ (Clayton et al., 2012). Phylogenetic analysis and comparative genomics have revealed that this organism is closely related to members of the Sodalis-allied clade of insect endosymbiotic bacteria, representatives of which are found in a wide range of insect hosts (Chrudimský et al., 2012; Clayton et al., 2012; Snyder et al., 2011) existing as both facultative and obligate mutualistic symbionts. The only exception to this rule is Biostraticola tofi, a basal representative of this clade that was isolated as part of a biofilm layer on a riverine limestone deposit (Verbarg et al., 2008). Although strain HS T was isolated from a hand wound in a human host, the original source of the wound was a dead tree branch, suggesting that strain HS T is capable of persisting in or on both plant and animal tissues. In addition, the genome sequences of two closely related insect symbionts, Sodalis glossinidius and ‘Candidatus Sodalis pierantonii’ SOPE (Oakeson et al., 2014), were found to be subsets of the genome sequence of strain HS T, suggesting that close relatives of strain HS may give rise to the Sodalis-allied insect symbionts in nature, possibly as a consequence of insects vectoring this bacterium between plant and animal hosts (Clayton et al., 2012).

To date, all insect-associated members of this clade have been found to maintain degenerated gene inventories that contain large numbers of pseudogenes and mobile DNA (Belda et al., 2010), or have substantially reduced genome size (McCutcheon & von Dohlen, 2011). Since the insect hosts provide a stable, highly nutritive and competition-free environment for the bacteria, relaxed selection leads to loss of bacterial genes that are not required to sustain the mutualistic relationship (Moran et al., 2008). The degree of genome degeneration is therefore predicted to be proportional to the age/timespan of the insect–bacterial association. Since the Sodalis-allied clade of symbionts contains representatives with relatively large (e.g. Sodalis glossinidius, ‘Candidatus Sodalis melophagi’) (Chrudimský et al., 2012) and small (e.g. Wigglesworthia glossinidia) genome sizes, it appears to include symbionts that are

The GenBank/EMBL/DDJB accession numbers for the draft genome of strain HS T are CP006569.1 (chromosome) and CP006570.1 (plasmid), those for the 16S rRNA and groEL gene are JX444565 and JX444566, respectively.

A supplementary list, one supplementary figure and one supplementary table are available with the online Supplementary Material.
both recent and ancient in origin (Moran et al., 2008). As might be expected, genome degeneration makes it difficult to culture insect symbionts in the laboratory because they become increasingly fastidious over time (Pontes & Dale, 2006). To date, only two members of the Sodalis-allied clade, Sodalis glossinidius and ‘Candidatus Sodalis melophagi’ have been successfully cultured in vitro and notably, they can only be grown in rich media (Chrudimský et al., 2012; Dale & Mauldin, 1999).

Whole genome sequencing demonstrated that strain HS\textsuperscript{T} possesses a relatively large genome, of 5.16 Mb (Clayton et al., 2012). It lacks any signs of the genome degradation that is apparent in insect-associated relatives (Clayton et al., 2012). This suggests that strain HS has a larger set of metabolic capabilities, including the capability to utilize a wider range of carbon and nitrogen sources. The current study shows that, compared with Sodalis glossinidius, strain HS\textsuperscript{T} does indeed possess more extensive metabolic capability and can grow in defined, minimal media with only a carbon source, nitrogen source and salts. Strain HS\textsuperscript{T} is also capable of utilizing carbon sources found in plants as well as animals. In this work we compare the biochemical properties of strain HS\textsuperscript{T} with a number of animal and plant pathogens, and close relatives including B. tofii and Sodalis glossinidius. We describe phenotypic characteristics that will allow researchers to identify strain HS\textsuperscript{T} in the environment. On the basis of results of the current study and comparative genome sequence analysis (Clayton et al., 2012), the name Sodalis praeactivus sp. nov. is proposed to accommodate strain HS\textsuperscript{T}.

Strain HS\textsuperscript{T} was isolated from serous fluid collected from a wound on the hand of a 71-year-old human patient. The patient’s hand was impaled with a branch from a dead crab apple tree. The organism was isolated on MacConkey agar plates and grown at 35°C, under an atmosphere of 5% CO\textsubscript{2}. The subsequent clinical course of treatment and aspiration of fluid from the wound were described previously (Clayton et al., 2012). Strain HS\textsuperscript{T} was able to grow on M9 minimal agar media (List S1, available in the online Supplementary Material), at 30 and 37°C, in an ambient atmosphere. Two variants of M9 media with different nitrogen sources (ammonium chloride and sodium nitrate) were utilized.

Unlike Sodalis glossinidius (Dale & Mauldin, 1999) and ‘Candidatus Sodalis melophagi’ (Chrudimský et al., 2012), strain HS\textsuperscript{T} is capable of growth in minimal media. The organism grew equally well on both types of M9 agar media, indicating the ability to utilize both ammonia and nitrate as nitrogen sources.

Phylogenetic analysis was performed using 16S rRNA gene and groEL sequences obtained during the sequencing of the Sodalis praeactivus sp. nov. draft genome (Clayton et al., 2012). These were aligned with 16S rRNA gene and groEL nucleotide sequences obtained from the GenBank database for a range of endosymbiotic and free-living Gram-stain-negative bacteria (Fig. 1a, b). Sequence alignments were generated using MUSCLE (Edgar, 2004) and PhyML (Guindon & Gascuel, 2003) and used to reconstruct phylogenetic trees using the HKY85 (Hasegawa et al., 1985) model of sequence evolution with 25 random starting trees and 100 bootstrap replicates. In accordance with previous findings (Clayton et al., 2012), the phylogenetic analysis based on 16S rRNA and groEL gene sequences placed strain HS\textsuperscript{T} in a clade with high bootstrap support with members of the Sodalis-allied insect symbionts (Fig. 1a, b). On the basis of 16S rRNA gene sequence analysis, the relationship between B. tofii and its most closely related insect endosymbiont (Sodalis glossinidius; 96.5 % sequence identity) is substantially more distant than that of strain HS\textsuperscript{T} and its closest insect-associated relative (‘Candidatus Sodalis prierantoniou’ SOPE; >99 % sequence identity). The placement of B. tofii on a relatively long branch corroborates this finding and stands in contrast to the placement of strain HS\textsuperscript{T} on a very short branch, indicating that B. tofii is more distantly related and that strain HS\textsuperscript{T} is more likely to have served as an evolutionary precursor of the Sodalis-allied insect symbionts; a proto-symbiont, as proposed previously (Clayton et al., 2012). In comparison with insect-associated members of this clade, Strain HS\textsuperscript{T} displays a relatively low rate of evolution of its 16S rRNA gene sequence. Other slow-evolving lineages are the symbionts of stinkbugs (Cantao ocellatus) and chestnut weevils (Circulio sikkimensis), which are predicted to have a recent origin in terms of their insect association (Kaiwa et al., 2010; Tojo & Fukatsu, 2011; Tojo et al., 2010). Notably, symbionts with small genome sizes, which are predicted to have a more ancient symbiotic origin, such as W. glossinidia and species of the genus Blochmannia, are localized on the longest branches in the tree. This is compatible with the notion that these bacteria are more ancient derivatives of a strain HS\textsuperscript{T}-like ancestor that have been subject to longer periods of accelerated sequence evolution in their insect hosts. However, we cannot exclude the possibility that the placement of these sequences in the Sodalis-allied clade is a consequence of long-branch attraction in the phylogenetic analysis.

The DNA G + C content of strain HS\textsuperscript{T} was extracted from the draft genome sequence and found to be 57.5 %.

MALDI-TOF (matrix assisted laser desorption ionization – time of flight) MS fingerprinting was performed on strain HS\textsuperscript{T} and Sodalis glossinidius essentially as previously described for identification of clinical isolates (Khot et al., 2012). Bacterial cells (~5 to 10 mg) were obtained from duplicate cultures on MM blood agar media (media composition in List S1) for Sodalis glossinidius and strain HS\textsuperscript{T} or directly extracted from insect host tissue (for the symbionts of grain weevils Sitophilus oryzae and Sitophilus zeamais), and suspended in 300 µl distilled water and mixed by inversion with 900 µl absolute ethyl alcohol. Cells were pelleted (16 000 g, 2 min), and the supernatant was discarded followed by a second centrifugation (16 000 g, 2 min) and aspiration of any remaining ethanol. Cells were resuspended in 50 µl 70 % formic acid.
acid, vortexed for 1 min and mixed by pipetting with 50 \(\mu\)l pure acetonitrile. Samples were centrifuged (16 000 g, 2 min), and 75 \(\mu\)l of supernatant (bacterial extract) was transferred to fresh tubes. Bacterial extract (1 \(\mu\)l) from each replicate culture was spotted in triplicate onto polished steel targets, air-dried and overlaid with 1 \(\mu\)l.

**Fig. 1.** Phylogeny of strain HST\(^1\) and related *Sodalis*-allied endosymbionts and free-living bacteria based on maximum-likelihood analyses of 16S rRNA (Fig. 1a) and groEL (Fig. 1b) nucleotide sequences. Insect endosymbionts that do not have formal nomenclature are designed by the prefix ‘E’, followed by the name of their insect host. Numbers adjacent to nodes indicate maximum-likelihood bootstrap values shown for nodes with bootstrap support greater than 70 \%. Bars, 0.1 (a) or 0.2 (b) substitutions per site.
HCCA matrix, which was allowed to air dry. Mass spectra were acquired, for generation of custom MS spectra with a total of 24 replicate sum spectra for each isolate, between 2000 and 20000 m/z in linear positive ionization mode (Microflex, Bruker Daltonics). MS profiles and MSP dendrograms were created with Biotyper 3.1 software (Database version 4.0.0.1, 5627 MS profiles, Bruker Daltonics) using the manufacturer’s recommended settings. The MALDI-TOF MS dendrogram analysis was supportive of the phylogenetic analysis, showing that strain HS\textsuperscript{T} grouped together with \textit{Sodalis glossinidius} and the closely related symbionts from the grain weevils, \textit{Sitophilus zeamais} and \textit{Sitophilus oryzae} (Fig. 2), which were placed in a clade separate from other members of the family \textit{Enterobacteriaceae}, including the phylogenetically distinct insect symbiont \textit{Arsenophonus nasoniae}. It is of interest to note that the MALDI-TOF analysis of the grain weevil symbionts was performed on bacterial cells isolated directly from insect tissues, highlighting the utility of this method for the identification of symbionts \textit{in vivo}. Furthermore, the addition of the current MS data to the Bruker Biotyper database should facilitate identification of relatives of strain HS\textsuperscript{T} in future clinical and environmental samples, potentially furthering our understanding of the ecology of these bacteria.

Biochemical characterization and substrate utilization tests were performed using the API 20E and API 50CH systems (bioMérieux), according to manufacturer’s recommendations, to assess enzyme activity and fermentation/utilization of carbon sources. Catalase activity was determined using 3 % (v/v) H\textsubscript{2}O\textsubscript{2} and chitinase activity was determined using a fluorimetric assay, CS 1030 (Sigma-Aldrich), according to the manufacturer’s recommendations. Since previously characterized members of the \textit{Sodalis}-allied clade of endosymbionts are metabolically dependent on their hosts, phenotypic tests have only been performed on \textit{Sodalis glossinidius}, which has been obtained in pure culture (Dale & Maudlin, 1999). Owing to this fact, the \textit{Sodalis}-allied clade is largely not amenable to phenotypic testing. Comparison of carbon source utilization traits showed that strain HS\textsuperscript{T} can be distinguished by at least three phenotypic characteristics from its closest relatives, \textit{Sodalis glossinidius} and \textit{B. tofi} (Table 1). Notably, strain HS\textsuperscript{T} could utilize sugars that are plant-based (e.g. cellobiose, xylose, rhamnose) and animal-based (e.g. lactose). In addition, strain HS\textsuperscript{T} could grow at 37\textdegree C whereas \textit{B. tofi} and \textit{Sodalis glossinidius} are incapable of growth at temperatures exceeding 30\textdegree C (Verbarg et al., 2008; Dale & Maudlin 1999). It is interesting to note that strain HS\textsuperscript{T} can utilize melezitose and trehalose, sugars that are predominantly found in aphids and insects, respectively (Owen, 1978; Elbein \textit{et al.}, 2003). This coupled with the fact that strain HS\textsuperscript{T} can produce chitinase as well as utilize N-acetylglucosamine, a building block of insect exoskeleton polymer chitin (Merzendorfer & Zimoch, 2003), indicates that strain HS\textsuperscript{T} has the metabolic potential to derive nutrition from an insect source. Notably, \textit{Sodalis}-allied symbionts are found in a wide range of insect hosts, known to feed either on animals (e.g. blood-feeding tsetse flies) or plants (e.g sap-feeding psyllids), which is consistent with the
notion that close relatives of strain HS\(^T\) have given rise to symbionts in both animal and plant-feeding insects.

As detailed above, unlike the insect-associated relatives of *Sodalis glossinidius*, strain HS\(^T\) is capable of growth on minimal media, consistent with its free-living/opportunistic lifestyle. Thus, for its formal nomenclature, selection of the species epithet (*prae captivus*) was based on the inference that strain HS\(^T\) is a free-living environmental precursor of the *Sodalis*-allied clade of insect symbionts.

**Description of *Sodalis prae captivus* sp. nov.**

*Sodalis prae captivus* (prae.cap.tivus, L. prep. prae before; L. masc. adj. captivus taken prisoner; N.L. masc. adj. prae captivus not yet taken prisoner).

Cells are aerobic, Gram-stain-negative, non-spore-forming short rods (2 μm in length, 1–1.5 μm in diameter). The colonies are white, rounded, mucoid and 1–2 mm in diameter after 24 h of growth at 30°C on LB agar plates. Growth is observed on LB agar, MacConkey agar, Columbia blood agar, MM blood agar and M9 agar from 25°C to 37°C. Exhibits swarming motility on LB agar (Fig. S1) and grows in LB broth at an optimal pH of 5.5 and a maximum NaCl concentration of 5.5 %. Utilizes the following carbon sources: glycerol, D-arabinose, L-arabinose, D-ribose, D-xylose, D-galactose, D-glucose, D-fructose, D-mannose, L-rhamnose, inositol, D-mannitol, D-sorbitol, methyl α-D-glucopyranoside, N-acetylglucosamine, amygdalin, aesculin ferric citrate, cellobiose, lactose, melibiose, sucrose, trehalose, raffinose, melezitose, inulin, starch, glycogen, xyitol, gentiobiose, turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, potassium gluconate and potassium-5-ketogluconate. Produces catalase, chitinase and β-galactosidase. Carbon source utilization test results are tabulated in Table S1.

The type strain is HS\(^T\) (= DSM 27494\(^T\) = ATCC BAA-2554\(^T\)), isolated from a human patient in the USA. The genomic G+C content of the type strain is 57.5 %.

**Acknowledgements**

This research was supported by National Institutes of Health (www.nih.gov) grant 1R01AI095736 (to C.D.) and the ARUP Institute for Clinical and Experimental Pathology (M.A.F.).

**References**


---

**Table 1. Comparison of carbon source fermentation**

<table>
<thead>
<tr>
<th>Carbon source</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-L-lyxose</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>D-Mannose</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>L-Rhamnose</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>D-Xylose</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>L-Arabinose</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Lactose</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Glycerol</td>
<td>W</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>D-Sorbitol</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Cellobiose</td>
<td>W</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>Melibiose</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>D-Tagatose</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Arbutin</td>
<td>–</td>
<td>+</td>
<td>ND</td>
</tr>
</tbody>
</table>


Phenotypic characterization of *Sodalis praecaptivus* sp. nov.