



# Draft Genome Sequence of *Helicobacter suis* Strain SNTW101, Isolated from a Japanese Patient with Nodular Gastritis

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We present here the draft whole-genome shotgun sequence of an uncultivated strain SNTW101 of *Helicobacter suis*, which has been maintained in the stomachs of mice. This strain was originally isolated from gastric biopsy specimens of a urea breath test-negative Japanese patient suffering from nodular gastritis.

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Helicobacter suis, naturally colonizing the stomach of pigs, is the most prevalent non-*H. pylori Helicobacter* species in humans with gastric diseases (1–4). We isolated *H. suis* strain SNTW101 from a urea breath test (UBT)-negative Japanese woman with nodular gastritis in 2008 (5). We have since maintained *H. suis* SNTW101 in the stomachs of female C57BL/6 mice, because this strain has not yet been successfully grown *in vitro* (5). The repeated inoculations with gastric mucosal homogenates from infected mice to uninfected mice have been performed at the intervals of three to six months (5).

The genomic DNA of *H. suis* SNTW101 was prepared from the mouse gastric mucosa by treatment with anti-H. pylori antibodycoated magnetic beads (5), because the infected mouse stomachs contained large quantities of endogenous Lactobacilli in addition to H. suis (6). A whole-genome shotgun library was generated from 68 ng of the purified genome using a TruSeq DNA sample preparation kit (Illumina Inc., San Diego, CA) following the manufacturer's protocol, but with four PCR cycles to reduce amplification bias. The library was sequenced in two lanes using HiSeq1500 with 151-bp paired-end readings, which yielded 255.6 million paired-end reads (77.2 Gb). After adapter trimming using Cutadapt 1.4.1 (7), the reads were mapped onto the mouse genome (GRCm38.p1) using Bowtie2 (8) to identify contaminations derived from the host mouse genome. The unmapped reads (4.7 million paired-end reads) were then assembled using Velvet 1/2/10 (9) with optimized parameters (-k 111 and -cov cutoff 12). The resulting assembly consisted of 672 contigs with a total length of 2,322,207 bp. To identify contigs derived from the H. suis genome, we conducted the following analyses: (i) the contigs were compared with the published draft genome sequences of *H. suis* HS1 and HS5 (10)

using BLASTn (11). The contigs satisfying the bidirectional best-hit criterion or an identity of  $\geq$ 90% were extracted as candidates. (ii) The contigs were searched against the NCBI-nr database using BLASTx, and whether the source organism of the best hit belonged to the genus *Helicobacter* was checked. (iii) The median read coverage of the contigs identified as *H. suis*-derived was 107×, and we eliminated those with low (<50) or high (>200) coverage. The final data set consisted of 42 contigs with a total length of 1,608,632 bp and N<sub>50</sub> of 132,024 bp, covering the entire lengths of existing the *H. suis* genomes of HS1 (1,635,292 bp) and HS5 (1,669,960 bp) (10).

Although *H. suis* SNTW101 was isolated from a UBTnegative patient, the putative gene cluster—including *ureA* and *ureB*, which encode the structural subunits of urease—was present in the chromosome. Indeed, *in vitro* studies of gastric mucosal homogenates from infected mice displayed urease activity. The *H. suis* SNTW101 genome sequence will contribute to the understanding of this pathogen's virulence mechanism.

Accession number(s). The draft whole-genome shotgun sequence of *H. suis* SNTW101 has been deposited in DDBJ/ENA/ GenBank under the accession no. BDAO000000000. The version described in this paper is the first version, BDAO00000000.1.

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## REFERENCES

- Flahou B, Rimbara E, Mori S, Haesebrouck F, Shibayama K. 2015. The other helicobacters. Helicobacter 20:62–67. http://dx.doi.org/10.1111/ hel.12259.
- Liang J, De Bruyne E, Ducatelle R, Smet A, Haesebrouck F, Flahou B. 2015. Purification of *Helicobacter suis* strains from biphasic cultures by single colony isolation: influence on strain characteristics. Helicobacter 20:206–216. http://dx.doi.org/10.1111/hel.12192.
- 3. Øverby A, Yamagata Murayama S, Matsui H, Nakamura M. 2016. In the aftermath of *Helicobacter pylori*: other helicobacters rising up to become the next gastric epidemic? Digestion 93:260–265. http://dx.doi.org/ 10.1159/000445399.
- 4. Nakamura M, Øverby A, Murayama SY, Suzuki H, Takahashi T, Takahashi S, Matsui H. 2016. Gastric non-*Helicobacter pylori* Helicobacter: its significance in human gastric diseases, p. 121–140. In Suzuki H, Warren R, Marshall B (ed), Helicobacter pylori, Part II. Springer, Japan, Tokyo. http://dx.doi.org/10.1007/978-4-431-55705-0\_8.
- Matsui H, Takahashi T, Murayama SY, Uchiyama I, Yamaguchi K, Shigenobu S, Matsumoto T, Kawakubo M, Horiuchi K, Ota H, Osaki T, Kamiya S, Smet A, Flahou B, Ducatelle R, Haesebrouck F, Takahashi S,

Nakamura S, Nakamura M. 2014. Development of new PCR primers by comparative genomics for the detection of *Helicobacter suis* in gastric biopsy specimens. Helicobacter 19:260–271. http://dx.doi.org/10.1111/hel.12127.

- 6. Matsui H, Takahashi T, Øverby A, Murayama SY, Yoshida H, Yamamoto Y, Nishiyama K, Seto Y, Takahashi T, Mukai T, Nakamura M. 2015. Mouse models for assessing the protective efficacy of *Lactobacillus gasseri* SBT2055 against *Helicobacter suis* infection associated with the development of gastric mucosa-associated lymphoid tissue lymphoma. Helicobacter 20:291–298. http://dx.doi.org/10.1111/hel.12203.
- Martin M. 2011. Cutadapt removes adapter sequences from highthroughput sequencing reads. EMBnet 17:10–12. http://dx.doi.org/ 10.14806/ej.17.1.200.
- 8. Langmead B, Salzberg SL. 2012. Fast gapped-read alignment with Bowtie 2. Nat Methods 9:357–359. http://dx.doi.org/10.1038/nmeth.1923.
- Zerbino DR, Birney E. 2008. Velvet: algorithms for *de novo* short read assembly using de Bruijn graphs. Genome Res 18:821–829. http:// dx.doi.org/10.1101/gr.074492.107.
- 10. Vermoote M, Vandekerckhove TT, Flahou B, Pasmans F, Smet A, De Groote D, Van Criekinge W, Ducatelle R, Haesebrouck F. 2011. Genome sequence of *Helicobacter suis* supports its role in gastric pathology. Vet Res 42:51. http://dx.doi.org/10.1186/1297-9716-42-51.
- Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25:3389–3402. http://dx.doi.org/10.1093/nar/25.17.3389.