SUPPLEMENTAL MATERIALS

Sampling

From each individual, samples from saliva as well as stomach and duodenum aspirates and biopsies were obtained. Saliva samples were collected from the oral cavity using a 5 mL BD Discardit™ II syringe. Aspirates were collected using a sterile 0.035´´ filiforme endoscopic retrograde cholangiopancreatography catheter (MTW® Endoscopy Manufacturer, Wesel, Germany), and a standard video gastroscope (GIF Q145, GIF 160, GIF 180HD; Olympus Medical, Hamburg, Germany). Biopsies were taken using a cup forceps with a needle (Olympus SwingJaw 2.8 mm FB-240 KA, Olympus Medical). After collection of aspirates from the descending part of the duodenum, biopsies were taken from this area. Subsequently, gastric juice was aspirated using a sterile catheter. Biopsies were taken from the antrum. Additional biopsies for histopathological examinations and rapid urease testing were taken from duodenum, antrum, angulus and corpus. Samples for RNA extraction were supplemented with 1 ml RNAlater (SIGMA- ALDRICH, St.Louis, MO, USA), kept at room temperature for 4-8 hours and stored at -80°C.

Clinical diagnosis of *H. pylori* infection

Classification of the *H. pylori* status was performed based on rapid urease test and histopathological assessment. Urease activity was tested in one antrum and one corpus biopsy during the endoscopy and assessed visually after 4h (HUT; Astra Zeneca, Wedel, Germany). The histopathological assessment was performed at the Institute of Pathology, University of Magdeburg, Germany by gastrointestinal pathologists. All sections were stained with hematoxylin and eosin and the modified Giemsa method to detect *H. pylori*. The Sydney pathologic classification for gastritis[1] and “operative link for gastritis assessment” and “operative link for gastric intestinal

metaplasia assessment" staging systems[2] were applied. *H. pylori* was positively diagnosed when one of the invasive tests (urease fast test, or histopathological assessment, or both) was positive. In addition, fasting venous blood samples were taken from each individual and anti-H.pylori IgG antibodies analyzed using the *H.pylori* IgG enzyme-linked immunosorbent assay (Biohit, Rosbach, Germany) according to the manufacturer´s instructions. A sample was classified as *H.pylori* positive if ≥ 30 enzyme immunounits of *H.pylori* specific IgG were observed.

RNA extraction, cDNA synthesis and library preparation

RNA was extracted using the RNeasy kit (Qiagen) following the manufacturer's instructions, but including a mechanical lysis step. Briefly, the biopsies were transferred from the RNAlater solution into a Lysing Matrix E tube containing 700 µl of RLT buffer with β-mercaptoethanol solution as specified by the manufacturer, whereas the saliva and aspirate samples were centrifuged for 10 min at 15.000 rpm and 4°C before the pellet was transferred and resuspended. Cells were lysed using a Fast Prep™-24 instrument (MP Biomedicas, Solon, OH, USA) at 6.0 m/s for twice 45 s. The samples were then centrifuged and the supernatant transferred to the column and DNA digested on column as previously described[3]. RNA was eluted with 32 µl of RNase free water. A second DNA digestion was performed using DNase Turbo (ThermoFisher Scientific) following the instructions of the provider. RNA was precipitated with 1:10 volume of sodium acetate (3M) and 3 volumes of ethanol. First-strand cDNA was synthesized using SuperScript® III First- Strand Synthesis System (Invitrogen™, Carlsbad, California, USA) and random primers, following the manufacturer´s instructions.

Amplicon libraries were generated as previously described[4] where the V1-V2 region of the 16S rRNA was amplified after 20 cycles PCR reaction using the 27F

and 338R primers[5]. Final PCR products were verified by agarose gel electrophoresis, purified using Macherey-Nagel 96-well plate purification kits (Macherey-Nagel, Düren, Germany) following manufacturer´s instructions and quantified with the Quant-iT PicoGreen dsDNA (Invitrogen). Equimolar ratios of amplicons were pooled and sequenced on a MiSeq (Illumina, Hayward, CA, USA).

Statistic analysis

To assess taxonomic diversity, funnel plots were constructed where delta+ and lambda+ were plotted as a function of species richness by sampling increments of 10 phylotypes from the masterlist of 687 phylotypes, with maximum random selections of 1000[6]. The funnel indicates the limits within 95% of the simulated TD values and the middle line represents the mean expected TD, thus providing a statistical framework to test whether these measures depart from expectation[7, 8].

References

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