Characterization of *Arcobacter* spp. isolated from human stool samples in Germany

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Aim

Arcobacter species are considered as emerging food- and waterborne pathogens. The two species *A. butzleri* and *A. cryaerophilus* have been classified as "serious hazard to human health" from the International Commission on Microbiological Specifications for Food (ICMSF) as they are associated with human diseases, like gastroenteritis. However, their role in human disease requires further studies especially regarding pathogenesis and underlying virulence factors. Thus, this study aimed at characterizing *Arcobacter* spp. isolates collected from human specimens regarding the genetic diversity, presence of virulence genes and cytotoxicity.

Methods

A total of 36 human *Arcobacter* isolates (24 *A. butzleri*, 10 *A. cryaerophilus* and 2 *A. lanthieri*) were included.

ERIC-PCR

For evaluating genetic diversity, the strains were characterized by ERIC-PCR (1). Analysis of fragment pattern was performed using BioNumerics v. 7.1 (Applied Maths, Sint-Martens-Latem, Belgium). For generation of dendrogram, the Dice coefficient and the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) were used.

Detection of virulence genes

The occurence of 10 putative virulence genes was investigated by PCR (2,3). For *A. lanthieri* additional primers were used including also primers for detection of cytolethal distending toxin (CDT) genes *cdtA*, *cdtB* and *cdtC* (4).

Cytotoxicity assay

Human colon adenocarcinoma cells HT-29/B6 were seeded in 96-well plates at a density of 3 x 10^5 cells/well and differentiated for 7 days. Cytotoxic effects were measured by the colorimetric WST-assay 48 h after bacterial inoculation with MOI of 100 (5).

Results

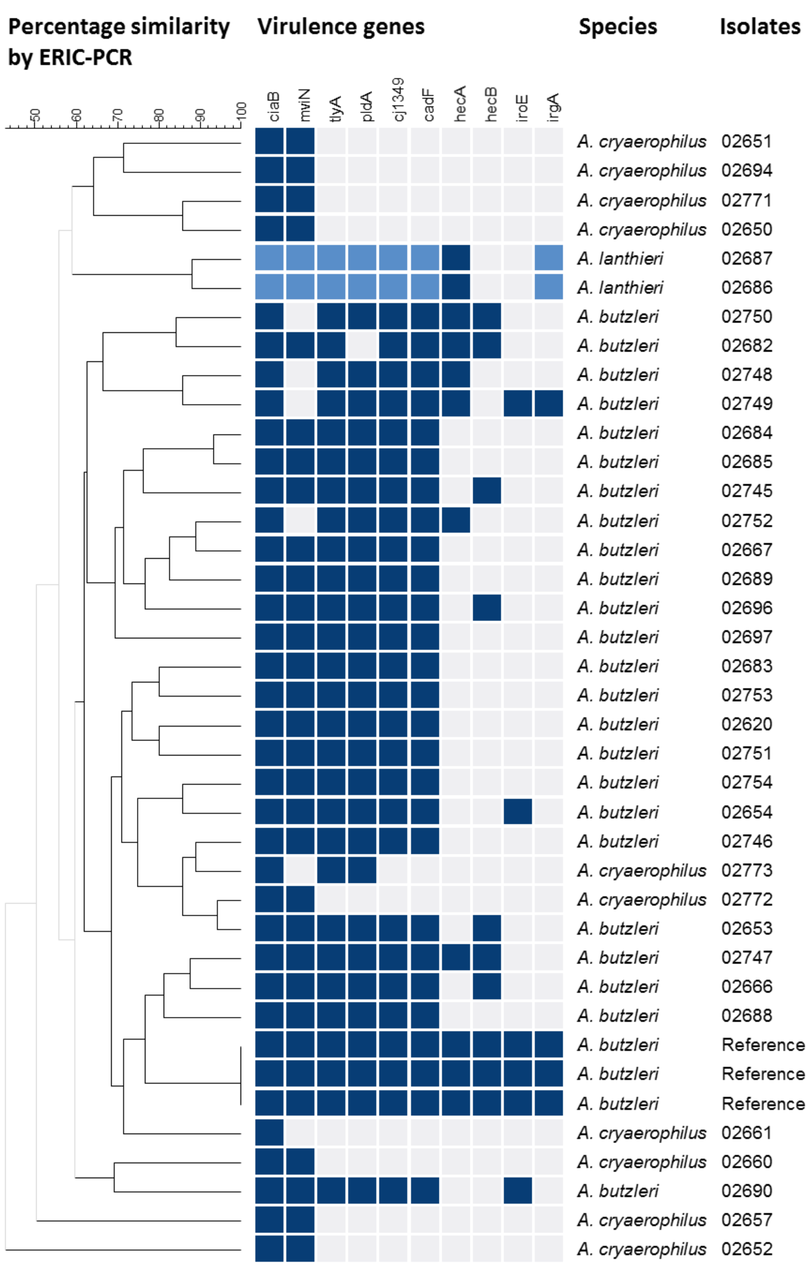

Figure 1: Genetic diversity of *Arcobacter* spp. and presence of virulence genes. The dendrogram is based on ERIC-PCR assay. Blue squares: gene detected by PCR; grey squares: gene not detected by PCR; light blue: gene detected by *A. lanthieri* specific primers. *A. butzleri* (CCUG 30485) was included as reference strain.

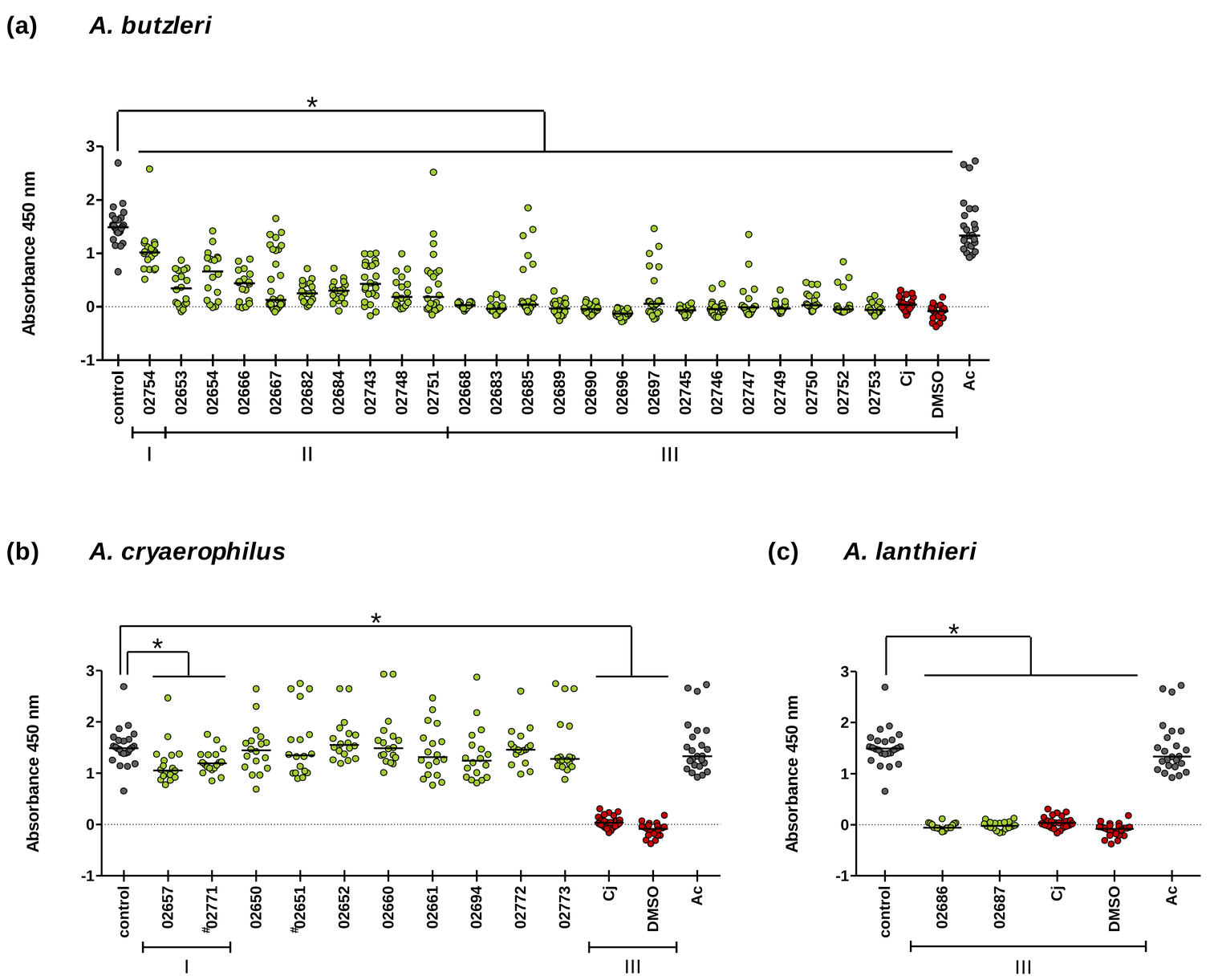

Figure 2: Viability of HT-29/B6 cells after inoculation with *Arcobacter* isolates. The level of toxicity was subjectively classified in three groups, including strains of low (I), moderate (II) and high cytotoxicity (III) with 20-49%, 50% to 94% and at least 95% reduction of absorbance compared to uninfected media control, respectively. At least three independent experiments were performed with six replicates each. Cj: *C. jejuni* 81-176, Ac: *A. cryaerophilus* ILSH 02659; # Inoculation with MOI 50; * $p < 0.05$ (Mann-Whitney U-test) compared with control.

ERIC-PCR

All investigated *Arcobacter* isolates had different genotypes indicating high genetic diversity. Dendrogram analysis revealed species-specific clusters for most *A. butzleri* (62% similarity) and both *A. lanthieri* strains (86%), whereas *A. cryaerophilus* isolates were widely distributed within the dendrogram (Fig. 1).

Detection of putative virulence genes

A large number of putative virulence genes was present in *A. butzleri* and *A. lanthieri*, while fewer virulence genes were detectable in *A. cryaerophilus* isolates (Fig. 1). Notably, the three CDT genes *cdtA*, *cdtB* and *cdtC* were abundant in both *A. lanthieri* isolates (data not shown).

Cytotoxicity assay

Our results indicate strain-specific cytotoxic effects among *A. butzleri* and *A. lanthieri* isolates, with most of them demonstrating high cytotoxicity on HT-29/B6 cells, whereas the majority of the investigated *A. cryaerophilus* isolates did not induce any cytotoxic effects (Fig. 2).

Conclusion

Our study provides evidence for the abundance of respective putative virulence genes and prominent *in vitro* cytotoxic effects of *A. butzleri* and *A. lanthieri*. Furthermore, the presence of the toxin genes *cdtA*, *cdtB*, *cdtC* in *A. lanthieri* may indicate the secretion of the exotoxin CDT as potential mechanism underlying cytotoxicity as opposed to *A. butzleri*. However, pathogenesis and virulence factors of *Arcobacter* are still poorly understood, which makes it difficult to assess the potential risk of this pathogen. Further investigation are required for a more in-depth evaluation of the role of *Arcobacter* in human disease.

References

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