Prevalence of *Arcobacter* spp. in seafood in retails of Germany

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Introduction

Arcobacter is an emerging zoonotic pathogen with a wide range of habitats and hosts such as animals, water and foods worldwide. Currently, among the 21 proposed *Arcobacter* species, *Arcobacter* (*A.*) *butzleri*, *A. cryaerophilus* and *A. skirrowii* have been classified as a serious hazard to human health. Consumption of contaminated food and water is considered to be the major transmission route to humans. This study aimed to evaluate the prevalence of *Arcobacter* spp. in retail seafood samples in Germany. Furthermore, the occurrence of putative virulence genes and genetic diversity of the isolates were also studied.

Material and methods

Sample collection

A total number of 230 samples consisting of mussels (81), shrimp (97), squid (39) and scallops (13) were collected from the local retail markets and supermarkets in Berlin.

Isolation of *Arcobacter* according to Ataby et al. (2003)

10 g of samples were homogenized in 90 ml *Arcobacter* Broth (Oxoid, Wesel) with CAT-supplement (Oxoid, Wesel) and incubated microaerobically for 48 h at 30 °C. The enrichment was diluted 1:10 in Brucella Broth (BD Biosciences, Heidelberg) and 300 µl dilutions were applied on a 0.6 µM filter (GE Healthcare Europe, Freiburg) placed on a Mueller Hinton agar plate (Oxoid) with 5% sheep blood (MHB). The filter was discarded after one hour aerobic incubation at 30 °C and further 100 µl Brucella Broth was dropped on the plates before streaking for better colony separation. The plates were then incubated for 48 h microaerobically at 30 °C and the suspected colonies were enriched on MHB plates microaerobically at 30 °C for further identification.

Molecular identification of isolates

DNA was extracted from the suspected isolates by Chelex method and identified to species level with mPCR according to Houf et al. (2000). The *rpoB* gene of the mPCR positive isolates were further sequenced (Korczak et al. 2006) and species confirmed by BLAST.

ERIC PCR for Genotyping

Genotyping of isolates belonging to the genus *Arcobacter* were further characterized by ERIC-PCR according to Houf et al. (2002). Band pattern were analysed using BioNumerics version 7.1 (Applied Maths, Sint-Martens-Latem, Belgium). After normalisation, the similarities between profiles, based on peak position, were calculated using Dice coefficient. For cluster analysis, the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) was used.

Detection of virulence genes

10 putative *Arcobacter* virulence genes (*cadF*, *pldA*, *irgA*, *hecA*, *hecB*, *cj1349*, *ciaB*, *mviN*, *tlyA* and *iroE*) of *A. butzleri* strains were detected using the PCR according to Karadas et al. (2013).

Results

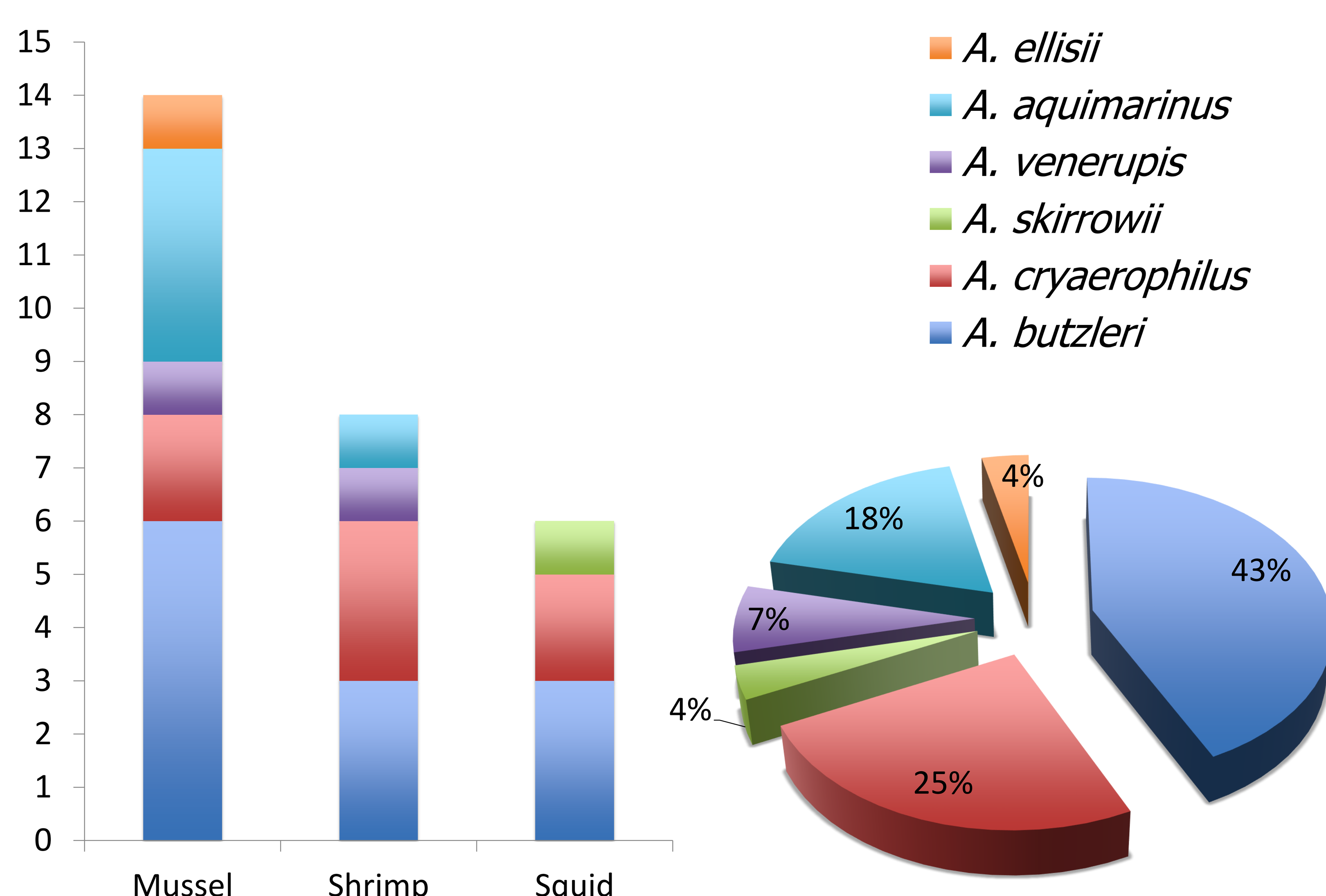

Fig. 1 *Arcobacter* spp. from different food matrix

Arcobacter spp. were found in 12% (28/230) of seafood samples (Fig. 1), consisting of 43% *A. butzleri*, 25% *A.cryaerophilus*, 18% *A. aquimarinus*, 7% *A. venerupis*, 4% *A. skirrowii* and *A. ellisii*, respectively. *A. butzleri* and *A. cryaerophilus* were isolated from all three food matrix (mussels, shrimp and squid), while *A. venerupis* and *A. aquimarinus* were found in both mussels and shrimp. Besides, only one *A. skirrowii* was found in squid and one *A. ellisii* in mussels. No *Arcobacter* spp. was found in scallops.

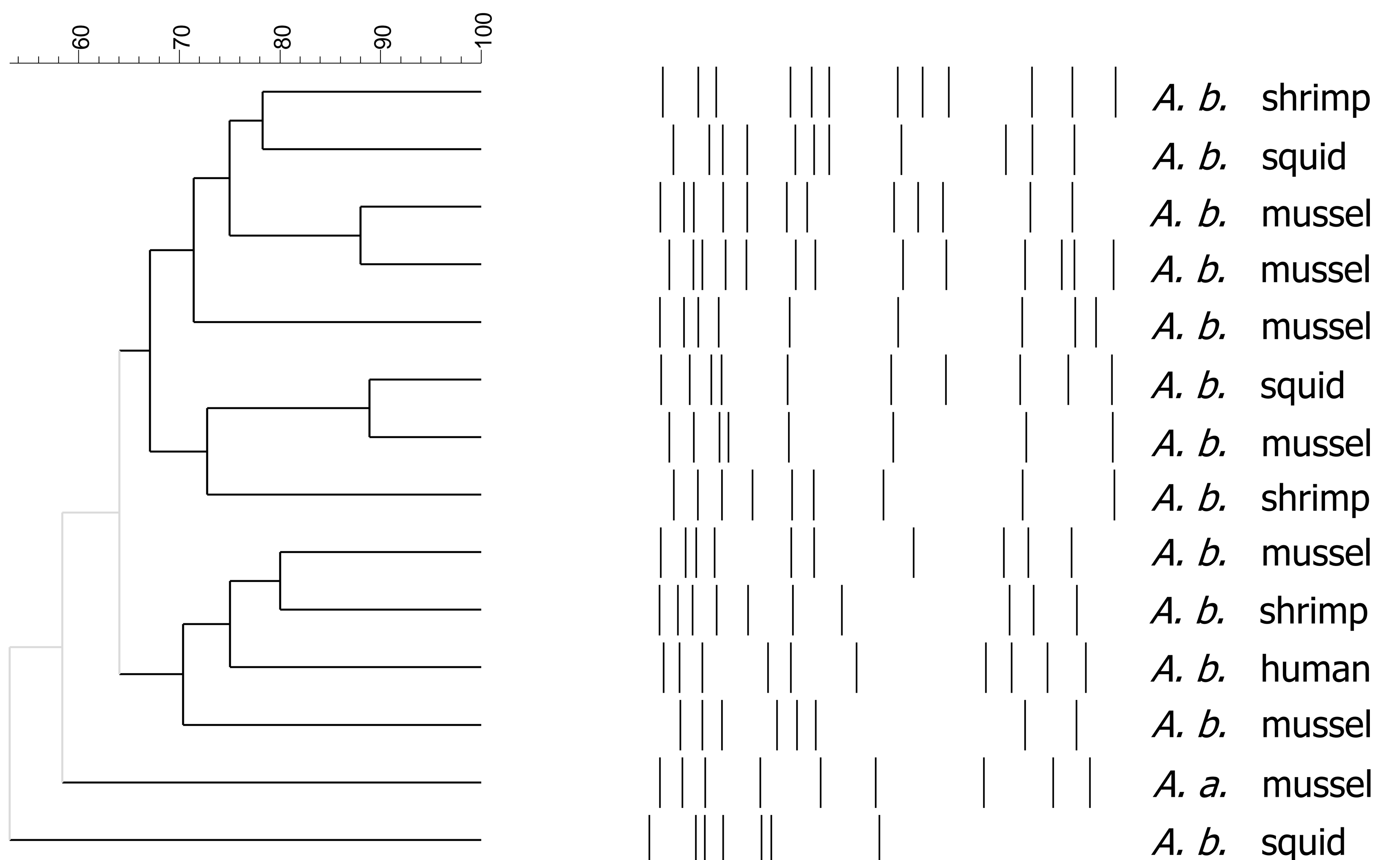

Fig. 2 Dendrogram of *A. butzleri* based on ERIC-PCR rooted to an *A. aquimarinus* strain
A. b. = Arcobacter butzleri ; *A. a. = Arcobacter aquimarinus*

ERIC-PCR assays were used to investigate the genetic diversity of *Arcobacter* spp. These assays demonstrated a high genetic diversity within *A. butzleri* strains including the reference strain CCUG 30485, isolated from a human sample (Fig. 2). A species differentiation of *Arcobacter* is not possible by ERIC-PCR (data not shown).

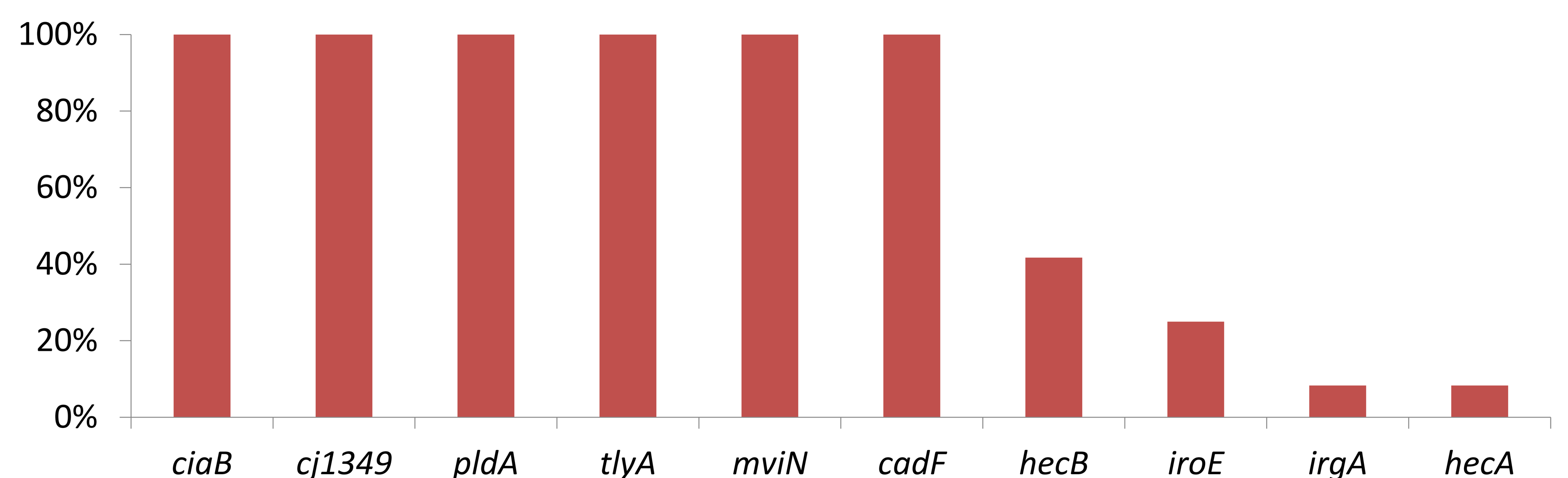

Fig. 3 Presence of putative virulence genes in *A. butzleri* strains isolated from seafoods (n = 12)

The distribution of putative virulence genes among the isolated *A. butzleri* strains is shown in Fig. 3. All investigated *A. butzleri* isolates carried the genes *ciaB*, *cj1349*, *pldA*, *tlyA*, *mviN* and *cadF*. Lower detection rates were observed for *hecB* (41.7%), *iroE* (25%), while both *irgA* and *hecA* were only found in 8% (1/12) of all *A. butzleri* strains.

Summary

In summary, *Arcobacter* spp. were isolated from 12% of the mussels, shrimp and squid samples at retail level in Berlin. Based on mPCR and *rpoB* sequence analysis, 6 different species were detected (*A. butzleri*, *A. cryaerophilus*, *A. aquimarinus*, *A. venerupis*, *A. skirrowii* and *A. ellisii*), while the majority of the *Arcobacter* spp. belong to the species *A. butzleri* and *A. cryaerophilus.* Comparable to other studies, all of our *A. butzleri* isolates encoded the six putative virulence genes: *ciaB*, *cj1349*, *pldA*, *tlyA*, *mviN* and *cadF*. The ERIC-PCR assays show high diversity within one species, however ERIC-PCR is not able to differentiate *Arcobacter* spp.

This study shows that consumption of contaminated raw seafood pose a risk of human infection with *Arcobacter*.

Literatur

Ataby et al 2003; Int J Food Microbiol
Houf et al 2000; FEMS Microbiol Lett
Karadas et al 2013; J Appl Microbiol
Coenye et al 1999; Int J of Syst Bacteriol

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