Community changes during the decontamination of hatching eggs using diazine derivates

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Introduction

Microbial interplay relies on communication molecules that are exchanged between interacting microbes. Diazine derivatives among other volatile organic compounds (VOCs) were recently shown to play an important role in microbial interactions. The underlying effects were recognized to be useful for stabilizing applications in food processing. Currently, several approaches of egg decontamination are used in the hatching industry. The most frequently used method is formaldehyde fumigation. Formaldehyde is known to be a carcinogenic hazard and many hatcheries are searching for formaldehyde-free and environmental friendly methods. We evaluated the application of diazines on contaminated egg shells and found that they can drastically reduce the bacterial count. Moreover, we demonstrated that low diazine concentrations lead to changes in the microbial community structure due to their varying anti-microbial effects on different organisms. These changes were visualized with amplicon sequencing and complementing techniques to further optimize application strategies.

Treatment efficiency accessed with NGS

The application of diazines on eggs from different producers was performed using two methods. The first approach is based on the evaporation of the volatile compounds (**passive treatment**), whereas in the second approach evaporation was accelerated using heat (**thermic treatment**). For a detailed look into the microbial communities on treated and untreated egg samples, next-generation sequencing (NGS) technologies were used. Subsequently, the **16S rRNA gene amplicons** from 64 samples were evaluated using the QIIME workbench and other bioinformatic tools. A high diversity of OTUs (operational taxonomic units) was detected in the utilized samples (**Fig. 2**). Moreover, structural community changes were observed during the treatments of the eggs (**Fig. 1**).

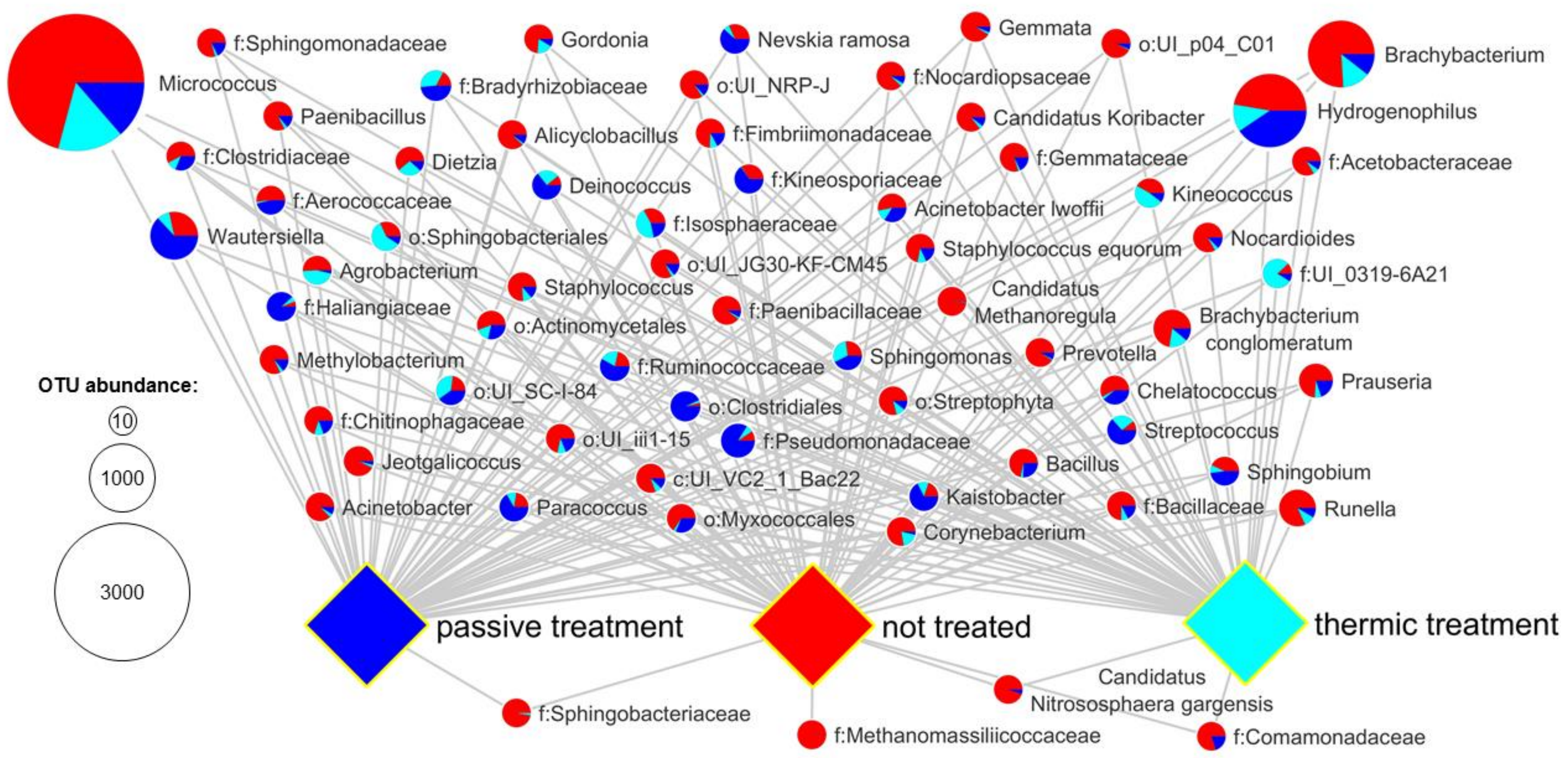

Figure 1: Abundance of OTUs on treated and untreated samples. The node size correlates to the total abundance. Pie charts indicate the fractions found in distinct samples.

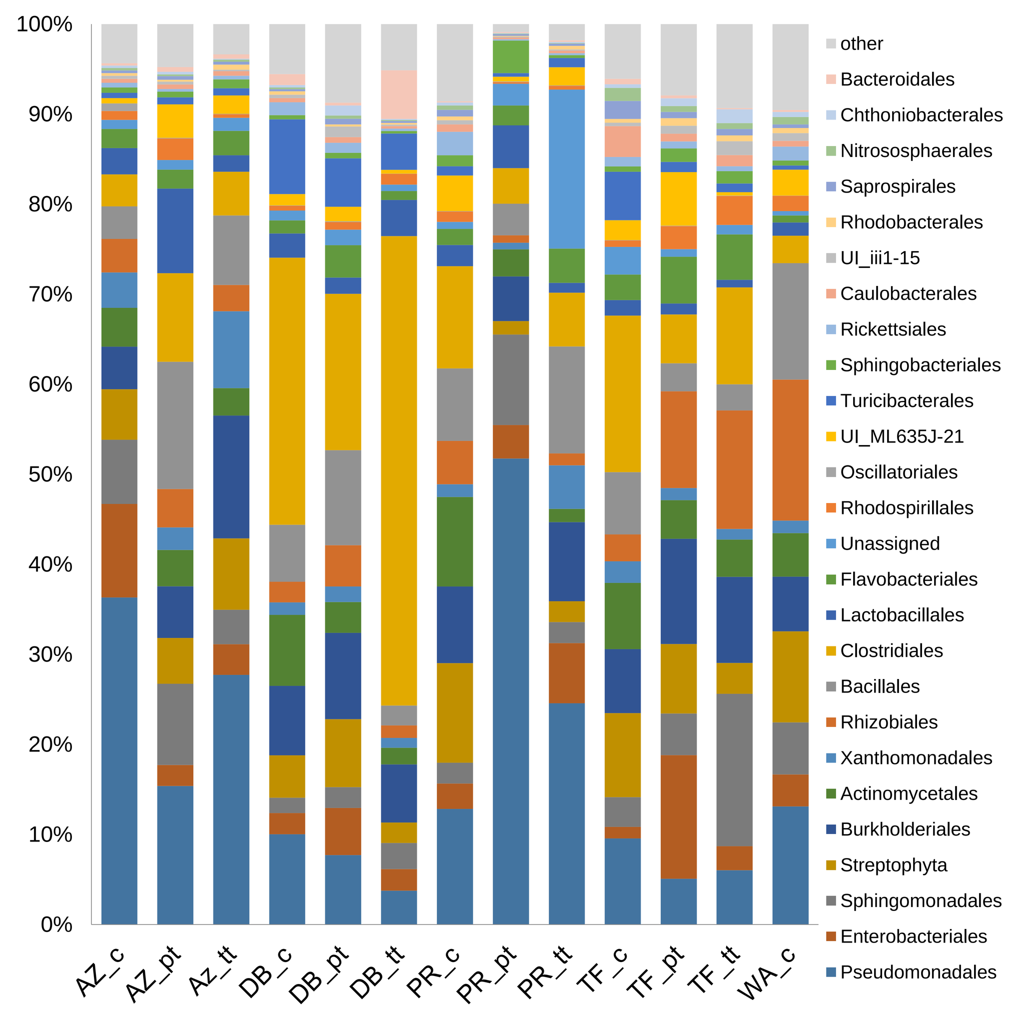

Figure 2: Observed diversity in all samples (order level). The bacterial diversity was very high between the samples due to varying residues on the eggs. **c**: **c**ontrol; **pt**: **p**assive **t**reatment; **tt**: **t**hermic **t**reatment

Using **Confocal Laser Scanning Microscopy (CLSM)** micrographs of the bacterial communities on eggshells were made (**Fig. 3**). LIVE/DEAD staining was used to detect the reduction of bacterial growth on the samples upon treatment with diazines. The decontamination efficiency on the sample was additionally analyzed using cultivation depended methods and **qPCR** with total DNA extracts bacteria-specific primers. Thermic treatment led to reduction rates >99% (**Fig. 4**), which corresponds to treatments with formaldehyde.

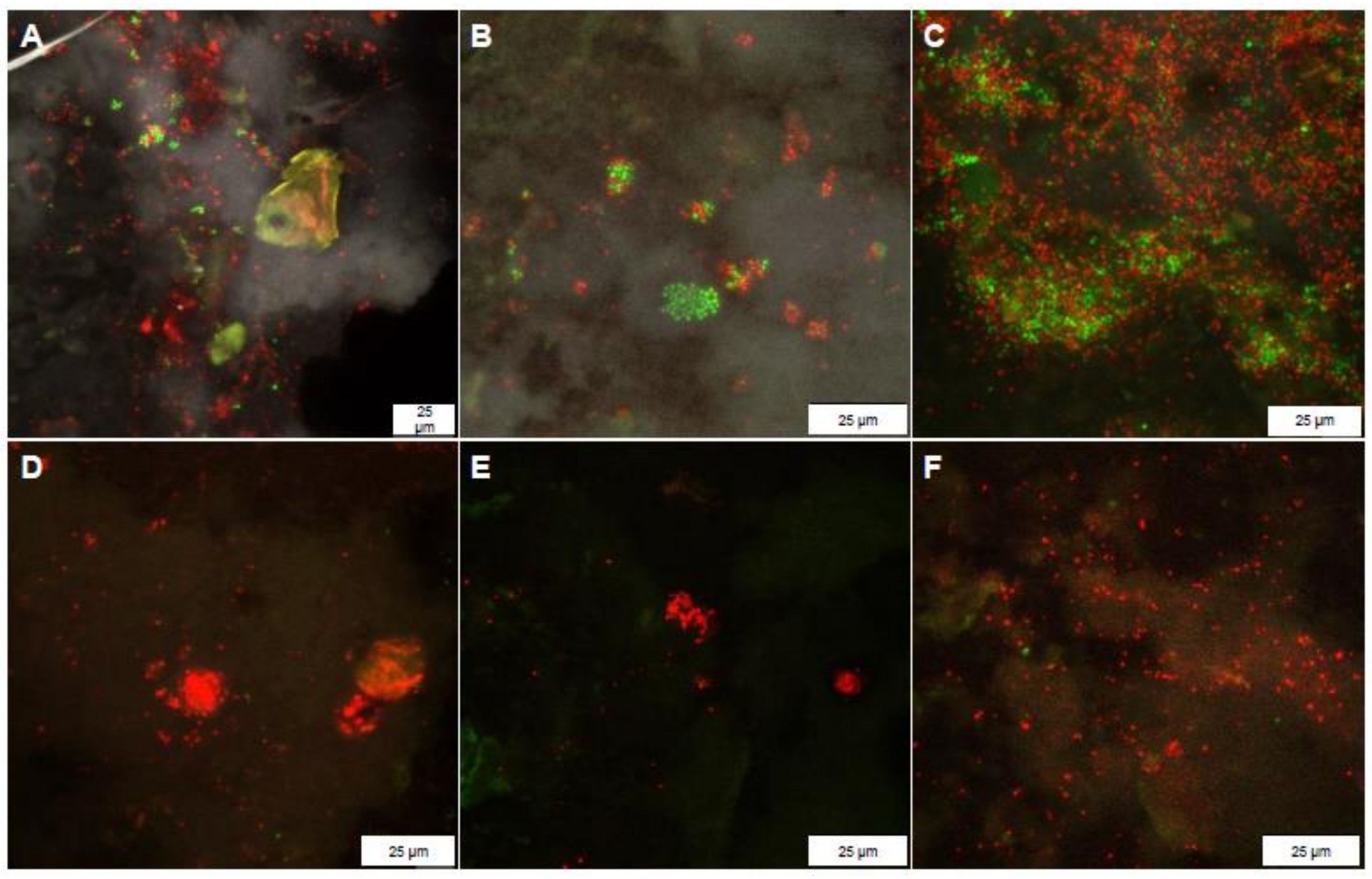

Figure 3: LIVE/DEAD staining of bacteria on egg shells. Green: living cells, red: dead cells. A-C: shells of untreated eggs, D-F: shells of treated eggs

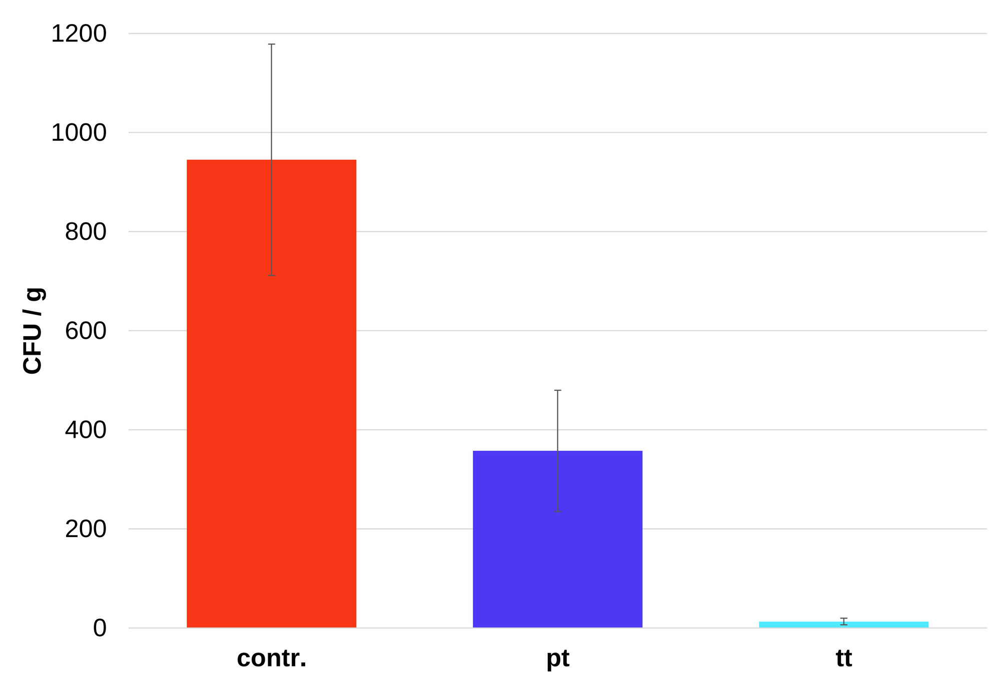

Figure 4: Cultivable bacteria were sampled from untreated (contr.) and diazine-treated eggs (pt; tt).

Objectives

- Assessment of diazine effectiveness and its impact on microbial communities for applications on natural products.
- Identification of resistant bacteria on egg shells and treatment optimization as a preparation for industrial applications.

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