

Oxytetracycline's Impact on Corn Silage Microbes and ARGs: Metagenomic and Metatranscriptomic Study

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Introduction

Antibiotic resistance genes (ARGs) are introduced into the soil from different sources especially animal dung and which are transferred to plants through various mechanisms (Huang et al., 2021). The crops grown on these soils are subsequently used for silage production and often contain several ARGs. Despite the significance of this ARGs transfer, there is a lack of comprehensive studies on the microbial communities and ARGs present in the silage. Therefore, addressing this cycle is crucial for mitigating the spread of antibiotic resistance.

Materials and methods

Freshly harvested and chopped whole crop corn (*Zea mays* L.) from Wuwei City, Gansu Province, China was divided into eighteen subsamples. Six of the subsamples were randomly assigned to one of the oxytetracycline (OTC) concentration treatments (0, 40 and 80 µg/kg fresh weight) and ensiled for 3 and 90 days in a vacuum-sealed polyethylene bags (30 × 23 cm). At each ensiling period, three replicates for each treatment were opened and samples were taken for OTC residual, metagenomic and metatranscriptomic analyses. Subsequently, microbial community analyses as well as ARGs annotation were conducted to track microbial succession, viable microbiota, ARGs and their expression.

Results

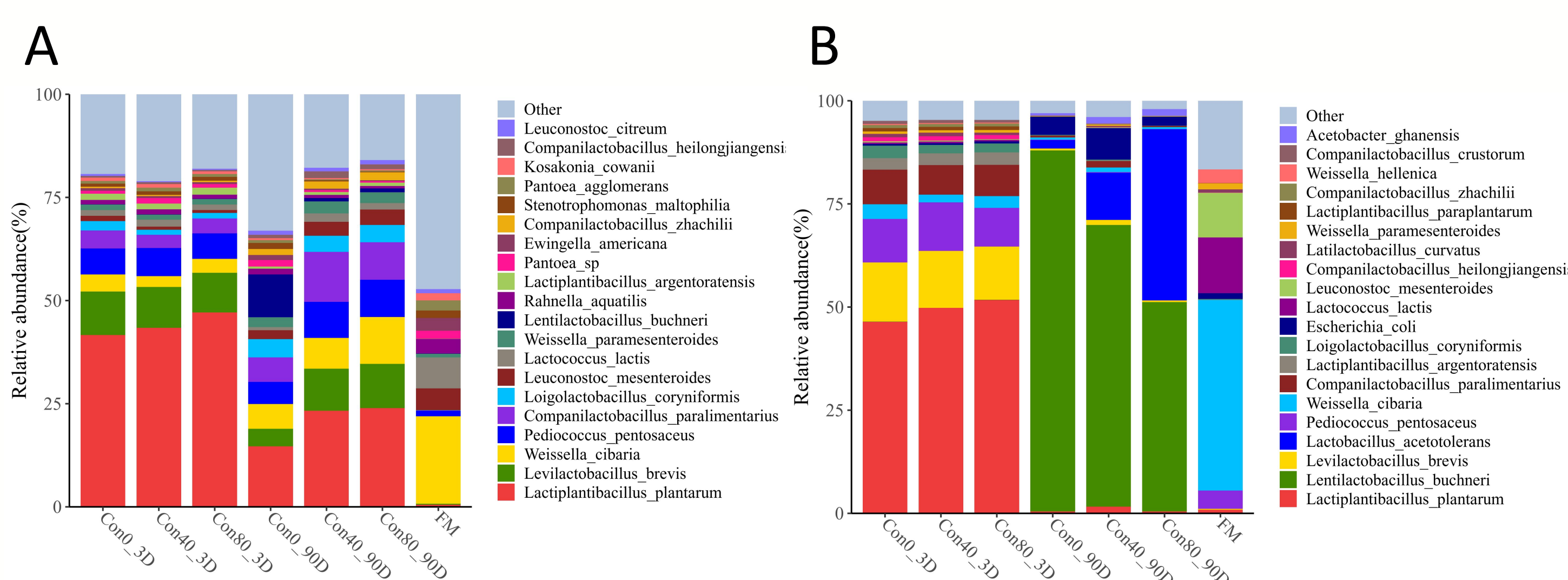


Fig 1 Bacterial community dissimilarities of corn silage (A, metagenomic ; B, metatranscriptomic)

The OTC has been degraded during the fermentation process, with a degradation rate of 55% in the control group and 85% in the OTC treatment group after 90 days of fermentation, while it had little effect on microbial diversity. *Weissella*, *Leuconostoc*, *Lactococcus*, and *Pediococcus* were shown to be high-abundant bacteria in prior to fermentation according to metagenomic (41.5%) and metatranscriptomic (86.2%). Metagenomic also discovered 17.7% unidentified and 39.5% undesirable bacteria. Both approaches identified *Lactiplantibacillus*, *Weissella*, *Levilactobacillus*, *Companilactobacillus*, *Pediococcus*, *Leuconostoc*, and *Loigolactobacillus* after three days of ensiling, which accounted for 96.3% from metatranscriptomic and 78% from metagenomic analyses. The largest difference in composition after 90 days was that majority of bacteria identified by metagenomic (52%–85%) belonged to the seven aforementioned genera, while *Lentilactobacillus* dominated the relative abundance (51%–88%) in metatranscriptomic analysis.

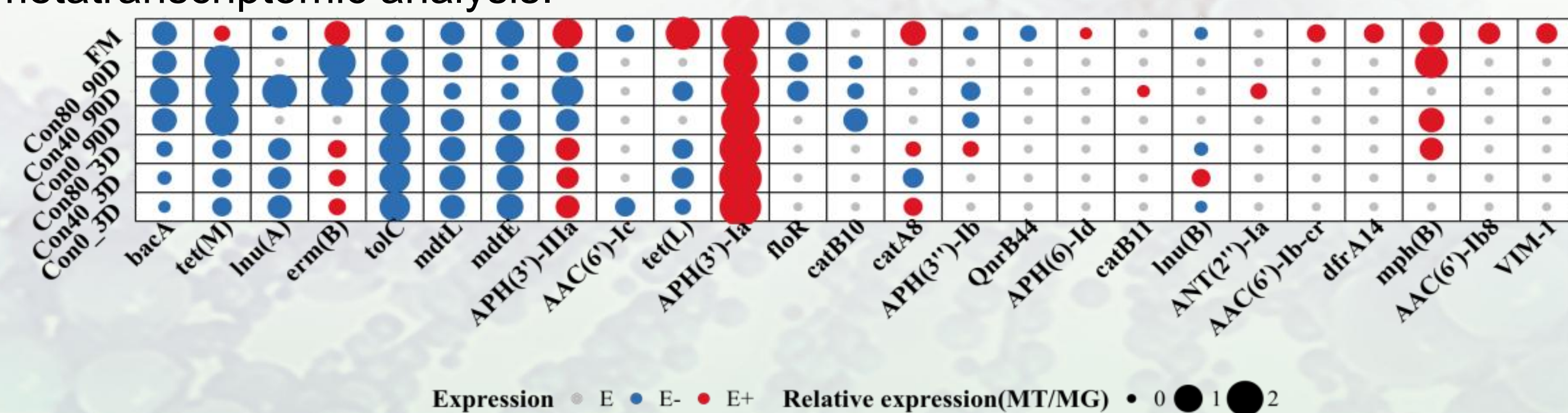


Fig 2 Relative expression of high-risk ARGs

The most abundant ARGs found in the corn silage were *bacA*, *tetM*, and *InuA* from metagenomics while *tetC*, *APH(3')-Ia* and *bacA* had higher expression during ensiling according to metatranscriptomic. Additionally, the most expressed ARGs were resistant to tetracyclines, novobiocin, bacteriocin, macrolide lincosamide streptomycin (MLS), multidrug, and aminoglycosides antibiotics.

Relative expression indicates how much a gene was overexpressed or underexpressed in the metatranscriptomic relative to the metagenomic, and it was calculated by dividing its relative activity by its relative abundance (metatranscriptomic/metagenomic). After fermentation, most ARGs' relative expression decreased except *ermB*. The relative expression of 'other major facilitator superfamily transporters' increased in control and 80 ug/kg groups after 90 days. High-risk ARGs like *tetM*, *ermB*, *APH(3')-IIIa* had high relative expression before fermentation, indicating potential increase after fermentation. However, after fermentation, the relative expression of the most high-risk resistance genes' significantly decreased or undetectable. Only *ermB* and *APH(3')-IIIa* showed high expression after 3 days. The 80 ug/kg group promoted *APH(6')-Ib* while 40 ug/kg group promoted *InuB*. After 90 days, only *APH(3')-Ia* and *catB11* showed high expression. The 80 ug/kg group reduced *APH(3')-Ia* but increased *mphB*, while 40 ug/kg group promoted *catB11* and *ANT(2'')-Ia* after 90 days.

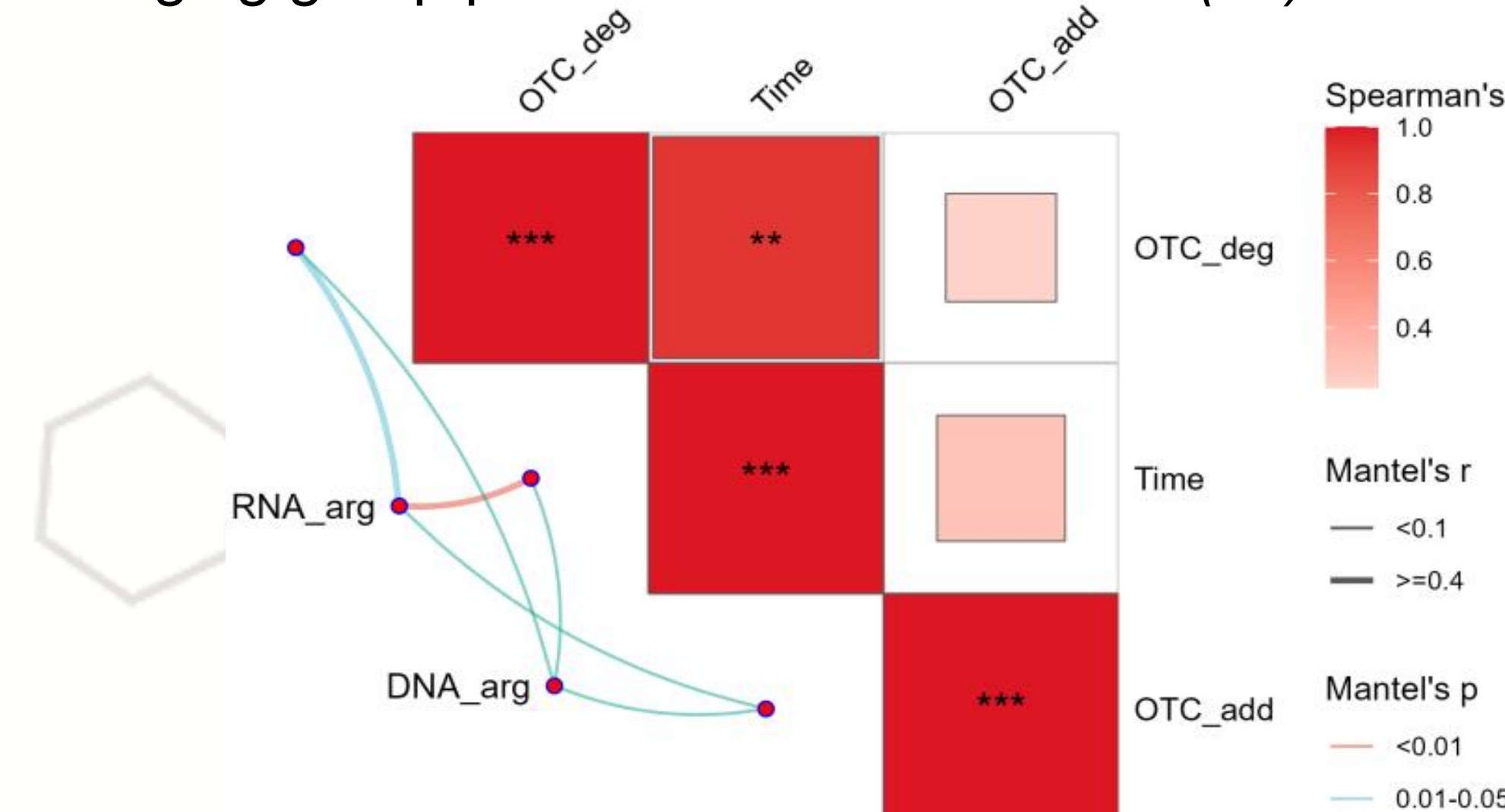


Fig 3 Effects of OTC, its degradation and fermentation time on tetracycline related ARG composition

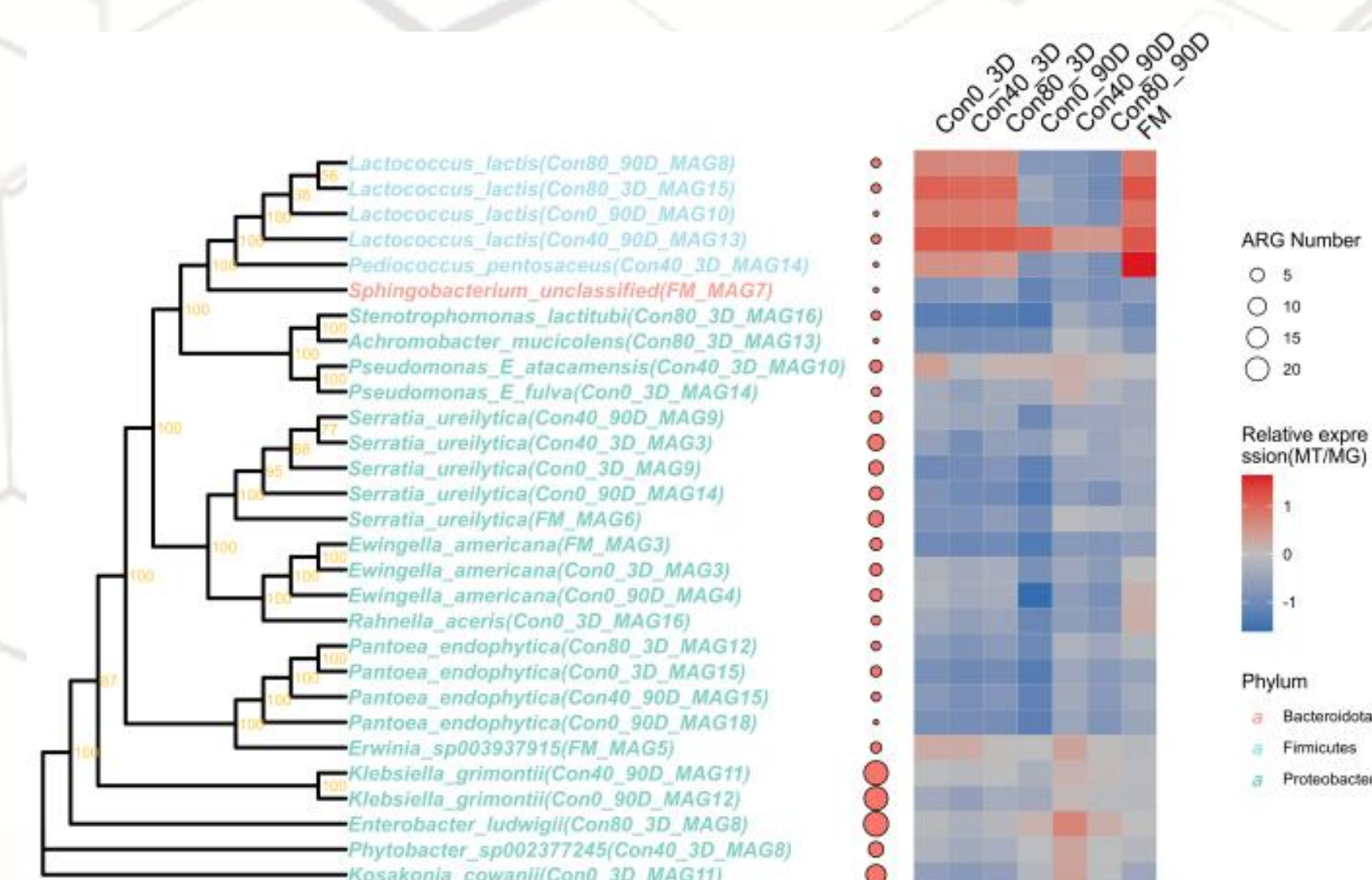


Fig 4 ARG hosts (Binging analysis)

Tetracycline-related ARGs in DNA and RNA were significantly impacted by OTC degradation, fermentation duration, and severe OTC addition. Pathogenic bacteria such as *Klebsiella*, *Enterobacter*, *Kosakonia*, *Pantoea*, *Serratia*, and others were the primary inhabitants of ARG hosts. ARGs were also hosted by *Lactococcus lactis* and *Pediococcus pentosaceus*, however their availability were greater in fresh and after three days of fermentation and falls as fermentation progresses.

Conclusions

The OTC has been degraded during the fermentation process with little effect on microbial diversity. This study is first to reveal the functional viable bacteria during ensiling by metatranscriptomic technology and the largest difference in composition after 90 days was found. Fermentation reduced the antibiotic resistance by lowering the relative expression of most ARGs, particularly high-risk ARGs. In addition, fermentation significantly affected tetracycline related ARGs and reduced potential resistant genes' hosts.

Reference

Huang R, Guo Z, Gao S, Ma L, Bu D. Assessment of veterinary antibiotics from animal manure-amended soil to growing alfalfa, alfalfa silage, and milk. *Ecotoxicology and Environmental Safety*, 2021, 224: 112699.