

# Changes in Fermentation Products, Bacterial Community Structure, & Their Functional Profiles During the Ensiling of Alfalfa (*Medicago Sativa* L.)

Siran Wang<sup>1\*</sup>, Mudasir Nazar<sup>1</sup>, Niaz Ali Kaka<sup>1</sup>

**Keywords:** alfalfa; silage; fermentation products; bacterial community; functional profile

**Background:** Alfalfa (*Medicago sativa* L.) is a perennial legume that presents a high nutritive value, protein content, productivity and digestibility, and it also contains high concentrations of vitamins and minerals. Ensiling of alfalfa is attaining high popularity in recent years. However, research on alfalfa silage production has mainly used culture-dependent methods or PCR-DGGE. Application of new techniques, such as high-throughput sequencing which reduces sequencing time and cost while providing abundant information for the study of microbial community structure, abundance, composition, genetic evolution and metabolic pathways shows great promise

**Aims:** To investigate the fermentation characteristics, bacterial community and predicted functional profiles during the ensiling of wilted alfalfa (*Medicago sativa* L.).

**Methods and Results:** First-cutting alfalfa was harvested at the early bloom stage, wilted for 6 h, and ensiled in laboratory-scale silos (1 L capacity). Triplicate silos were sampled after 1, 3, 7, 15, 30 and 60 days of ensiling, respectively. The bacterial communities of wilted alfalfa and silages on day 3 and 60 were assessed through high throughput sequencing technology, and their functional characteristics were evaluated according to the Kyoto Encyclopedia of Genes and Genomes (KEGG) using Tax4Fun. After 60 days of ensiling, alfalfa silage showed a moderate fermentation quality, indicated by high lactic acid (56.7 g/kg dry matter (DM)) and acetic acid (39.4 g/kg DM) contents, and low concentrations of butyric acid (2.12 g/kg DM) and ammonia nitrogen (128 g/kg total nitrogen). *Lactobacillus* rapidly became predominant on day 3, and increased to 60.4% on day 60. Results of functional prediction analyses showed that the metabolism of amino acid, energy, cofactors and vitamins were reduced, while metabolism of nucleotide and carbohydrate were increased during ensiling. Fructokinase, 1-phosphofructokinase and pyruvate kinase played important roles in producing lactic acid. The production of acetic acid may be correlated with the enhancement of 6-phosphogluconate dehydrogenase and acetyl-CoA synthetase.

**Conclusions:** Knowledge regarding bacterial dynamics and their metabolic pathways during alfalfa ensiling is important for understanding the fermentation process and may contribute to the production of nutritious and stable alfalfa silage.

**Significance and Impact of the Study:** High throughput sequencing technology combined with 16S rRNA gene-predicted functional analyses could provide a new and comprehensive insight into bacterial community dynamics and functional profiles to further improve the silage quality of alfalfa.

---

<sup>1</sup>Institute of Ensiling and Processing of Grass, College of Agro-Grassland Science, Nanjing Agricultural University, Nanjing 210095, China.

\* Corresponding author: [835343877@qq.com](mailto:835343877@qq.com)