

## Screening of lactic acid bacteria with high-antioxidant activity and its effects on fermentation, biochemical composition and antioxidant profiles of alfalfa silage

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**Introduction:** The antioxidant substances present in silages can improve the antioxidant capacity of ruminants and the quality of livestock products. However, studies on the application of antioxidant lactic acid bacteria (LAB) in silage and its effects on antioxidant profiles of its products have not been reported. Therefore, the objectives of this study were to screen a LAB strain with high-antioxidant activity and to test its effects on fermentation, biochemical composition and antioxidant profile of alfalfa silage ensiled at two different dry matter (DM) contents.

**Materials and Methods:** The fresh chopped alfalfa was wilted to the different DM contents of 300 and 400 g/kg fresh weight, and then ensiled with treatments of distilled water (control), *L. plantarum* MTD-1 and antioxidant *P. acidilactici* J17. Then vacuum-sealed entirely, with 3 replicates for each treatment. The bag silos were deposited at an environmental-temperature and on day 60 d, it was analyzed for fermentation characteristics, fatty acid compositions and antioxidant properties.

**Results and Discussion:** The application of J17 strain resulted in lower proportion of saturated fatty acid (SFA) and higher proportions of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) at different DM contents after ensiling (Table 1). Compared with the control and MTD-1 groups, J17-treated silages showed a higher levels of total anti-oxidation competence (T-AOC), glutathione peroxidase (GSH-Px) and catalase (CAT) activity, whereas a lower tendency of total superoxide dismutase GSH-PX (T-SOD) activity (Table 2). T-SOD activity decreased with the application of J17 strain. One of the possible explanations is that silage fermentation is an anaerobic fermentation process and that the consequently the oxygen pathway is close, resulting in a rapid declined in pH in the presence of LAB.

**Table 1** Total FA content (g/kg of DM) and FA compositions (g/100g of Total FA) of freshly chopped alfalfa and alfalfa silages ensiled at different forage DM for 60 d

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| Item         | Dry Matter (30%)   |                    |                    | Dry Matter (40%)   |                    |                    | SEM  | ANOVA ( <i>P</i> -value) |        |        |
|--------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|------|--------------------------|--------|--------|
|              | Control            | MTD-1              | J17                | Control            | MTD-1              | J17                |      | I                        | DM     | I×DM   |
| TFA, g/kg DM | 12.97 <sup>e</sup> | 10.33 <sup>d</sup> | 10.90 <sup>c</sup> | 10.42 <sup>e</sup> | 9.60 <sup>b</sup>  | 10.89 <sup>a</sup> | 0.08 | <0.001                   | <0.001 | <0.001 |
| SFA          | 69.87 <sup>a</sup> | 68.26 <sup>b</sup> | 64.31 <sup>c</sup> | 70.30 <sup>a</sup> | 62.60 <sup>d</sup> | 54.94 <sup>c</sup> | 0.15 | <0.001                   | <0.001 | <0.001 |
| MUFA         | 5.08 <sup>a</sup>  | 4.73 <sup>b</sup>  | 5.31 <sup>a</sup>  | 4.18 <sup>d</sup>  | 4.25 <sup>cd</sup> | 4.46 <sup>c</sup>  | 0.04 | <0.001                   | 0.002  | 0.062  |
| PUFA         | 25.05 <sup>e</sup> | 27.00 <sup>d</sup> | 30.38 <sup>c</sup> | 25.52 <sup>e</sup> | 33.15 <sup>b</sup> | 40.60 <sup>a</sup> | 0.14 | <0.001                   | <0.001 | <0.001 |

<sup>a-c</sup>Means of inoculation treatment within a row with different superscripts differ ( $P < 0.05$ ); TFA, total fatty acids; SFA, saturated fatty acid; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SEM, standard error mean.

**Table 2** Antioxidant activity of alfalfa silages ensiled at different forage DM for 60 d.

| Item         | Dry Matter (30%)    |                     |                     | Dry Matter (40%)   |                     |                     | SEM  | ANOVA ( <i>P</i> -value) |        |        |
|--------------|---------------------|---------------------|---------------------|--------------------|---------------------|---------------------|------|--------------------------|--------|--------|
|              | Control             | MTD-1               | J17                 | Control            | MTD-1               | J17                 |      | I                        | DM     | I×DM   |
| T-AOC(U/mg)  | 14.74 <sup>f</sup>  | 17.21 <sup>e</sup>  | 19.98 <sup>d</sup>  | 26.27 <sup>c</sup> | 27.44 <sup>b</sup>  | 30.44 <sup>a</sup>  | 0.10 | <0.001                   | <0.001 | 0.055  |
| T-SOD(U/mg)  | 48.57 <sup>a</sup>  | 48.51 <sup>a</sup>  | 46.83 <sup>b</sup>  | 49.11 <sup>a</sup> | 46.62 <sup>b</sup>  | 46.35 <sup>b</sup>  | 0.09 | <0.001                   | 0.004  | <0.001 |
| GSH-PX(U/mg) | 101.72 <sup>e</sup> | 137.41 <sup>b</sup> | 113.45 <sup>d</sup> | 126.9 <sup>c</sup> | 127.24 <sup>c</sup> | 174.83 <sup>a</sup> | 0.13 | <0.001                   | <0.001 | <0.001 |
| CAT(U/mg)    | 1.67 <sup>d</sup>   | 2.18 <sup>c</sup>   | 3.97 <sup>a</sup>   | 1.74 <sup>d</sup>  | 2.64 <sup>b</sup>   | 4.25 <sup>a</sup>   | 0.03 | 0.001                    | <0.001 | 0.065  |

<sup>a,b,c</sup>Means of DM content at ensiling within a row with different superscripts differ ( $P < 0.05$ ); T-AOC, total anti-oxidation competence; T-SOD, total superoxide dismutase; GSH-PX, glutathione peroxidase; CAT, catalase; SEM, standard error mean.

**Conclusions:** Application of the antioxidant producing strain *Pediococcus acidilactici* J17 effectively improved alfalfa silage antioxidant activity, MUFA and PUFA, and reduce proportion of SFA compared to the control and MTD-1 groups, which has the potential to be an ideal silage inoculum.