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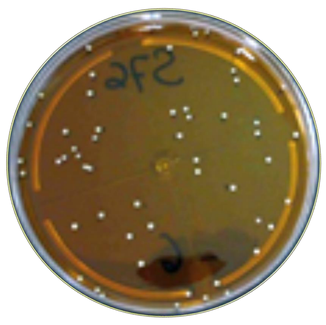

Screening heterofermentative lactic acid bacteria for osmotolerance

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Introduction

Rapid wilting forages to > 35 % DM prior to ensiling is recommended:

⊕ Advantages	⊖ Challenges
+ ⬇ Proteolysis	– ⬇ compaction
+ ⬇ Effluent	– ⬆ risk for aerobic deterioration
+ ⬆ Nutrient concentration	

➢ Use of **heterofermentative lactic acid bacteria** (LAB) → chance to increase aerobic stability.

How to screen strains suitable for high dry matter (DM), i.e. tolerant to ⬆ osmotic pressure, *in vitro*?

Materials and methods

- Microbiology laboratory – *in vitro*:
  Inoculated standard MRS broth for control (low osmolality*) and High Sugar (HS ≙ high osmolality) MRS broth approved for homofermentative LAB as culture medium (Martens et al. 2024)
  *osmolality quantifies the molar concentration of osmotically active particles per kg weight
- Grass silages – *in situ* for validation:
  At 5 different locations in Germany, grass wilted to 35-49 % DM as target, ensiled in triplicates on small scale, uninoculated control and inoculants:
- 5 different silage additives:
  - LAB1-4: strains of *Lentilactobacillus buchneri*
  - LAB5: *L. buchneri, L. diolivorans, Lacticaseibacillus rhamnosus*
- Indicator for LAB activity - pH decrease:
  - *In vitro*: pH after 0, 24, 36 and 48 h of incubation at 30 °C
  - *In situ*: pH after 0 and 5 d of ensiling at ambient temperature

Fig 1. From left to right hand: *In vitro* – *in situ* testing – pH measurement

Results and discussion

- *In situ*:
  - Actual DM content 30.7-52.6 % DM
  - pH after 5 d similar in control and inoculated treatments (in 4 out of 5 cases) → effect of inoculant hardly distinguishable
  - Effect of DM on pH (Fig. 2a), but small gradient angle in contrast to homofermentative LAB (Fig. 2b)

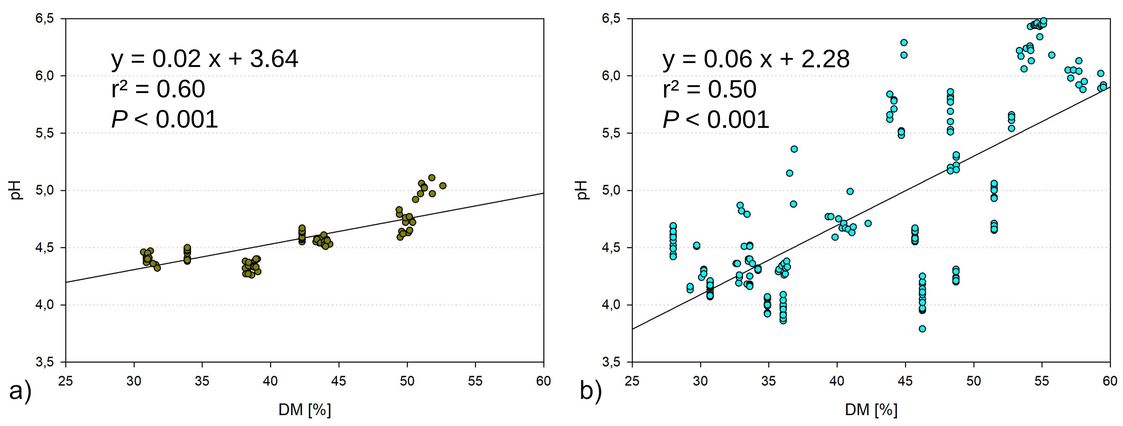

Fig 2. Linear regression between DM content and pH value of grass silages inoculated a) with heterofermentative LAB, b) with homofermentative LAB (Martens et al. 2024).

Conclusions

- The selected HS medium is suitable to test osmotolerance of heterofermentative LAB.
- The metabolic activity of heterofermentative LAB is barely affected by high osmolality prevailing in wilted forages.

- *In vitro*:
  - Initial inhibition of lactic acid fermentation:
    - 3 out of 5 products exhibited similar behavior in standard and HS MRS (Fig. 3a-b)
    - The two more efficient products decelerated acid production in HS MRS (Fig. 3b)
  - After 36 h, the initial impediment has been overcome by all strains independent of osmolality, similar to *in situ*.

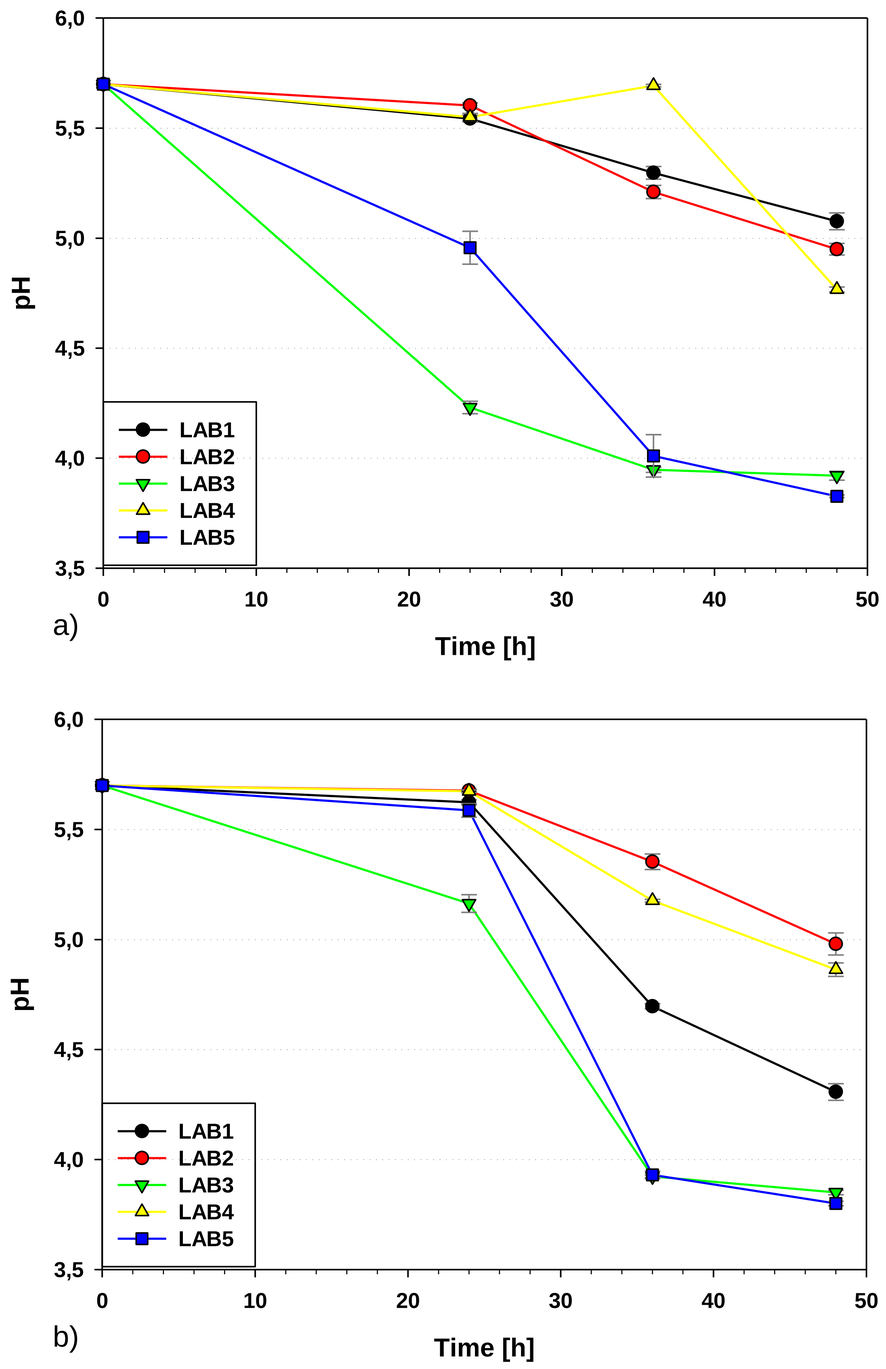

Fig 3. pH development *in vitro* for 48 h. Media inoculated with 5 different additives (LAB1-5). a) Standard MRS, b) High Sugar MRS [commas in the graphs are decimal separators]

➢ **Increased DM does hardly affect fermentative capacity of heterofermentative LAB in contrast to homofermentative LAB.**

➢ **Opposite to the latter, the *in vitro* test for heterofermentative LAB is rather a control tool for osmotolerance than necessary for selection.**