

Modulation of bacterial community and metabolome in whole crop corn silage by inoculating homo- or heterofermenters

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Background

The reasons for detecting the dynamics of silage microbial community and metabolome



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Silage fermentation: microbial communities

- Ensiling : epiphytic lactic acid bacteria fermentation under anaerobic conditions
- Silage fermentation quality: microbial communities present and their succession during ensiling.

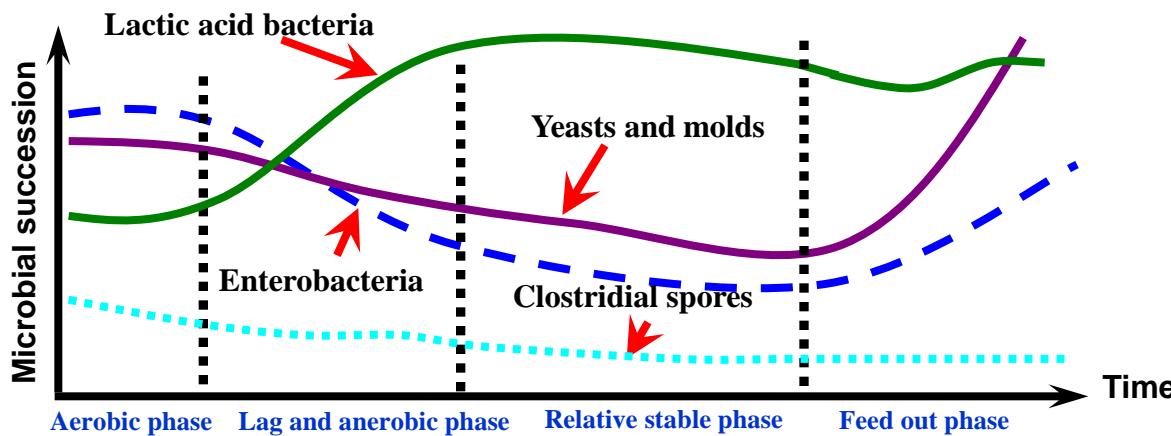
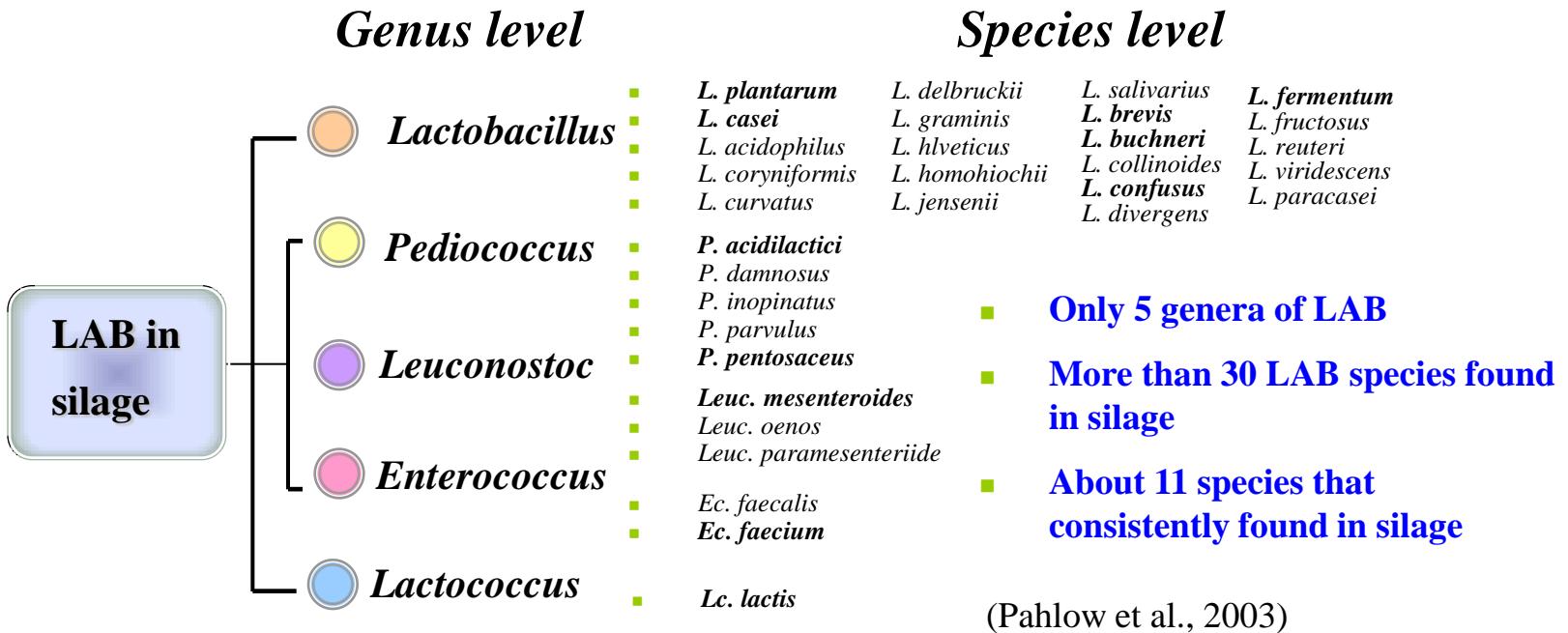


Fig. 1 General model of microbial succession during silage fermentation

- Better understanding of microbial communities and their succession underlying silage formation could provide valuable information for **modulating** the ensiling process and **improving** silage fermentation quality.

Lactic acid bacteria (LAB) in silage

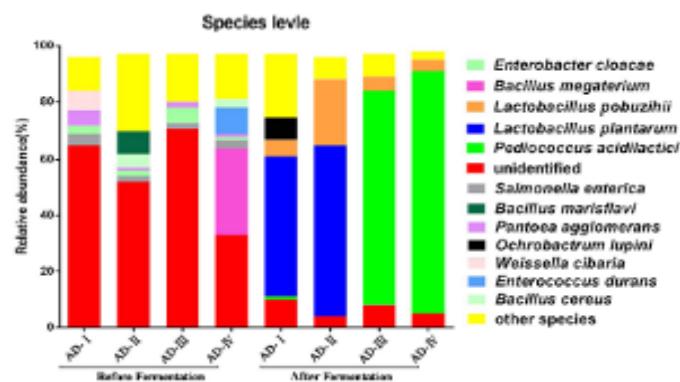


Detecting the bacterial community in silage at species level!



PacBio RSII (SMRT)

Size: 10-15 Kb; almost whole length 16S rRNA genes



Typing microbes at **species** level



High throughout sequencing (NGS)

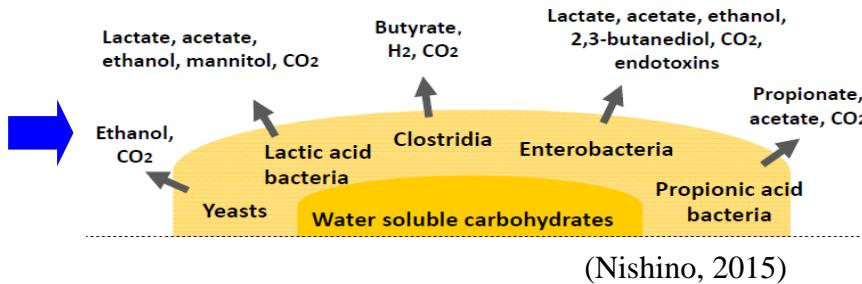
- ***SMRT is a more powerful tool for revealing microbial communities in silage!!!***

Silage fermentation: metabolites

- Since year 1945, the Nobel Price owner Dr. A. Virtanen
- Research on silage fermentation metabolites are mainly focused on:
**organic acid (lactate, acetate, propionate, butyrate), ethanol,
1,2 –propanediol, etc., to evaluate silage fermentation and aerobic stability.**



Artturi Virtanen



(Nishino, 2015)



Fermentation metabolism

LAB

Amino acids
Fatty acids
Oligosaccharides
Vitamins
Small peptides
flavoring agents
Aromatic compounds

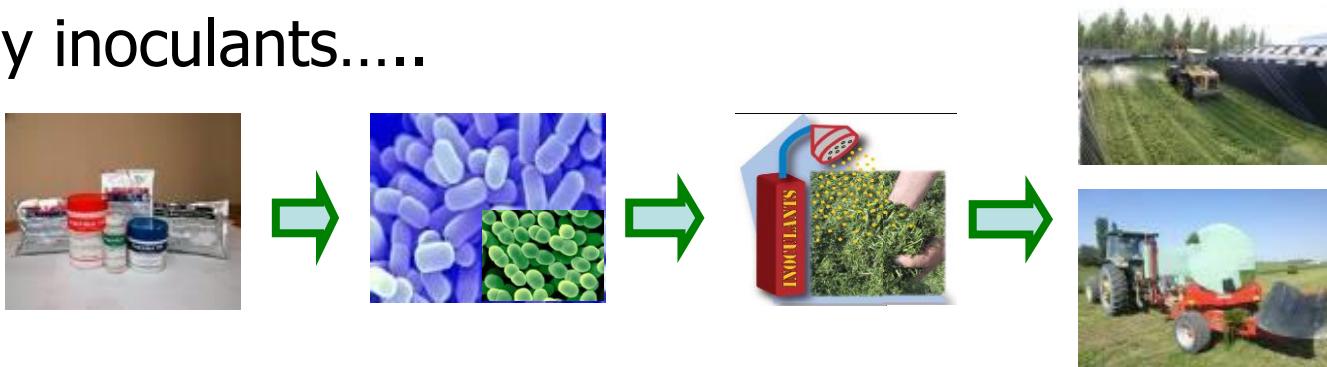
Many other metabolites have not been identified in ensiled forage???



Silage metabolome, a new version on silage fermentation?

Modulation of Silage fermentation

By inoculants.....



Homofermenter **vs.** Heterofermenter

(*L. plantarum*)

1 6-C Sugar → 2 Lactic Acid

(*L. buchneri*)

1 6-C Sugar → 1 Lactate + 1 Acetate + CO₂

1 6-C Sugar → 1 Lactate + 1 Ethanol + CO₂

- ***How these two kinds of inoculants modulate the bacterial community and metabolome in silage?***

A study was conducted to investigate.....

■ Modulation of bacterial community and metabolome in whole crop corn silage by inoculating homofermentative *L. plantarum* or heterofermentative *L. buchneri*



- ◆ Control
- ◆ *L. plantarum*
- ◆ *L. buchneri*

Sampled at
different days

(3, 7, 14, 30, 45, 90 d)



PacBio RSII (SMRT)



- ◆ Bacterial community
- ◆ Metabolome

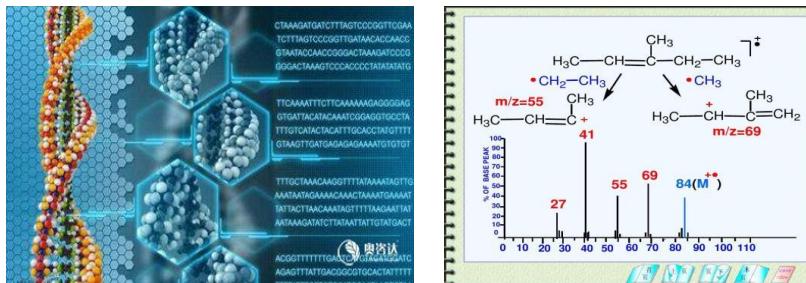


GC/TOF-MS

Results

Modulation of Bacterial Community and Metabolome in **whole crop corn** Silage

by *L. plantarum* or *L. buchneri*



Bacterial community dynamics in corn silage

Table 1. Sequence and bacterial diversity estimation of fresh forage and silages

Sample ID	Observed species	Avg length(bp)	Chao1	Shannon	Simpson	Coverage
FM	1139.0	1462.7	3155.4	7.91	0.99	0.876
C3	627.3	1487.3	1133.1	6.22	0.95	0.967
C7	695.3	1496.7	1368.3	5.58	0.92	0.959
C14	997.3	1476.0	2249.1	6.25	0.93	0.922
C30	626.7	1496.3	1228.5	5.07	0.88	0.952
C45	816.0	1495.3	1658.5	5.94	0.92	0.934
C90	290.3	1471.3	679.0	1.16	0.30	0.995
P3	696.0	1484.0	1361.4	6.12	0.95	0.944
P7	726.0	1490.3	1402.4	5.51	0.89	0.951
P14	1021.3	1503.3	2313.5	5.96	0.91	0.925
P30	742.3	1491.0	1521.6	4.94	0.82	0.946
P45	823.7	1499.3	1677.9	5.00	0.83	0.949
P90	442.0	1464.3	871.8	2.03	0.46	0.991
B3	765.3	1474.7	1557.0	6.20	0.95	0.963
B7	783.3	1482.0	1625.0	6.34	0.95	0.934
B14	865.3	1482.7	1790.0	6.04	0.94	0.927
B30	812.0	1500.0	1784.3	5.84	0.93	0.925
B45	863.3	1499.3	1767.7	5.26	0.86	0.948
B90	372.0	1464.3	800.6	2.37	0.59	0.991

***C.** control; **B**, *L. buchneri* inoculated group; **P**, *L. plantarum* inoculated group; numbers are the fermentation times of 3, 7, 14, 30, 45 and 90 days. **FM**, fresh forage.

Bacterial community dynamics in corn silage

■ Genus level

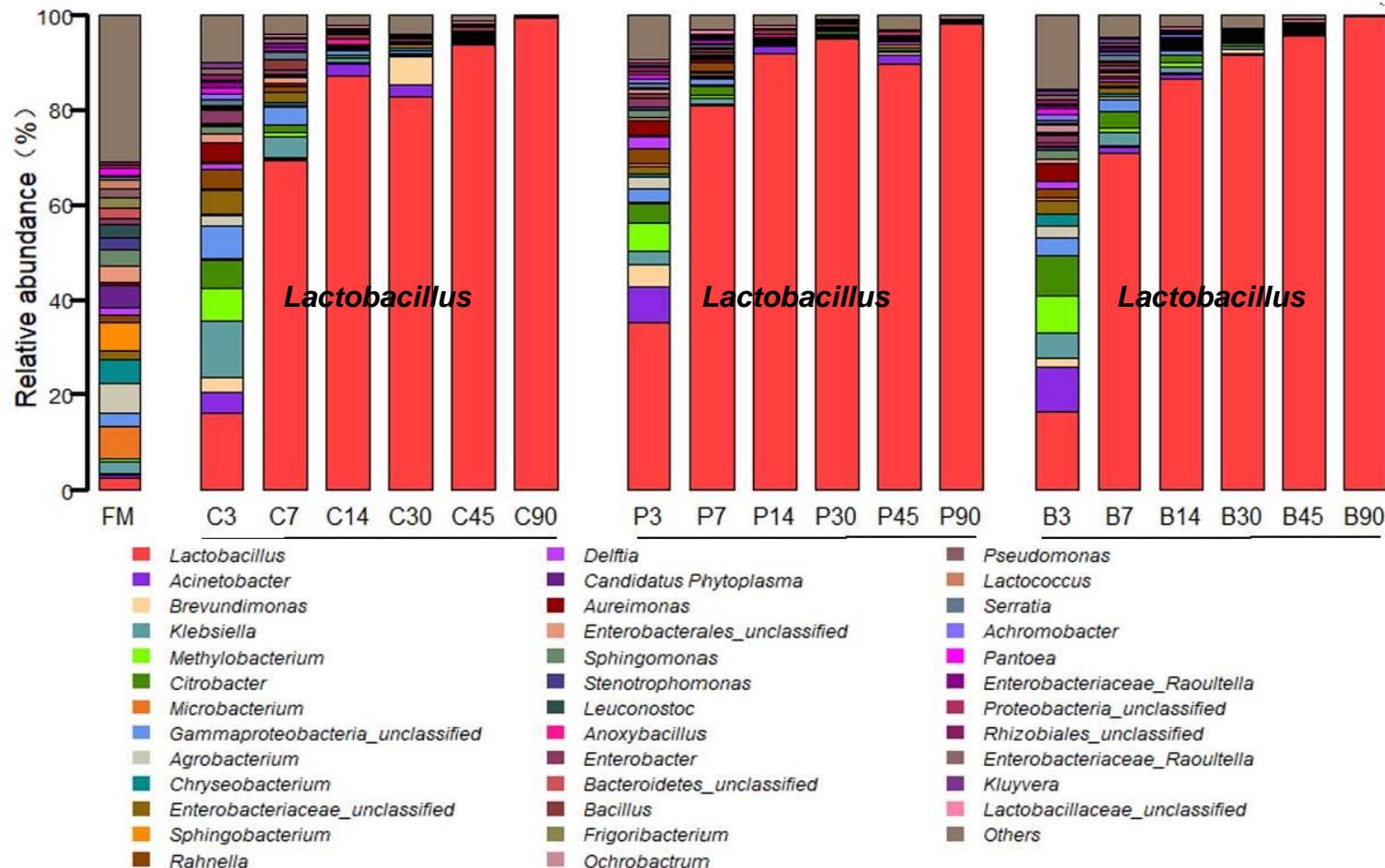
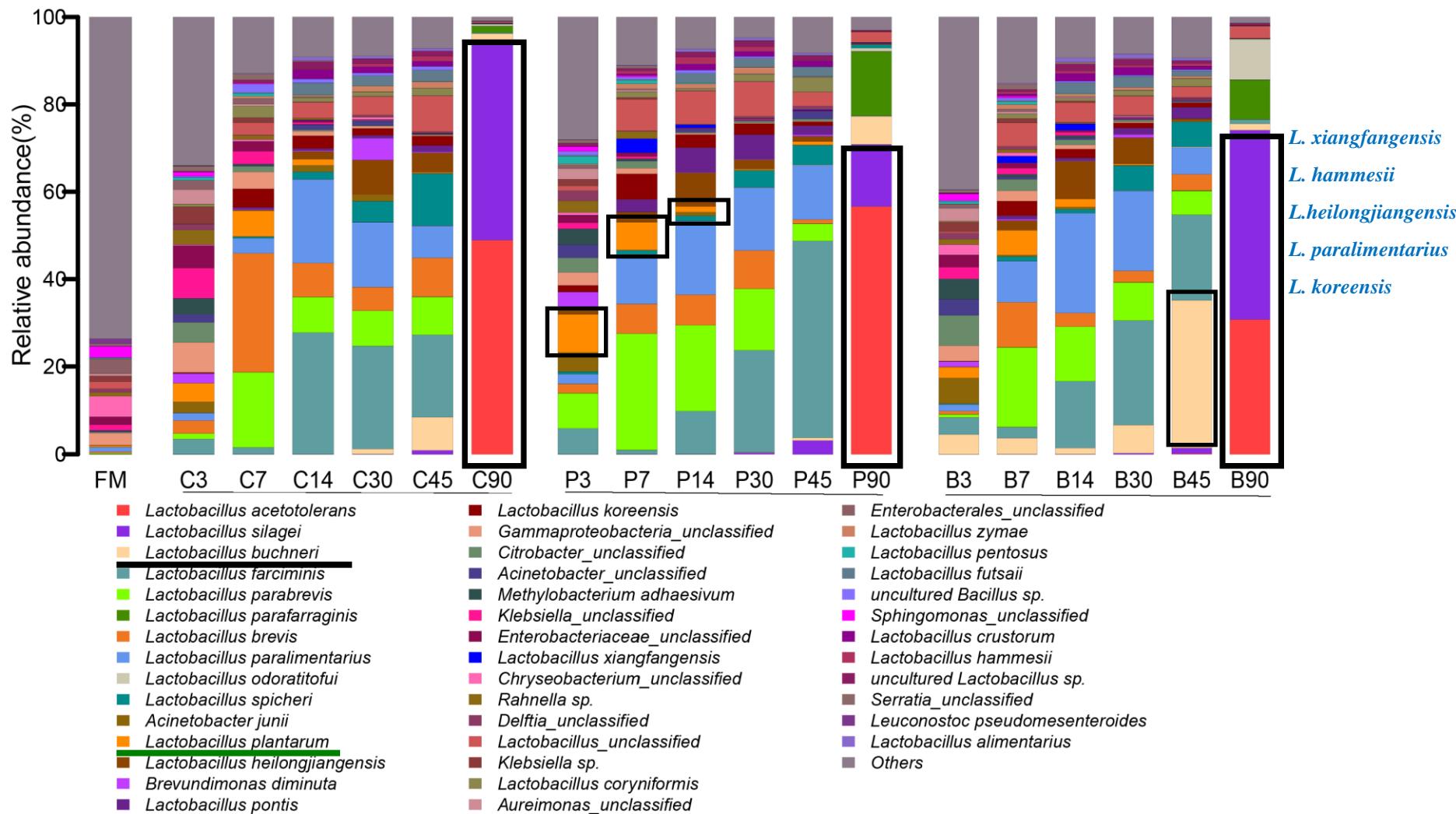


Figure 1. Bacteria community dynamics in corn silages ensiled at different days. **C**, control; **B**, *L. buchneri*; **P**, *L. plantarum*



24 *Lactobacillus* species!!!

Fig. 2 Bacterial community dynamics *at species level* in fresh and ensiled whole crop corn

C. control; **B.** *L. buchneri*; **P.** *L. plantarum*; numbers are the fermentation times of 3, 7, 14, 30 , 45 and 90 days.

Metabolome in whole crop corn silage

- Based on the GC-TOF-MS of 48 samples, a total **608** substances were detected, and **259** different metabolites were identified with their relative concentrations.

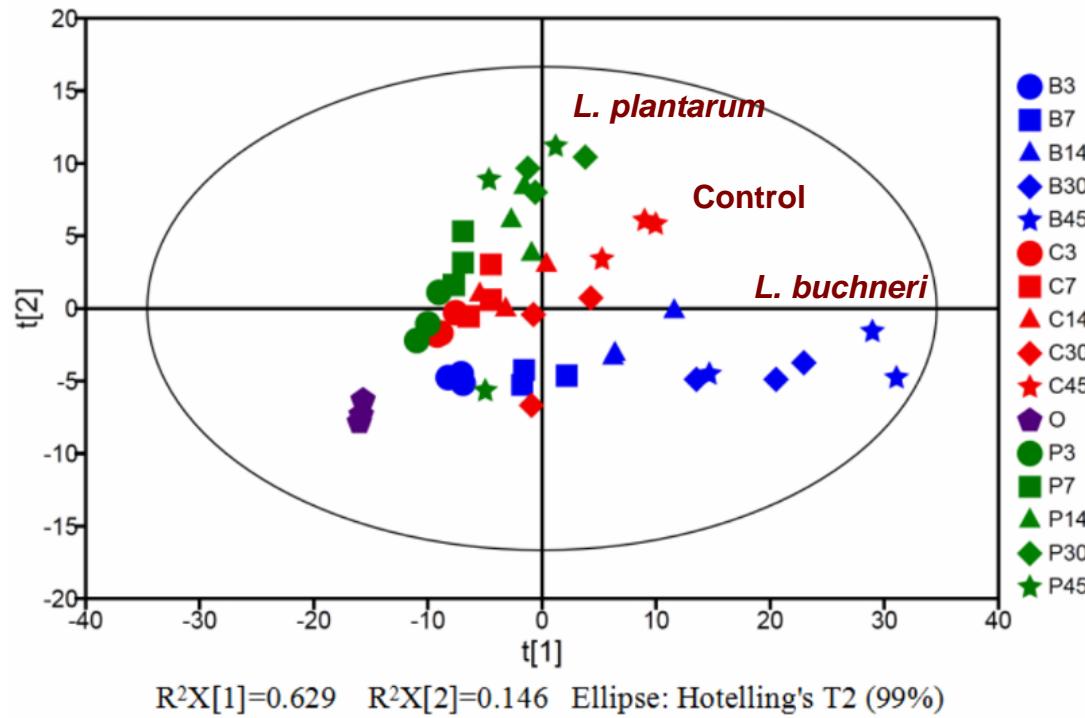


Fig. 3 Principal component analysis (PCA) of metabolic profiles in corn silage with or without inoculation of *L. plantarum* or *L. buchneri* ($n=3$).

*C. control group; B, *L. buchneri* inoculated group; P, *L. plantarum* inoculated group. Numbers are the fermentation days.

Table 2. Relative concentrations of a partial of major differential metabolites in corn silages without or with inoculations of *L. plantarum* or *L. buchneri* for 90 days

Metabolite name	Relative concentration ¹			Fold-changes ²		
	Control	<i>L. buchneri</i>	<i>L. plantarum</i>	$\text{Log}_2(\text{B/C})$	$\text{Log}_2(\text{P/C})$	$\text{Log}_2(\text{P/B})$
Phenylalanine	0.000	0.014	0.054	-13.162	-15.104**	-1.942*
Lysine	0.000	0.325	0.741	-17.705	-18.894**	-1.189
Tyrosine	0.000	0.087	0.217	-15.809**	-17.125*	-1.315*
Glycine	0.000	0.006	0.006	-11.917**	-12.057**	-0.141
Oxamic acid	0.000	0.055	0.059	-14.701**	-14.794**	-0.093
2-Hydroxybutanoic acid	0.109	0.508	0.091	-2.218*	0.264	2.482*
3-Hydroxypropionic acid	0.000	0.010	0.010	-15.023**	-15.012**	0.011
2-Methylglutaric acid	0.018	0.001	0.003	4.883	2.858	-2.025*
Lauric acid	0.000	0.021	0.026	-13.724*	-14.069*	-0.345
Isocitric acid	0.000	0.029	0.110	-14.231	-16.145*	-1.914
4-Hydroxycinnamic acid	0.000	0.299	0.323	-17.586**	-17.696**	-0.110
3,4-Dihydroxycinnamic acid	0.000	0.028	0.029	-14.160*	-14.197*	-0.037
Mannose	0.000	0.080	0.000	-15.677*	-5.911	9.766*
Cellobiose	0.000	0.031	0.039	-14.304**	-14.637**	-0.333*
Saccharic acid	0.025	0.217	0.035	-3.128*	-0.513	2.615*
2-Keto-L-gulonic acid	0.000	0.054	0.000	-15.127**	3.169	18.297**
Cytosin	0.000	0.000	0.158	3.211	-16.669**	-19.880**
Pyrogallol	0.000	0.000	0.008	3.211	-12.415*	-15.625*
Gluconic lactone	0.000	0.005	0.005	-11.753**	-11.543**	0.210*
D-glucosamine	0.000	0.361	0.000	-17.859*	3.169	21.028*
4-Methyl-5-thiazoleethanol	0.000	0.017	0.004	-13.414*	-11.367*	2.047*
Conduritol- β - epoxide	0.000	0.016	0.010	-13.32*3	-12.753**	0.570
N-Acetyl-D-galactosamine	0.818	0.012	0.090	6.134	3.187	-2.947*
Phytosphingosine	0.005	0.001	0.003	2.503*	0.807	-1.696
Tetrahydrocorticosterone	0.000	0.001	0.002	-9.059	-10.500**	-1.440
Stigmasterol	0.005	0.000	0.000	3.936	14.764	10.829**

Correlations between main metabolites and bacteria

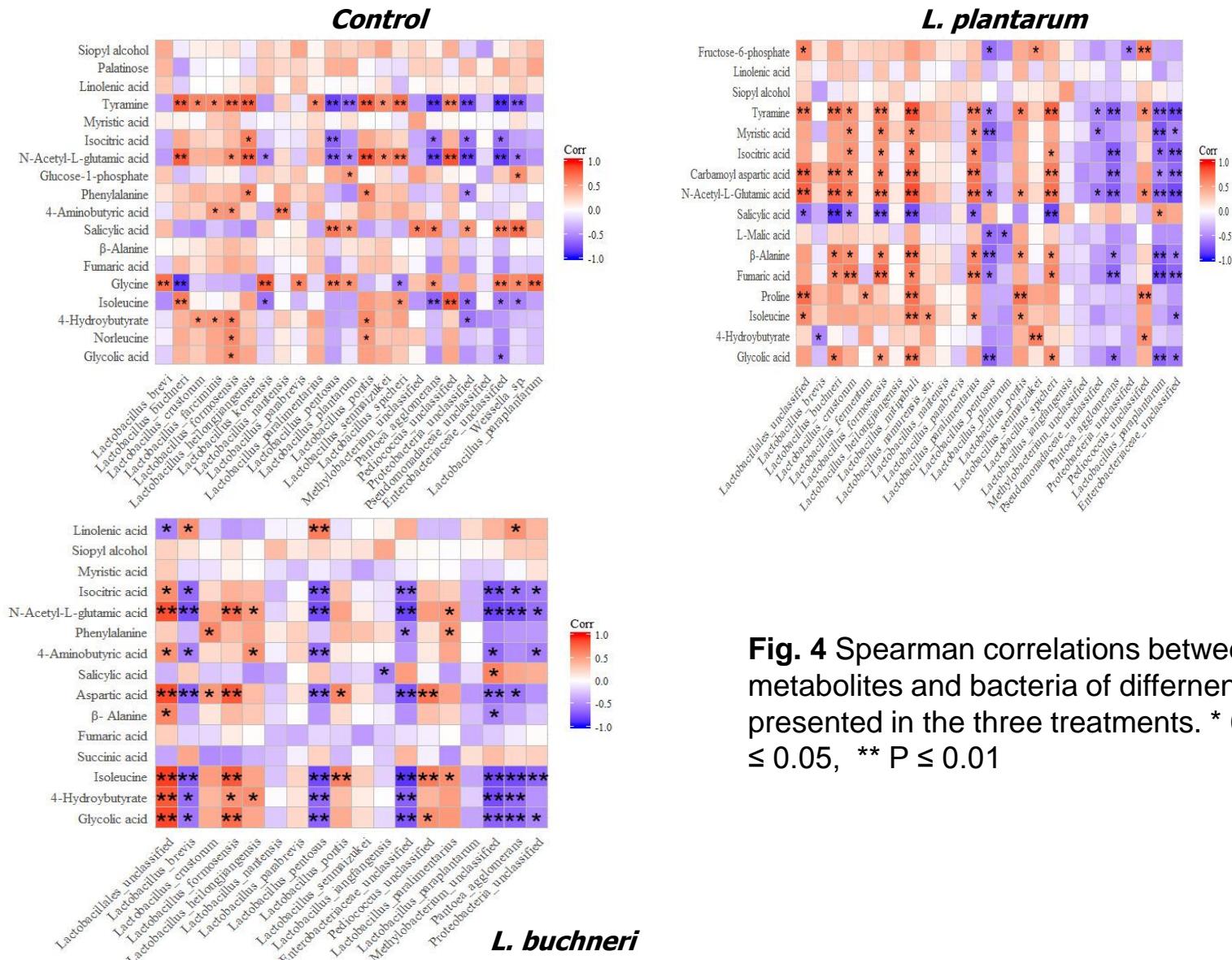


Fig. 4 Spearman correlations between main metabolites and bacteria of differentially presented in the three treatments. * $0.01 < P \leq 0.05$, ** $P \leq 0.01$

Biological implications of evaluating silage microbial community and metabolome

- Revealing the microbial community dynamics at species level in ensiled forages provides us valuable information to modulate silage fermentation process
- Profiling of metabolome encourages us to re-evaluate ensiled forages with perspectives of not only nutritvie value and fermentation quality but also animal health and welfare.
- Integration of silage microbiome and metabolome will provide important information on screening targeted LAB with biological functions.



**Thank you for your
attention!**