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Comparison between a *Clostridium tyrobutyricum* - specific quantitative polymerase chain reaction (qPCR) method and a traditional method for determining total spore-forming bacteria in clover-grass silage

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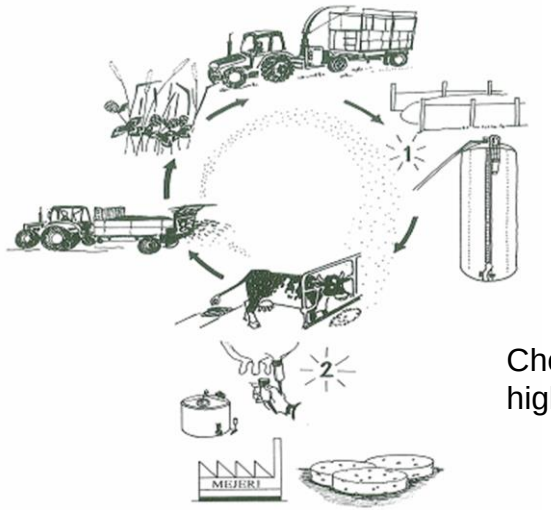
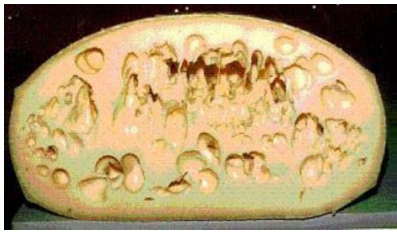
Introduction

- The most probable number (MPN) method is commonly used to estimate total spore counts (Jonsson 1990).
- MPN method is time consuming and not specific for *C. tyrobutyricum*.

- The qPCR method can quickly quantify and identify microorganisms by their DNA sequences, such as *C. tyrobutyricum*.





Cheese made from milk with high count of *C. tyrobutyricum*

Objectives

- To investigate potential differences and relationships in the clostridia estimate of silage between the qPCR method and the MPN method.

- To evaluate relationships between silage fermentation characteristics and

1) *C. tyrobutyricum* copies (qPCR)
2) total spore-forming bacteria count (MPN)

Material and Methods

Sward of 75% red clover/25% grass
Harvest September 4, 2011 in third cut
at Skara, southwest Sweden

Wilted to 28-35% DM
16% crude protein of DM
10% WSC of DM

Harvest

Inoculants applied at chopper

Homofermentative LAB

Heterofermentative LAB

Homo/Hetero LAB

Control without inoculant

14 silage jars stored for 142 d

Methods for clostridia estimate

qPCR method TaqMan

- Fast DNA kit for soil and lysozyme to disrupt cell walls

The Probe, forward- and reverse primer used (Bassi et al., 2013)

Primer/probe	Sequence
Primer forward (ptaF)	5'- AAG GGA AGT GCA CAA CAT GA - 3'
Primer reverse (ptaR)	5'- ACT ACC AGG TGC TTT TAA ATT TGC - 3'
Probe (ptaP)	5'- FAM - TGC TAA ACA AAT GCA ACC AGA – TAMRA - 3'

- Number of gene copies/g silage was calculated

MPN

- Bryant Burkey broth (BBB) as substrate, 37°C for 7 days

Statistical analysis

- Least-square (LS) means between methods were compared in PROC GLM of SAS

- Linear regression analysis in PROC REG of SAS to investigate relationships between total spore forming bacteria count by the MPN method and the *C. tyrobutyricum* numbers by the qPCR method

- Stepwise regressions in PROC REG of SAS to investigate relationships between silage fermentation characteristics and

1) *C. tyrobutyricum* by qPCR
2) total spore count by MPN

$P < 0.15$ as the significance level for inclusion of a variable in the model

Results

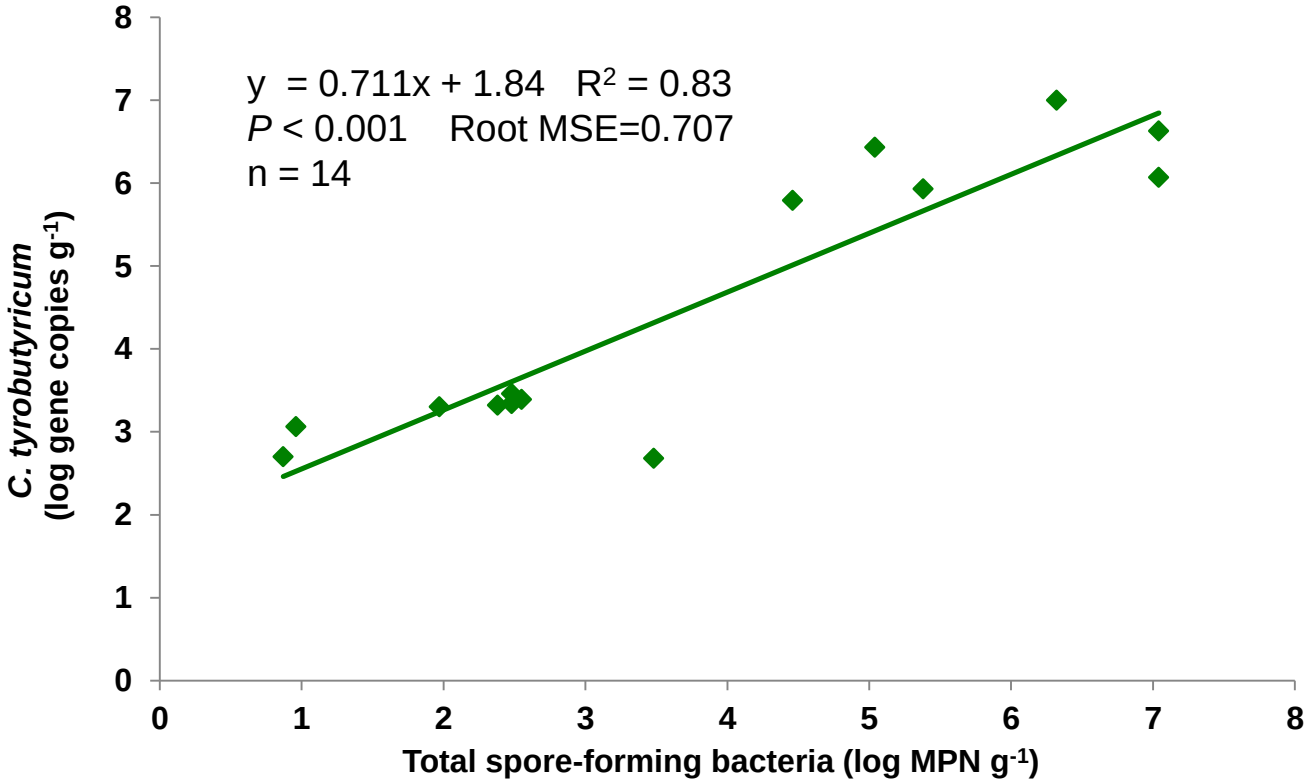
Composition	Range of 14 samples
C. tyrobutyricum, log gene copies g^{-1}	2.7 – 7.0
Total spore-forming bacteria, log MPN g^{-1}	0.9 – 7.0
pH 3 days	4.38 – 5.18
Final pH	4.19 – 4.94
Lactic acid, % DM	3.0 – 11.3
Acetic acid, % DM	2.0 – 4.9
Propionic acid, % DM	0.00 – 0.96
Butyric acid, % DM	0.00 – 1.05
Ethanol, % DM	0.24 – 0.78
1,2 – Propandiol, % DM	0.03 – 0.43
NH_3-N, % of total N	8.0 – 15.1
WSC, % of DM	0.31 – 0.81

Comparison between methods

LS means for each method n = 14 silage samples

MPN	qPCR	SEM	*P*
3.7 log MPN g^{-1}	4.5 log copies g^{-1}	0.51	0.301

Relationship between clostridia estimates by MPN and qPCR



Relationships between fermentation characteristics and *C. tyrobutyricum* and total spore-forming bacteria

Method	Fermentation parameter	*P*	Slope	Root MSE	R^2
qPCR	pH 3 days	< 0.001	+		
	Lactic acid	< 0.001	-		
	Acetic acid	< 0.05	+		
	1,2 - Propanediol	0.107	-	0.239	0.98
MPN	Butyric acid	< 0.001	+		
	Butanol	0.113	+	0.719	0.90

Conclusions

- The qPCR method for specific detection of *C. tyrobutyricum* in clover-grass silage seems promising but needs to be investigated further in comparison to the MPN method.

- Using a larger data set, more robust relationships with the fermentation products can be developed.

THANK YOU FOR YOUR ATTENTION!!