



# **Species specific quantification of dairy propionic acid bacteria in milk and cheese by quantitative real-time PCR**

**Meral Turgay**

Agroscope, Institute for Food Sciences (IFS)  
Bern-Liebefeld, Switzerland

**IDF Cheese Science & Technology 2016, Dublin**



# Dairy propionic acid bacteria (PAB)

- Four typical dairy propionic acid bacteria:  
*P. freudenreichii*, *P. jensenii*, *P. acidipropionici*, *P. thoenii*
- Gram-positive, non sporulating bacteria
- Naturally present in raw milk
- Grows only poorly in milk
- Ferments lactate into propionate, acetate and CO<sub>2</sub>
- In Swiss-type cheese *P. freudenreichii* is used to evoke the typical eye-formation and the nutty and sweet flavour

# Cheese defects associated with PAB

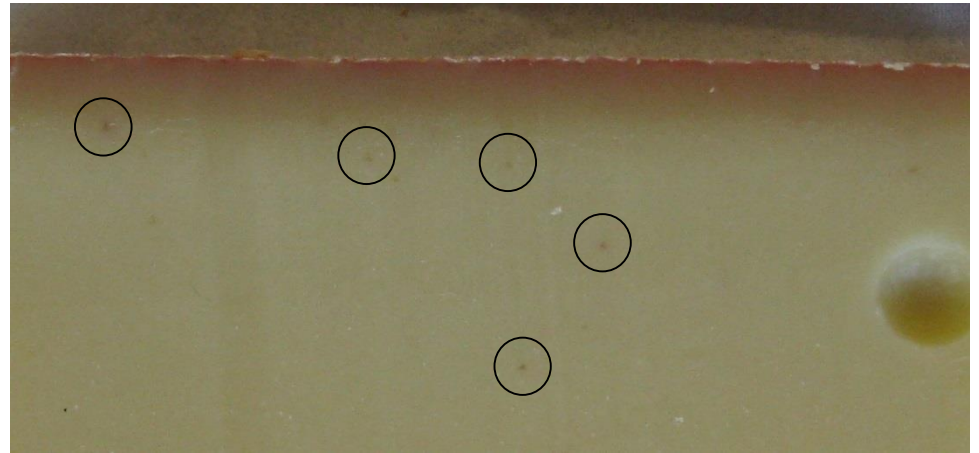
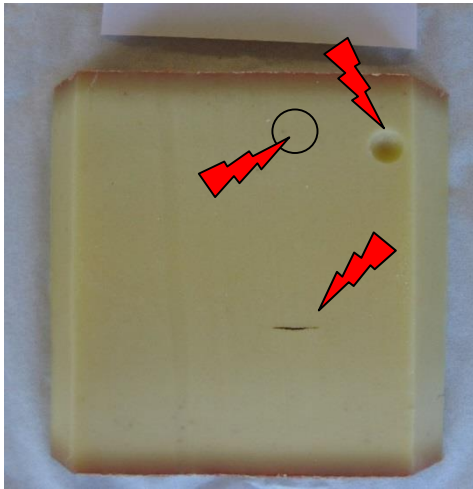
## Emmental Switzerland



Formation of cracks and splits in Swiss-type cheeses due to late fermentation

# Cheese defects associated with PAB

## Gruyère

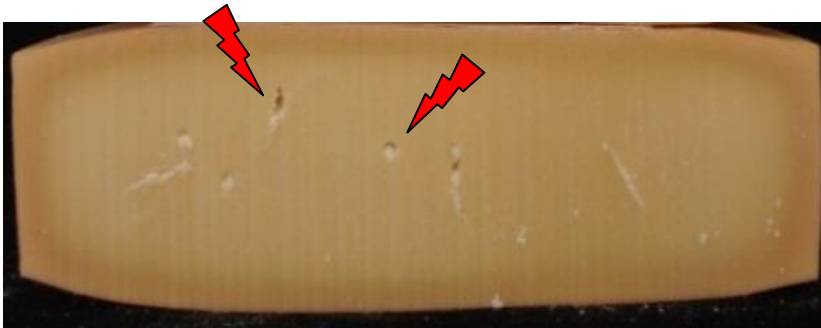


Splits, atypical eye-formation, formation of spots and undesired sweetish flavour

# Cheese defects associated with PAB



Sbrinz



# PAB in manufacture of raw milk cheeses

- Absence of PAB in vat milk is crucial
- Requirement: PAB concentration in raw milk < 30 cfu/mL  
passing this threshold entails a milk delivery ban
- Fast evidence of the milk quality is essentially
- Raw milk quality is verified with plate count method  
PAB grow slowly (6 -10 days)
- In practice detection of PAB is done with one plate per  
sample



# qPCR based approach

- Extraction method for DNA from milk without previous enrichment (Graber et al. 2007)
- Four genes with a high divergence between the four species of dairy PAB were selected
- Primers and hydrolysis probe design to amplify in the target gene the species specific sequence

Dairy PAB	targeted gene
<i>P. freudenreichii</i>	groL2
<i>P. acidipropionici</i>	pduP
<i>P. jensenii</i>	ppk
<i>P. thoenii</i>	aroE



# Strains used for validation

Dairy PAB	Strains	Other taxa in raw milk flora	Species
<i>P. freudenreichii</i>	22	<i>Lactobacillus</i>	9
<i>P. acidipropionici</i>	20	<i>Streptococcus</i>	4
<i>P. jensenii</i>	26	<i>Lactococcus</i>	2
<i>P. thoenii</i>	20	<i>Pediococcus</i>	2
		<i>Leuconostoc</i>	2
		<i>Brevibacterium</i>	1
		<i>Enterococcus</i>	2
		<i>Luteococcus</i>	2
		<i>Clostridium</i>	4
		<i>Staphylococcus</i>	4
		<i>Bifidobacterium</i>	2
		<b>Total strains</b>	<b>36</b>

Phylogenetically related species of dairy PAB	Strains
<i>P. cyclohexanicum</i>	1
<i>P. australiense</i>	1
<i>P. acidifaciens</i>	1
<i>P. microaerophilum</i>	1
<i>P. olivae</i>	1
<i>P. damnosum</i>	1





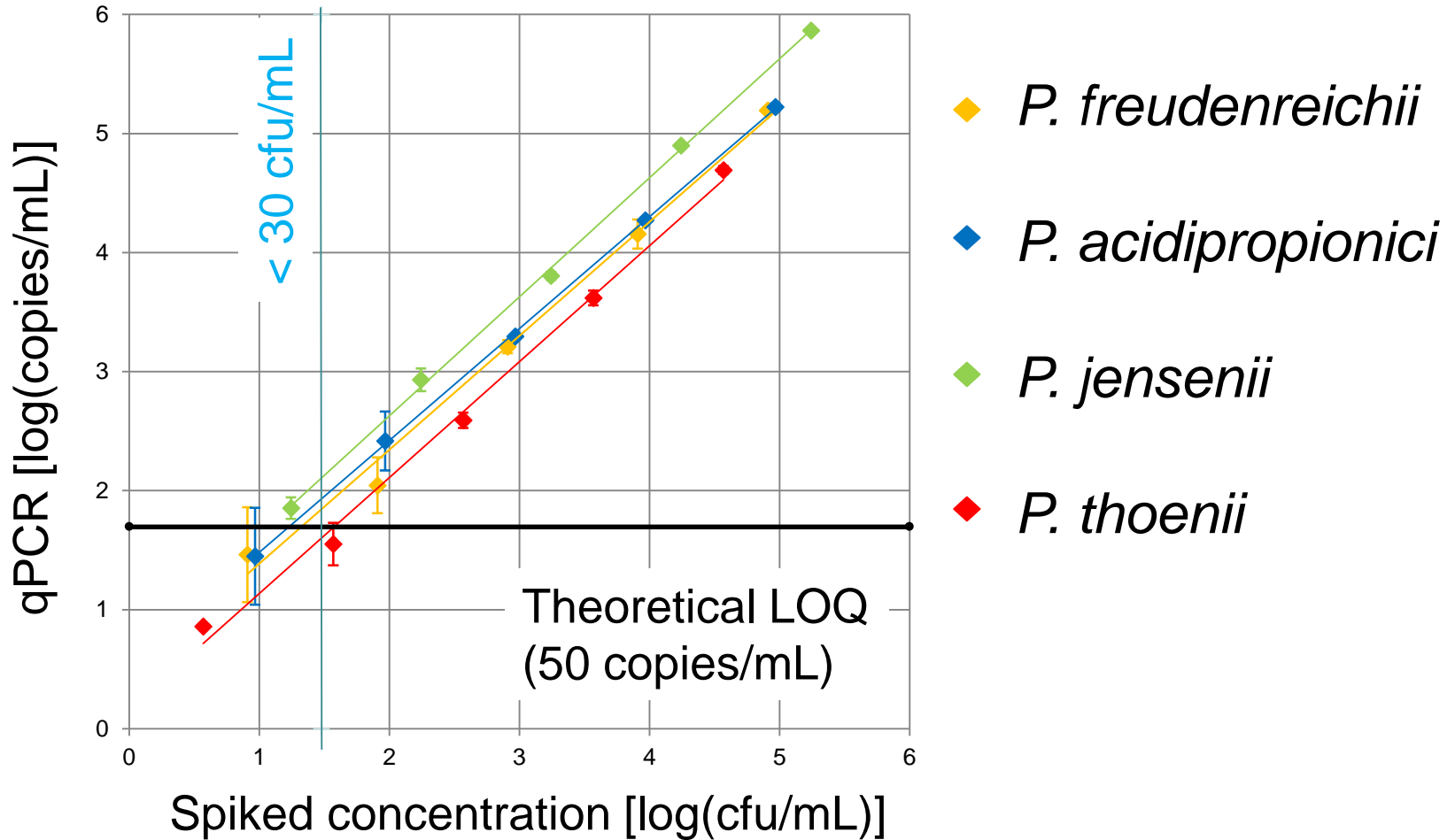
# Results: Sensitivity and specificity

Species	Strains	groL2	pduP	ppk	aroE
<i>P. freudenreichii</i>	<b>22</b>	<b>22</b>	0	0	0
<i>P. acidipropionici</i>	<b>20</b>	0	<b>19</b>	0	1*
<i>P. jensenii</i>	<b>26</b>	1*	0	<b>26</b>	1*
<i>P. thoenii</i>	<b>20</b>	1*	0	0	<b>20</b>
Propionibacterium ssp.	<b>6</b>	0	0	0	0
Species of other taxa	<b>36</b>	0	0	0	0

Primer specificity, expressed in cycle threshold:

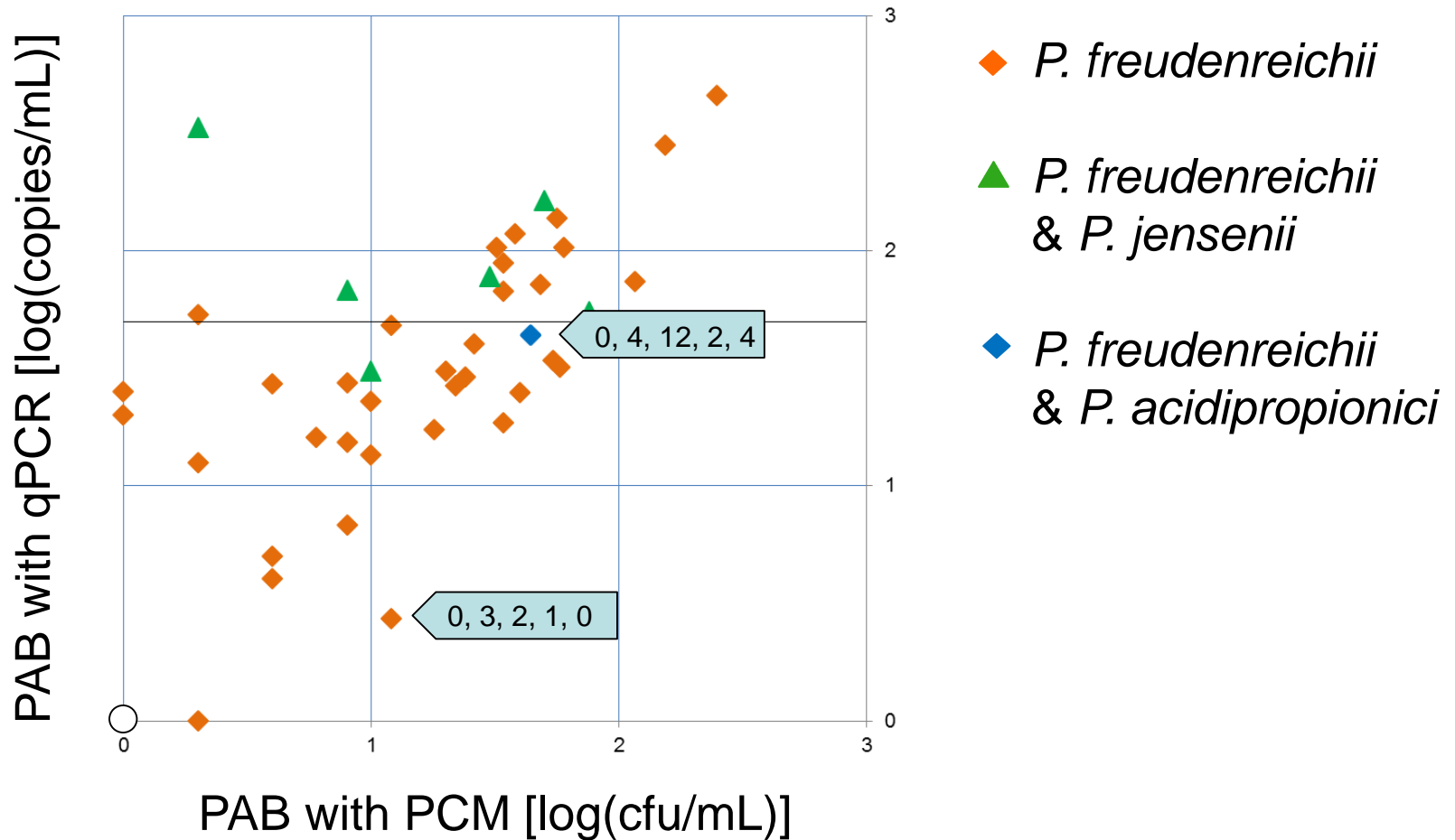
groL2	<i>P. freudenreichii</i>	18.75 ± 0.65 (n = 22)
aroE	<i>P. thoenii</i>	20.33 ± 0.81 (n = 20)
	1*	> 33

# Assay performance (spiked raw milk)





# Application: 51 vat milk samples



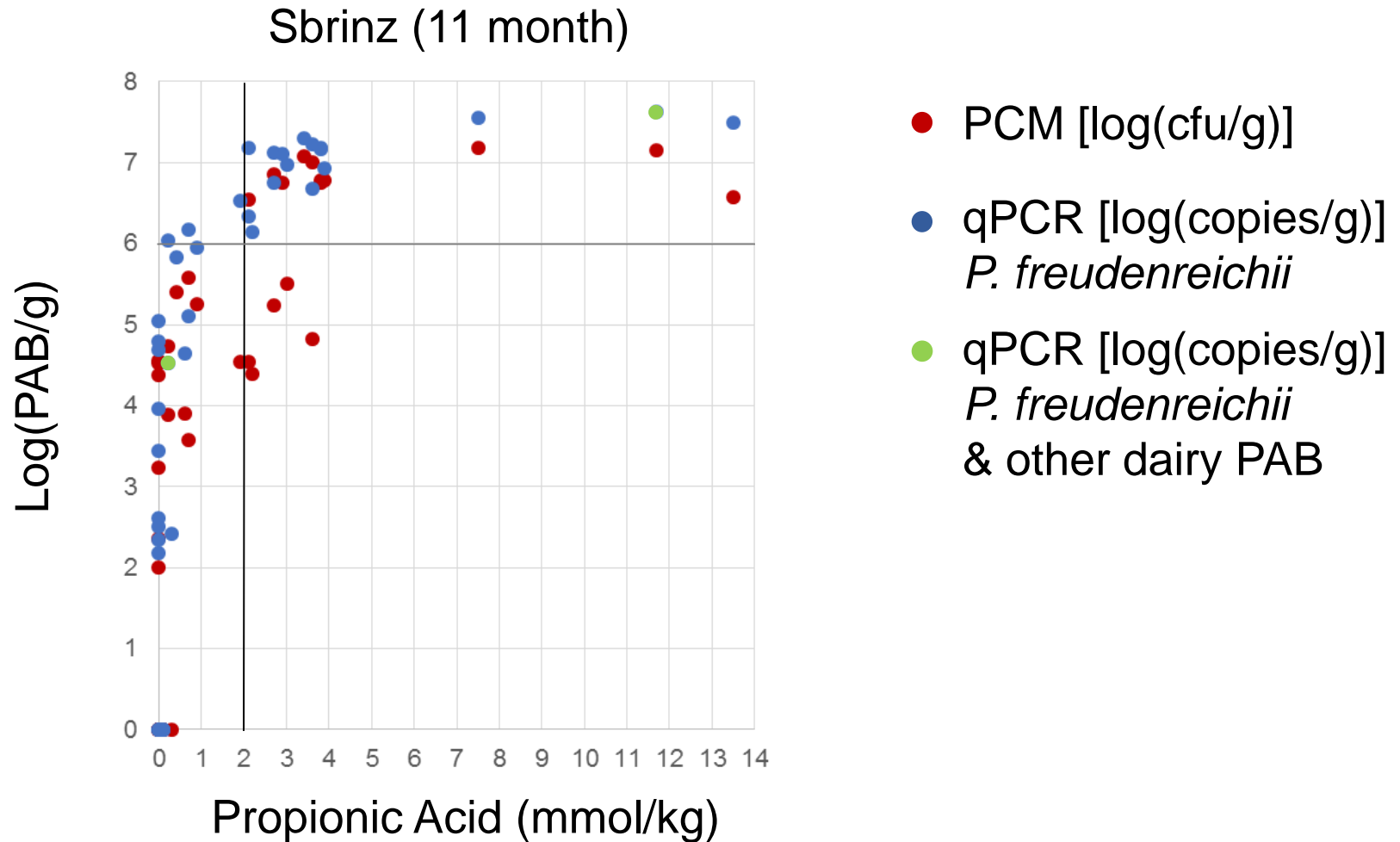


# Case Study: Sbrinz cheese

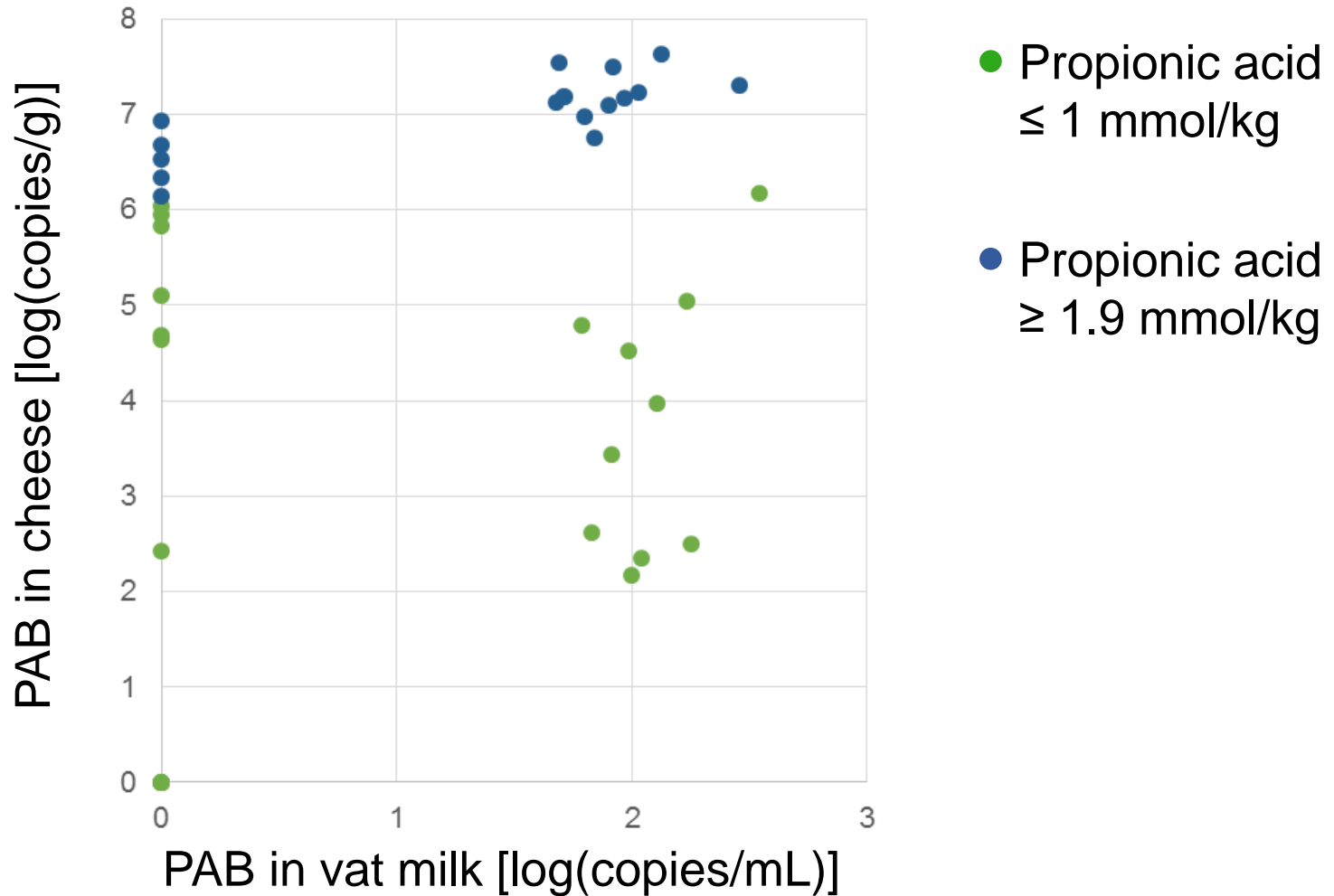
- Tracing dairy PAB in 40 vat milk samples
- Concentration of dairy PAB and propionic acid (PA) in corresponding 40 cheeses



# Concentration of PAB and PA in cheese



# PAB in vat milk and cheese (11 months)





# Conclusions

The newly developed qPCR based method

- is suitable for the control of vat and bulk tank milk
- enables species-specific detection of the dairy PAB
- delivers results in 1-2 days instead of 6-10 days

But, it is more expensive than plate count method

Future Work: Automatization of the method

Application (of the method) in the field



# Team

Hans Graber

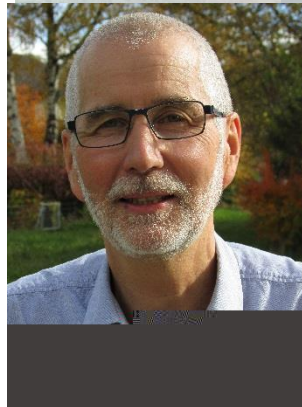
Walter Schaeren

Ruedi Amrein

Daniel Wechsler

Ueli Bütikofer

Elvira Wagner







# Thank you for your attention



**Agroscope** good food, healthy environment