

Comparison of qPCR with FISH-based enumeration of undefined starter cultures

FRIEDRICH, U., SCHNEIDER, H., AND FRANZEN, K.

Research & Development, Danisco Deutschland GmbH, Niebüll, Germany
udo.friedrich@danisco.com

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Introduction

In many dairies undefined multiple-strain cultures of lactic acid bacteria are the most extensively used starter cultures. Compared to defined cultures undefined ones offer significant advantages in terms of their technological properties such as aroma formation and phage resistance. The bacterial composition is a key factor for their subsequent performance in food fermentations. Therefore, technologies are required to enumerate the abundance of key component bacteria. Conventional plate counting has been the chosen method in most microbiological laboratories. One of the major drawbacks of cultivation-based assays is their high time-consumption of about 5 days. In this study, quantitative PCR was compared with FISH to enumerate important lactic acid bacteria of undefined starter cultures.



Fig. 1: PROBAT™ starter cultures are used for various dairy products including different types of cheese and butter.

Methods

DNA of PROBAT™ cultures was isolated after a combined treatment with lysozyme and mutanolysin following recommendations of the manufacturer (DNeasy tissue kit, Qiagen).

qPCR was carried out using TaqMan® and Molecular Beacon® probes targeting the lactate dehydrogenase (*ldh*) and 16S rRNA-encoding genes using a Rotor-Gene 3000™ model qPCR system. DNA proportions of the taxa *Lactococcus lactis* subsp. *lactis*, *L. lactis* subsp. *cremoris*, and *Leuconostoc spec.* were determined in duplex assays relative to total 16S rDNA. For quantification the equation of Pfaffl (2001) was applied using pooled DNA of pure cultures of several strains of each taxon as controls.

Whole cell fluorescence *in situ* hybridization (FISH) was carried out applying 16S rRNA-targeting probes for the same taxa, essentially as described previously (Friedrich et al., 2003). Cell area percentages were determined using digital image analysis.

Results and Discussion

Target gene evaluation

The validity of the *ldh* gene to discriminate the two subspecies *L. lactis* subsp. *lactis* and subsp. *cremoris* was shown previously (Urbach et al., 1997). The larger sequence variation as compared to the 16S rDNA-sequences facilitated primer and probe design for the two subspecies of *Lactococcus lactis*. *Leuconostoc spec.* was detected based on the 16S rDNA sequence.

TaqMan® vs. Molecular Beacon® Probes

As expected, TaqMan probes yielded significantly higher fluorescence increases than Molecular Beacons (Fig. 2). Although both probe formats allowed discrimination of the two *ldh*-genotypes TaqMan probes were used subsequently because of the higher fluorescence yields.

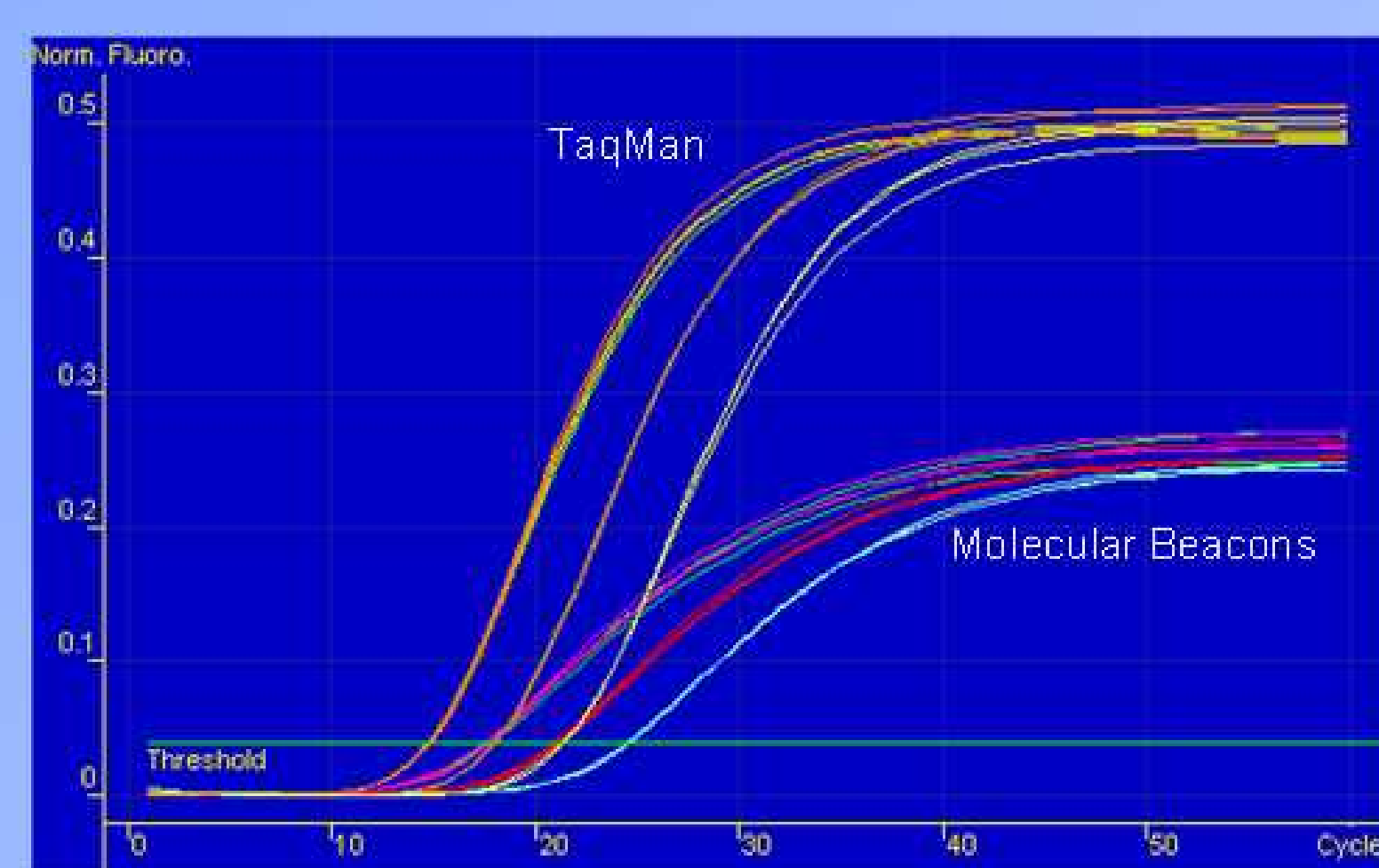


Fig. 2: Linear plots of fluorescence increases measured using a Rotor-Gene qPCR-system. TaqMan and Molecular Beacon probes were both labelled with FAM and BHQ1. Optimised PCR reaction conditions were applied for the comparison.

Fluorescence *in situ* hybridization (FISH)

Genus- and subspecies-specific 16S rRNA-targeting probes were designed and established using reference strains.

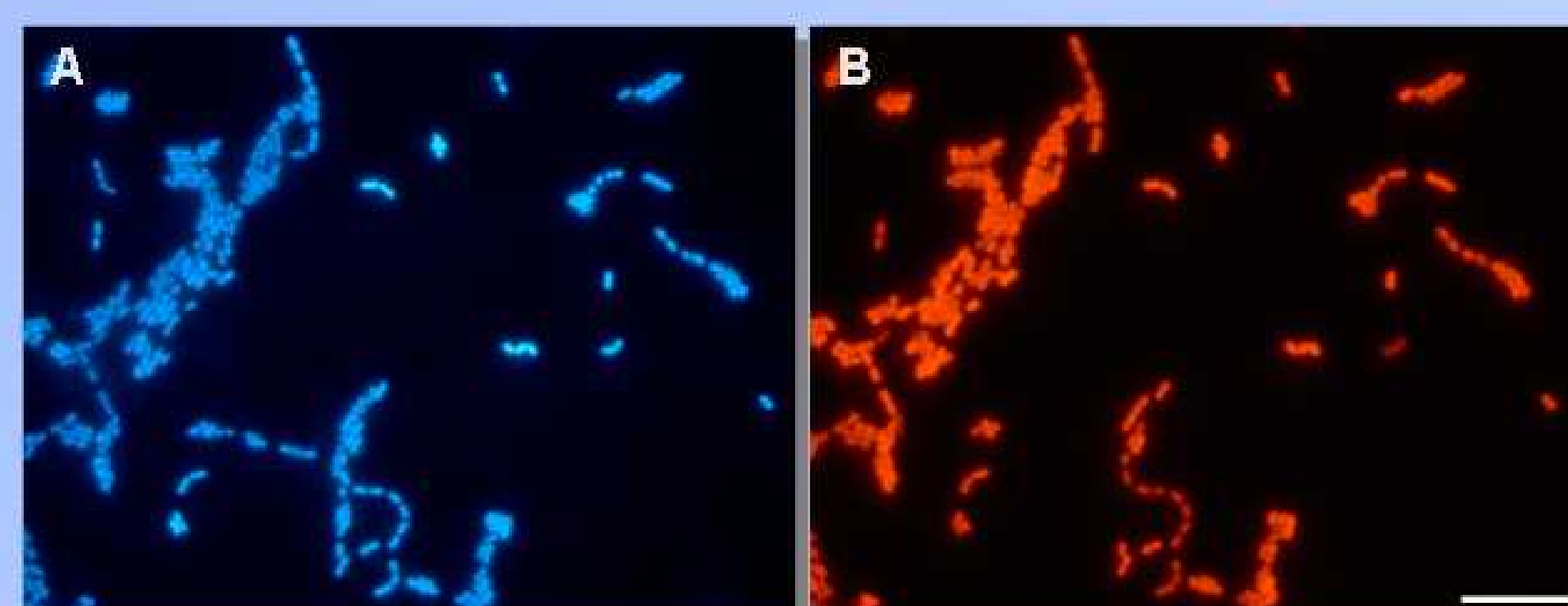


Fig. 3: Microscopic photographs of cells of a PROBAT™ 505 culture. (A) Image obtained after DAPI-staining showing total cells. (B) Same field of view as (A) showing cells after hybridization with Cy3-labelled probe targeting the 16S rRNA of *L. lactis* subsp. *cremoris*.

Comparison of qPCR with FISH

Both qPCR and FISH revealed very similar culture compositions with *L. lactis* subsp. *cremoris* being the most dominant taxon in the PROBAT cultures. For PROBAT 606, fairly large proportions of *Leuconostoc spec.* were detected by FISH (6%). These bacteria accounted for only 1-2% by qPCR.

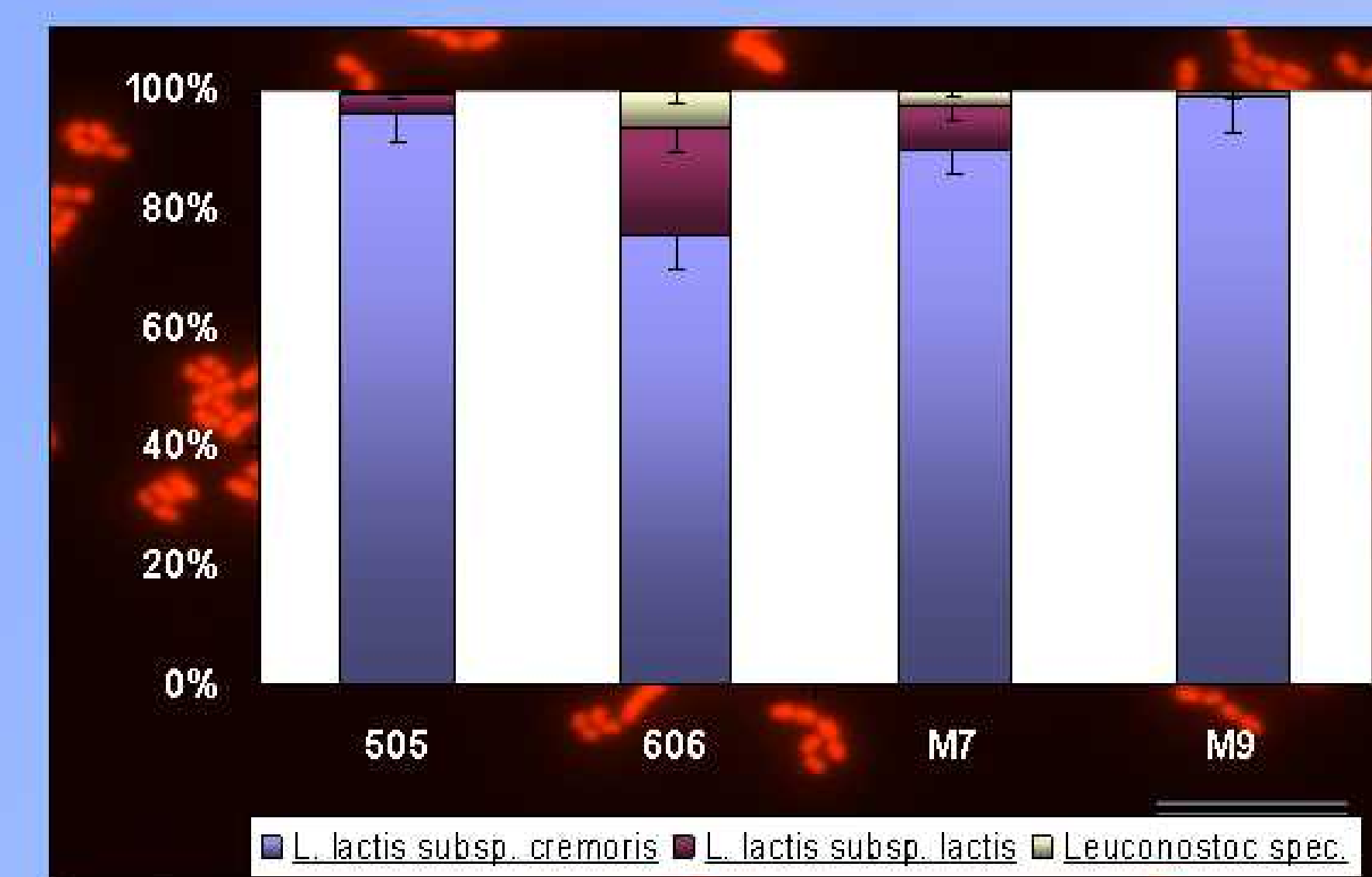


Fig. 4: FISH-results of different PROBAT cultures: Probe-specific percentages relative to the *Bacteria*-specific probe EUB338. Proportions were calculated as ratio of the areas covered by probe-labelled vs. DAPI-stained cells.

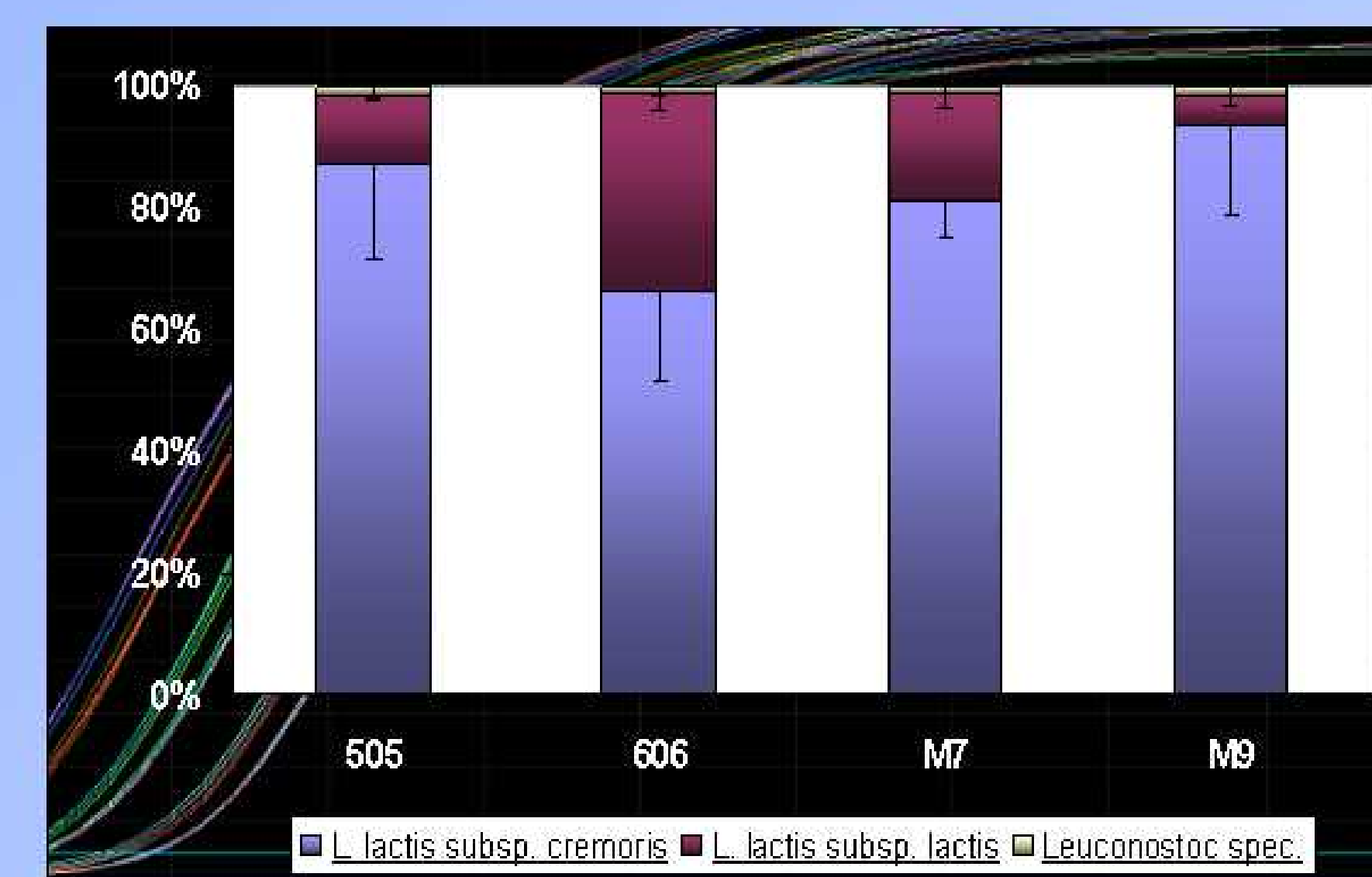


Fig. 5: qPCR-results: Percentages of each of the three genotypes relative to total 16S rDNA. A similar trend as for FISH was observed. While mean proportions of *L. lactis* subsp. *cremoris* were lower than by FISH portions of subsp. *lactis* were higher. Standard deviations of replicates seen for qPCR were generally larger than those for FISH.

Summary

- A qPCR assay based on relative quantification was developed to circumvent the bias inherent in absolute quantification.
- Calculation of relative proportions of specific bacterial genotypes was PCR efficiency-corrected (Pfaffl, 2001).
- Both FISH and qPCR showed similar patterns of the bacterial composition of PROBAT™ starter cultures. Similar results were also obtained using selective plate-counting (no data shown).
- qPCR and FISH were less time-consuming than the laborious cultivation-dependent plate-counting technique.
- The qPCR approach presented will be developed further for routine analyses.

References

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- Urbach, E., Daniels, B., Salama, M. S., Sandine, W. E., and Giovannoni, S. J. (1997). *Appl Environ Microbiol* 63, 694-702.