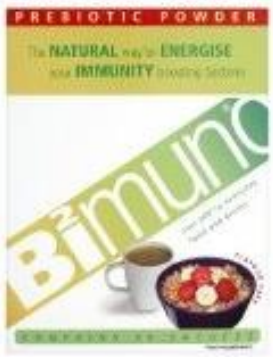
GOS and FOS in infant formulas



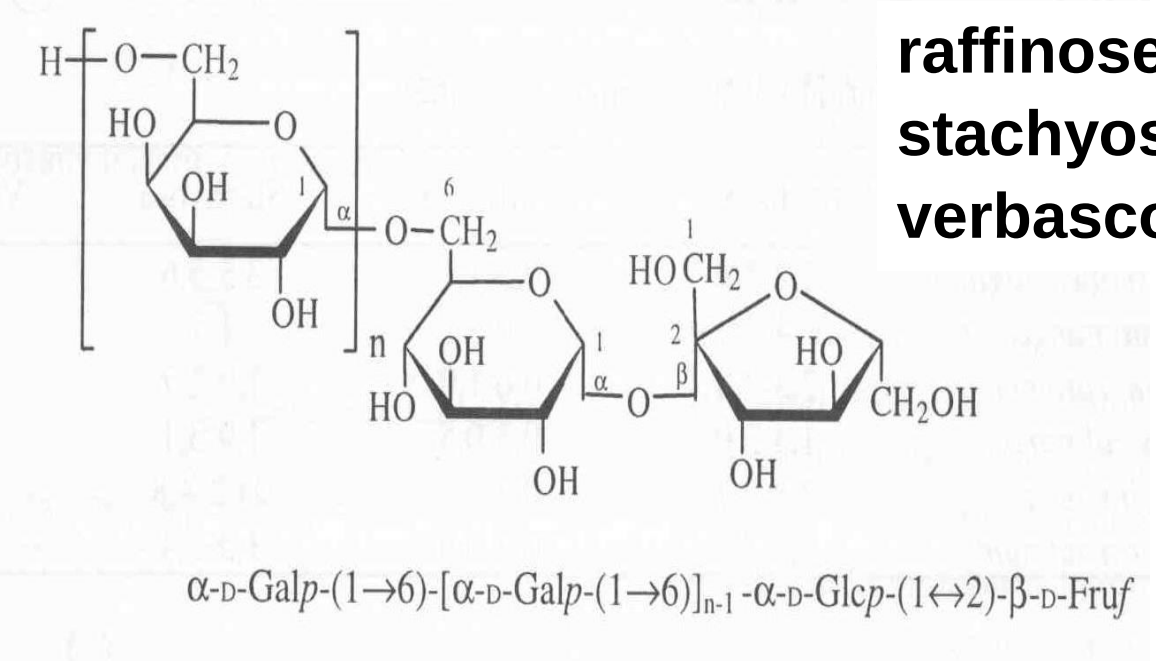
GOS : FOS = 9 : 1 (8g/L)

SOS – Soya oligosaccharides

- Bifidogenic properties
- Extraction from soya whey

Soya whey → (Protein and salts removing) → Soya oligosaccharides

Raffinose series oligosaccharides (RSO)



raffinose (n = 1)
stachyose (n = 2)
verbascose (n = 3)

SOS (RSO) content in leguminoses (% in dry matter)

(Velíšek, 1999)

Leguminose	Raffinose	Stachyose
Bean	0.3 – 1.1	3.5 – 5.6
Pea	0.6 – 1.0	1.9 – 2.7
Lentil	0.3 – 0.5	1.9 – 3.1
Soya	0.2 – 0.8	0.02 – 4.8

Human Milk Oligosaccharides (HMOs)

- Neutral - do not contain sialic acid
- Acidic – contain sialic acid
- Main monomers: glucose, galactose, fucose, N-acetylglukosamine and sialic acid
- HMOs: disacharides(lactose), di-,**tri-,tetra, penta**, …okta-,…dekasaccharides……. (up to date, more then 200 structures identified)

Differences between the human milk and cow's milk (g/l)

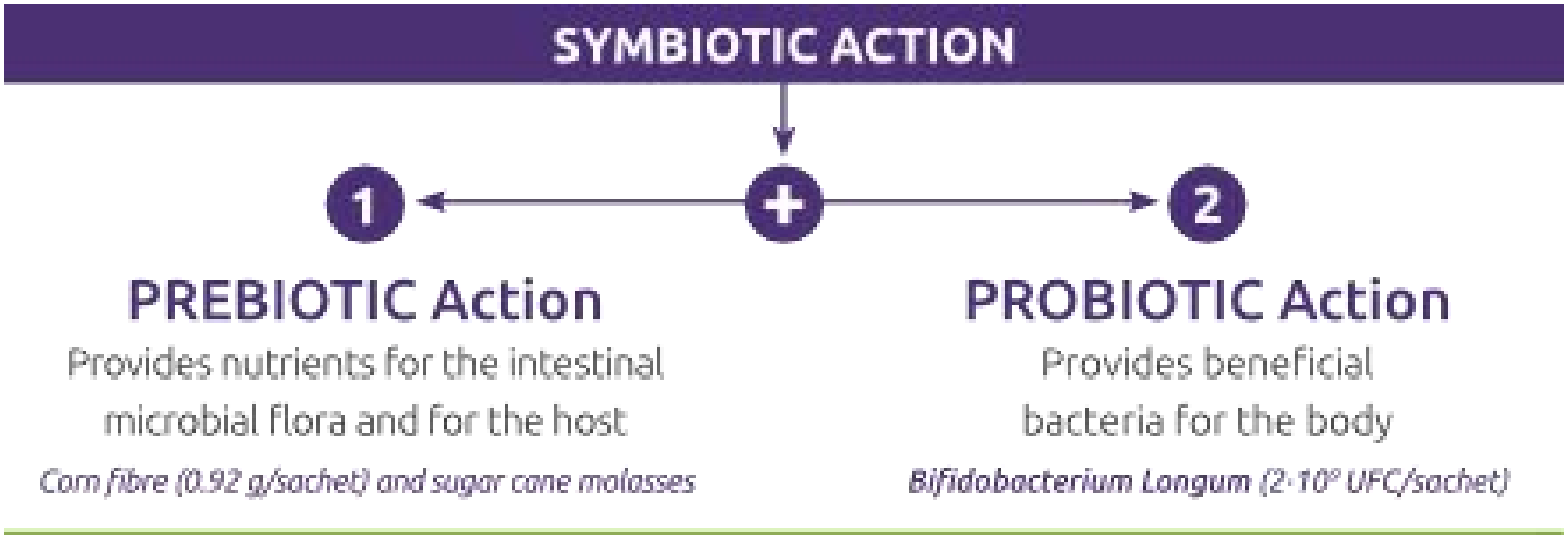
Component	Human milk	Cow milk
Total solids	129	125
Casein	4	28
Albumin	5	7
Lactoferrin	2	0,03
Fat	38	31
Oligosaccharides	8 – 12	0.03 – 0.06
Lysozyme (µg/ml)	400	0.4
Riboflavin	0.43	1.57
Calcium	0.34	1.14
Phosphorus	0.4	0.93

Mechanisms of Human Milk Oligosaccharides Action

- Prebiotic effects
- Development of central nervous system(sialic acids)
- Prevention of adhesion of pathogens
- Absorption of minerals (Ca, P)

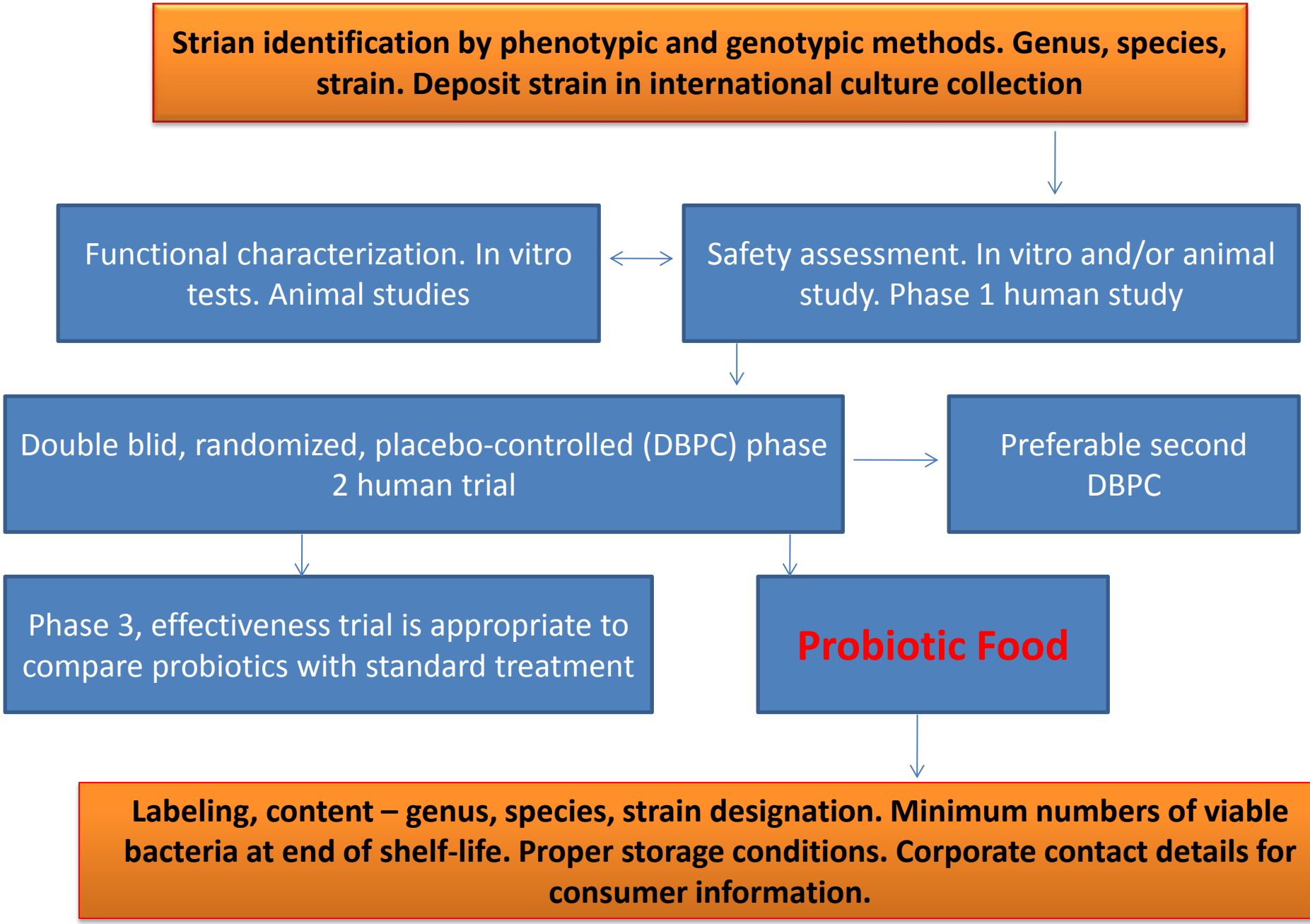
Synbiotics - definition

Combination of probiotics and prebiotics (synergy)



ENUMERATION AND IDENTIFICATION OF PROBIOTICS AND PREBIOTICS IN FOOD

Guidelines for the evaluation of Probiotics for Food Use (FAO/WHO 2002)

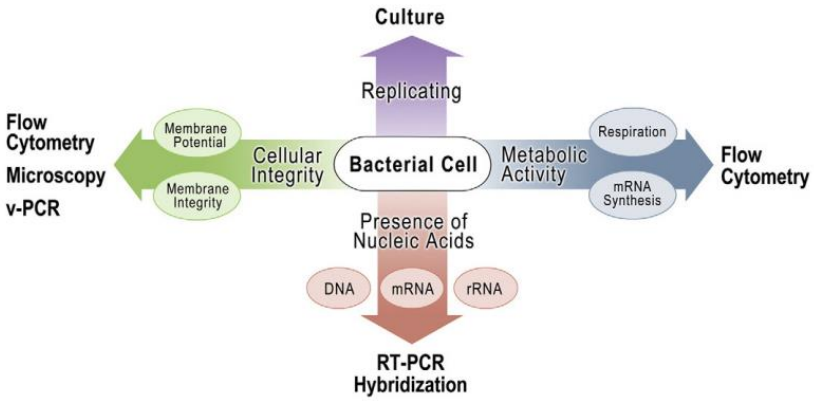


Methods for bacterial identification

Method	Family	Genus	Species	Strain
Phenotypic tests	+	+	+	
FISH		+	+	
Amplificaion of DNA		+	+	+
DNA – DNA Hybridization		+	+	
Genetical fingerprinting			+	+
Gene sequencing, Full genome sequencing			+	+

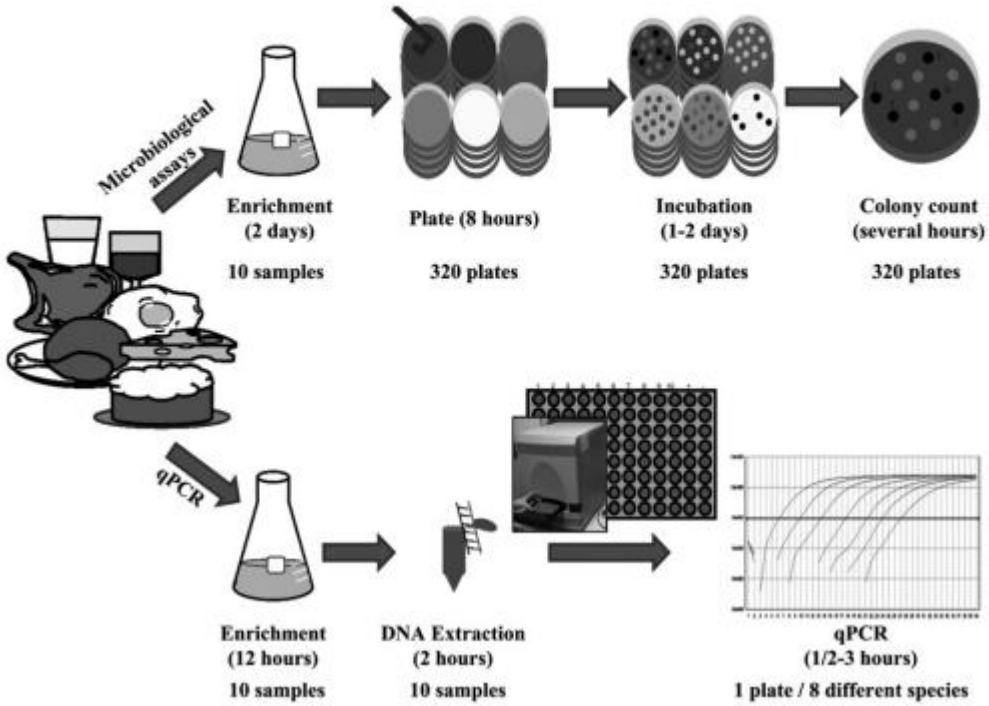
Culture and non-culture based techniques

C. Davis / Journal of Microbiological Methods 103 (2014) 9–17



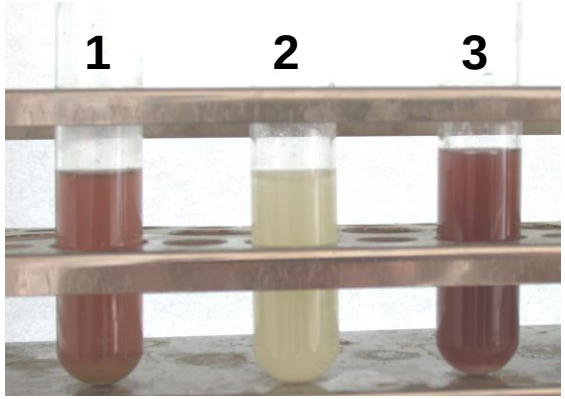
Summary of the facets of a probiotic bacterial cell that may be probed via various culture and non-culture based techniques to as

N. Martínez et al. / Trends in Food Science & Technology 22 (2011) 367–376



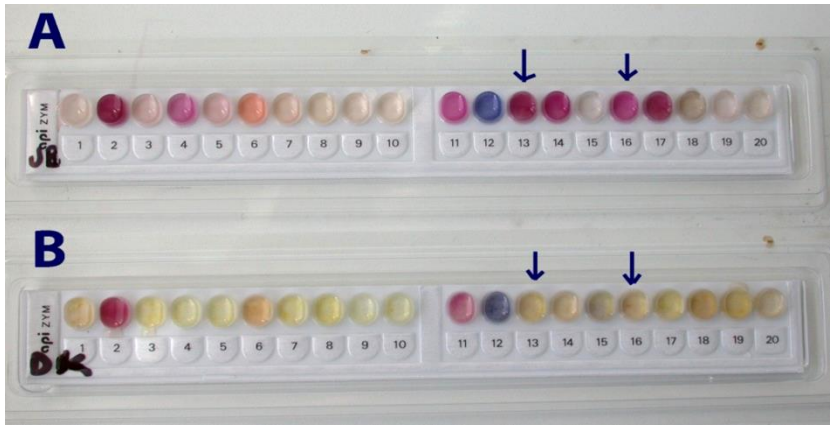
Enzymatic and biochemical methods for bifidobacteria detection and identification

fructose-6-phosphate phosphoketolase activity



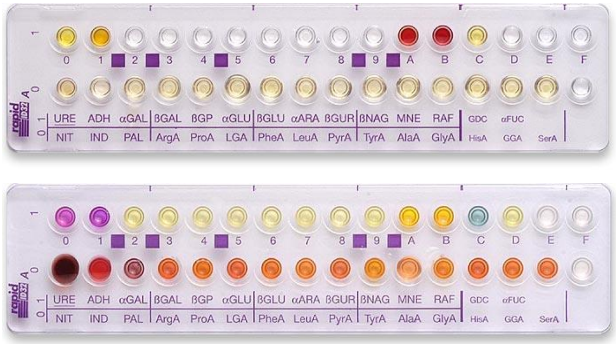
Bifidobacteria-positive (1) and bifidobacteria-negative (2) faecal samples (3 – positive control)

α-galactosidase and α-glucosidase activity (API ZYM, BioMérieux, Francie)



A - bifidobacteria-positive faecal sample, B - bifidobacteria-negative faecal sample (No13 - α-galactosidase, No16 - α-glucosidase)

Biochemical kits API 50 CHL a API ID 32A Rapid (BioMérieux, Francie)

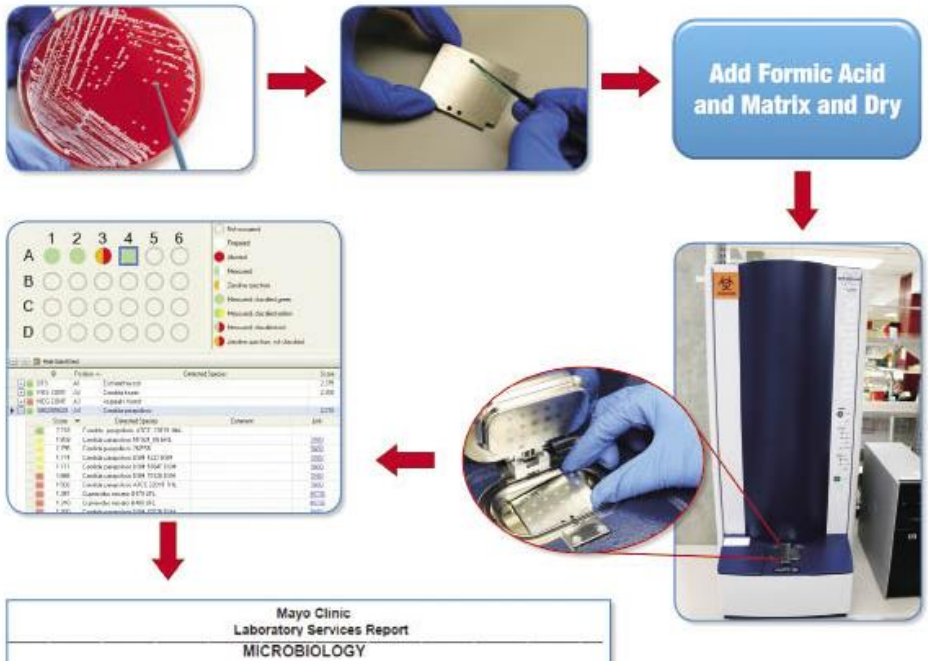


Vlková et al. (2005) J. Microbiol. Meth. 60, 365-373

Use of MALDI-TOF mass spectrometry for identification of bacteria

Matrix-assisted laser desorption/ionization (MALDI) is a soft ionization technique use in mass spectrometry allowig the analysis of biomolecules and large organic molecules.

➢MALDI-TOF-MS is a powerful, rapid, precise, and cost-effective method for identification of intact bacteria



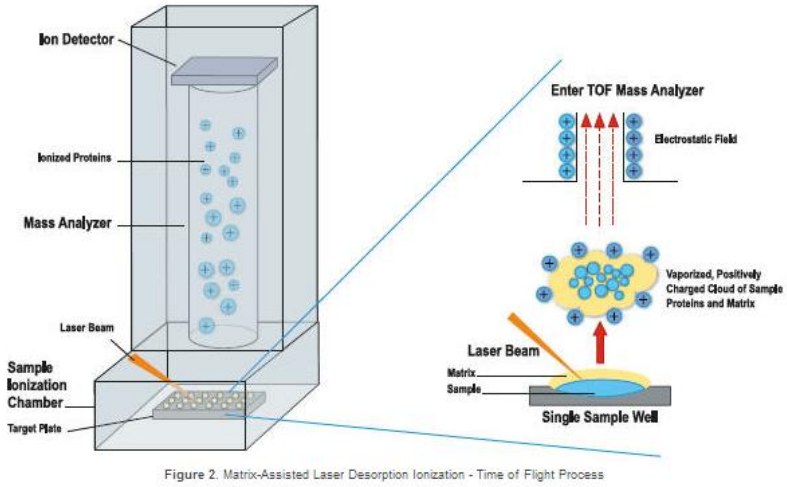
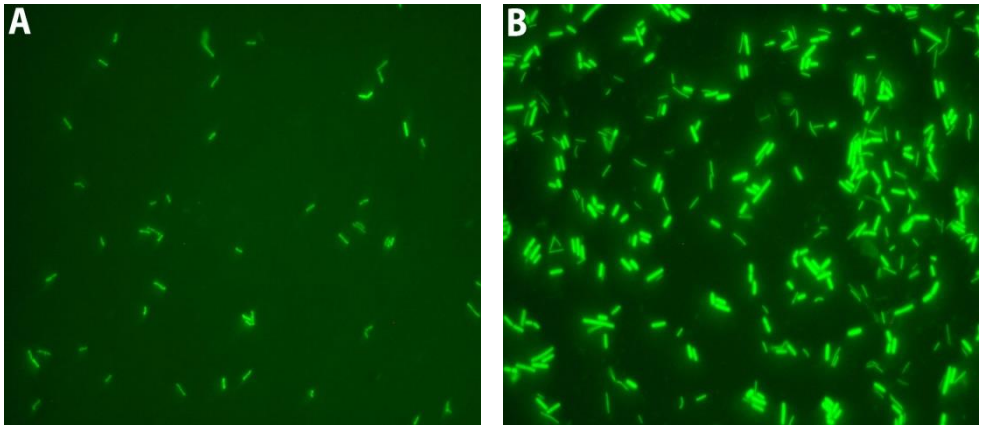


Figure 2. Matrix-Assisted Laser Desorption Ionization - Time of Flight Process

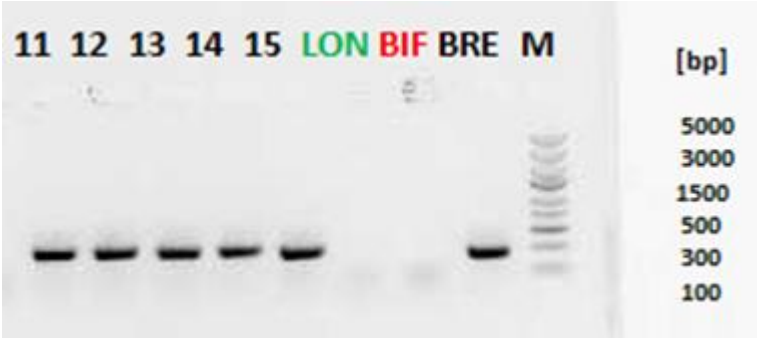
http://www.mayomedicallaboratories.com/articles/communique/2013/01-maldi-tof-mass-spectrometry/

Molecular methods for detection and identification of intestinal bacteria

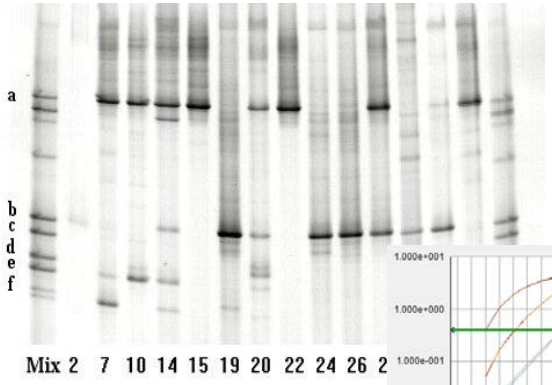
FISH procedure using bifidobacteria-specific (A) and clostridia-specific (B) probes



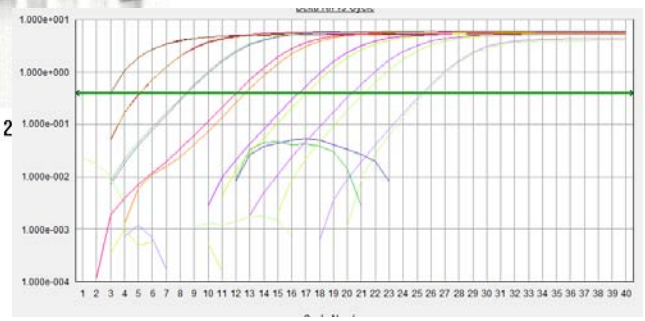
genus- or species-specific PCR



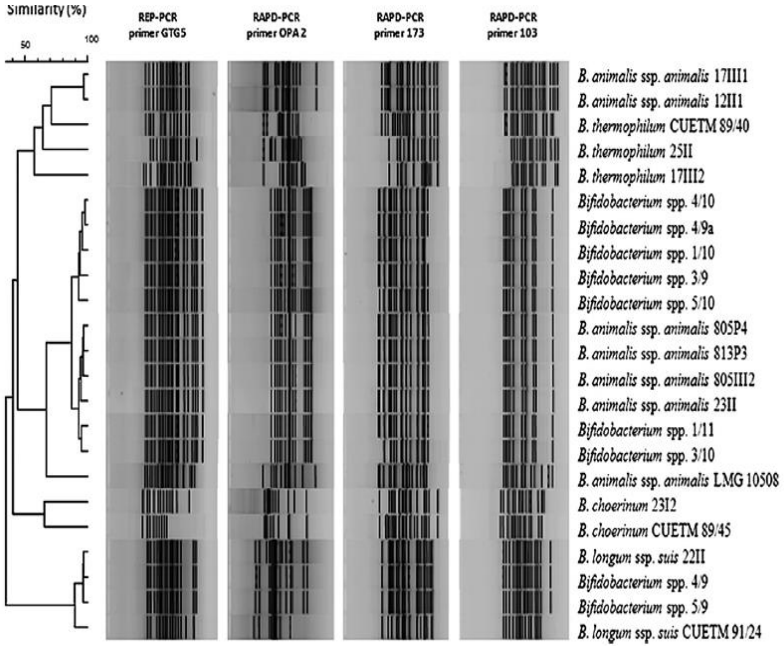
Nested-DGGE



qPCR

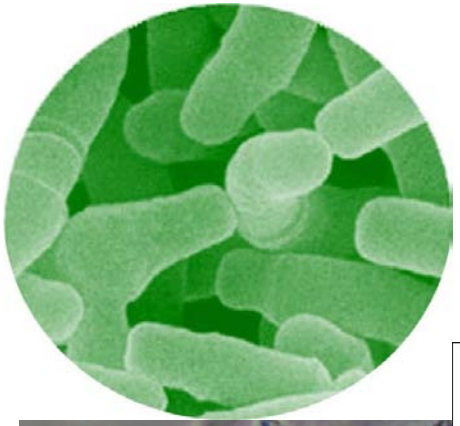


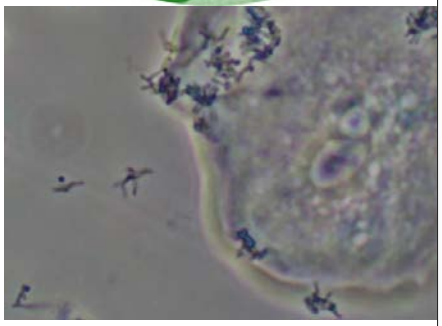
PCR – RAPD

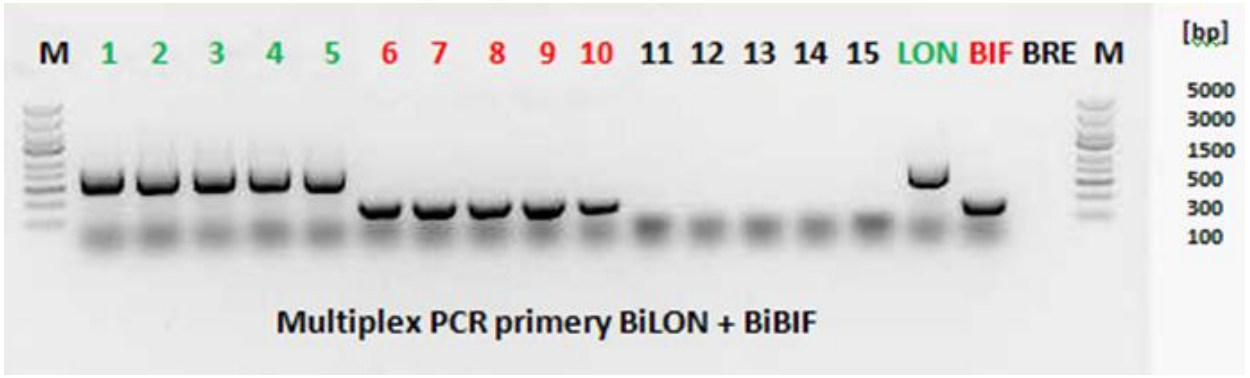


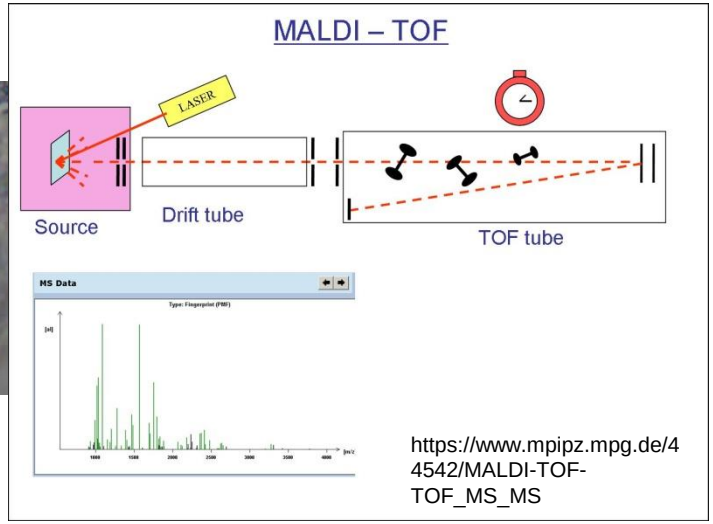
Polyphasic identification

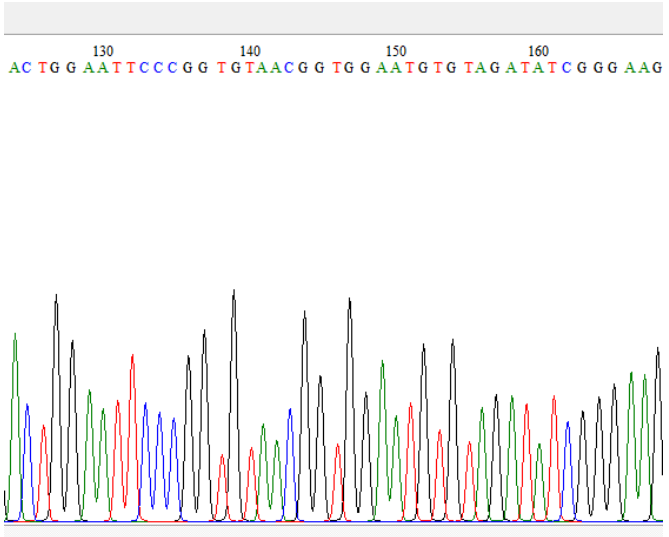
- Combination of morphological, physiological, biochemical, serological, molecular and other methods











General demand (IDF, EU)

- Probiotic fermented milk product should contain 10^6 CFU/g of probiotic bacteria at the time of sale.

ISO/IDF Standards for Probiotic Bacteria

- ISO 7218 Food Microbiology
- ISO 7889:2003 (IDF 117) Enumeration of yoghurt bacteria
- ISO 9232:2004 (IDF 146) Identification of youghurt bacteria
- ISO 20128:2006 (IDF 192) Enumeration of *Lactobacillus acidophilus* in fermented milk products
- ISO 29981:2010 (IDF 220) enumeration of bifidobacteria i n fermented milk products

Enumeration of Bifidobacteria

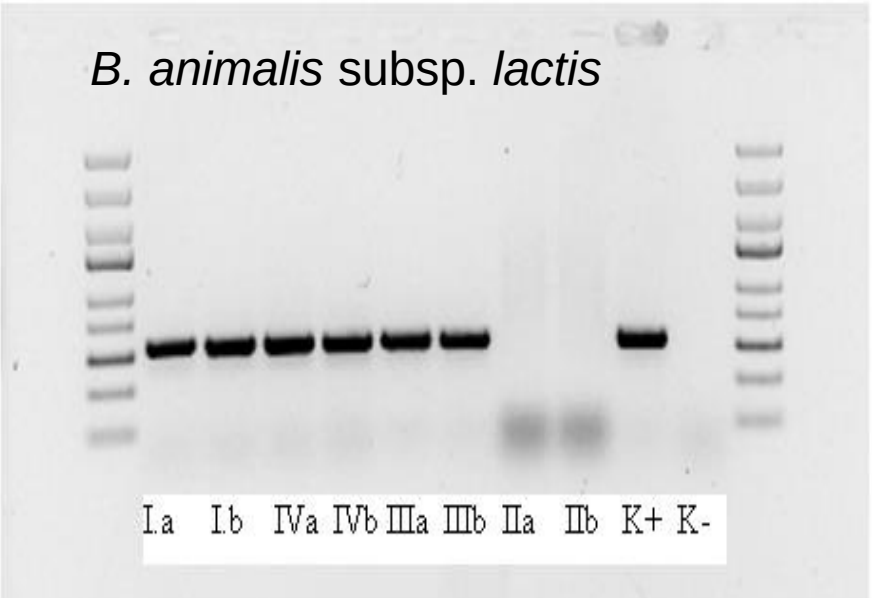
- ISO 29981:2010 (IDF 220)
- TOS medium with mupirocin (50 mg/L)
- Reliable for milk products
- Not suitable for isolation of bifidobacteria from faecal samples
- Not suitable for the enumeration of *B. bifidum* species

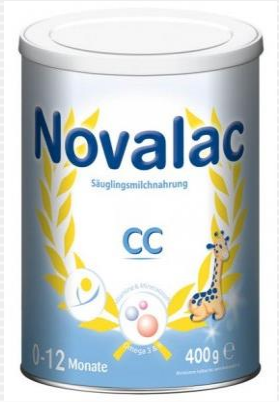
Detection of bifidobacteria

Product	Declared count (logCFU/g)	Measured count	Species identified
Activia (fermented milk drink)	6.00	8.45 ± 0.05	*Bifidobacterium animalis* subsp. *lactis*
Activia (youghurt) (Danone)	6.00	8.41 ± 0.28	*Bifidobacterium animalis* subsp. *lactis*
Probio fix (S & D Pharma CZ; probiotic capsules)	9.43	10.38 ± 0.12	*Bifidobacterium animalis* subsp. *lactis*
Probio fix imun (S & D Pharma CZ; probiotics capsules)	9.43	10.51 ± 0.08	*Bifidobacterium animalis* subsp. *lactis*

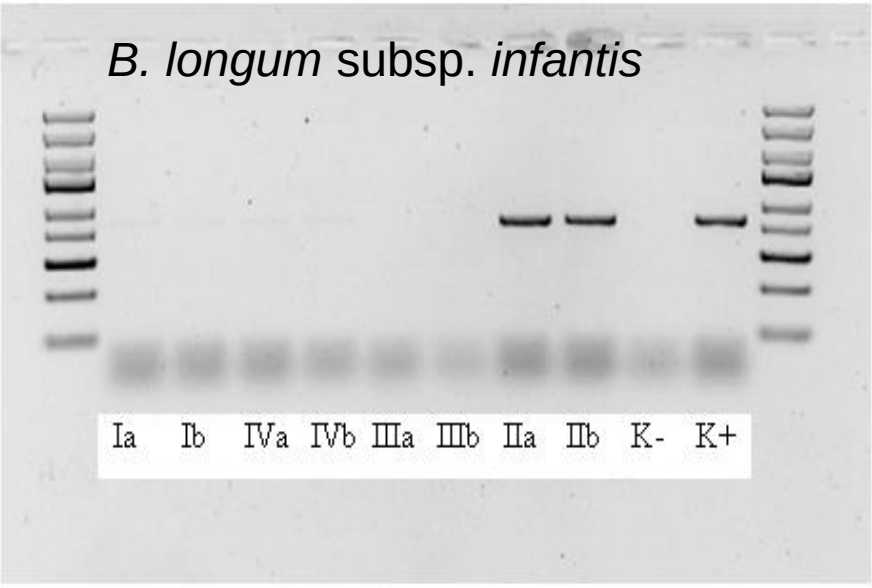
Bifidobacteria species in infat formulas

B. animalis subsp. *lactis*

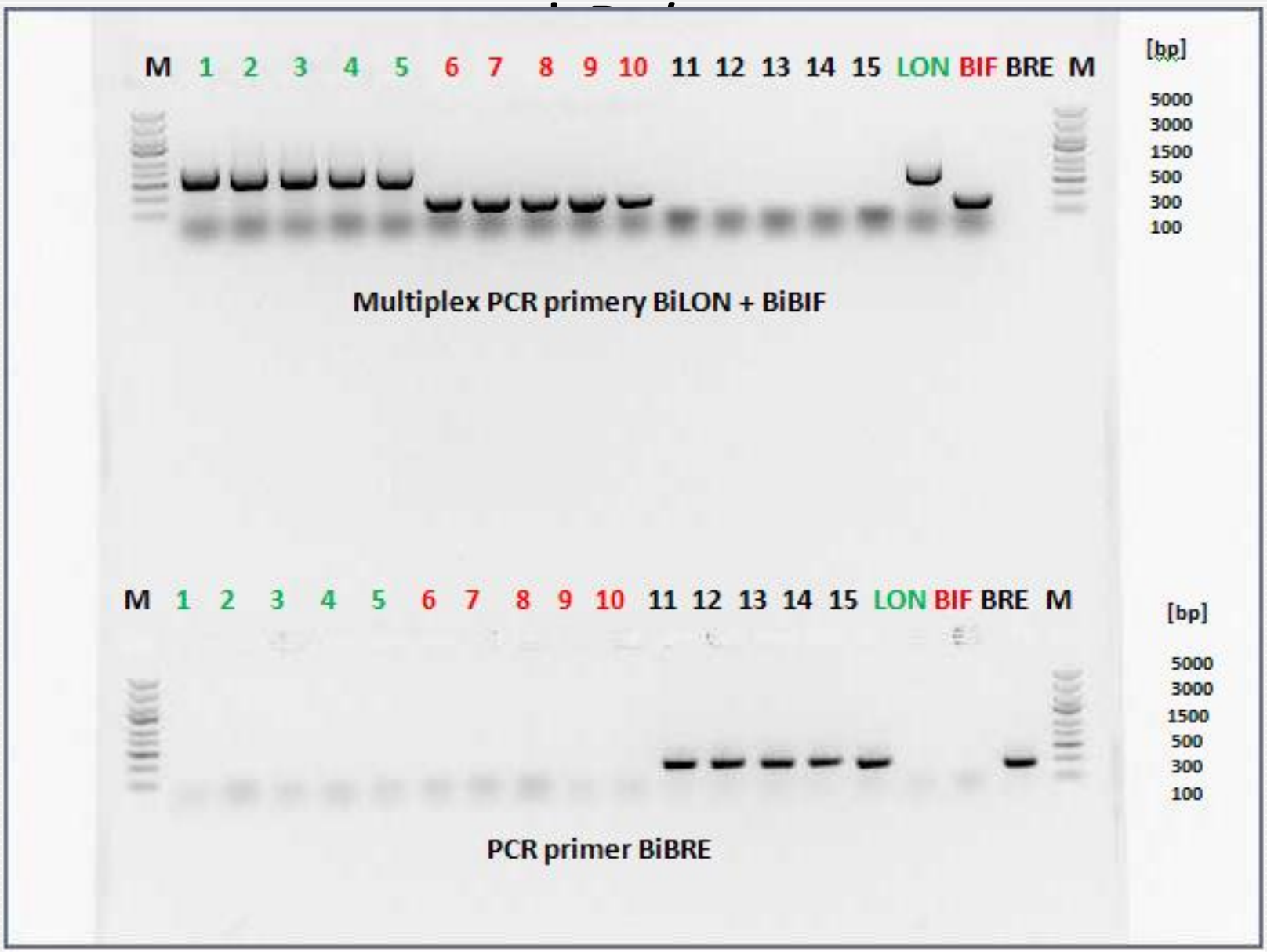




B. longum subsp. *infantis*

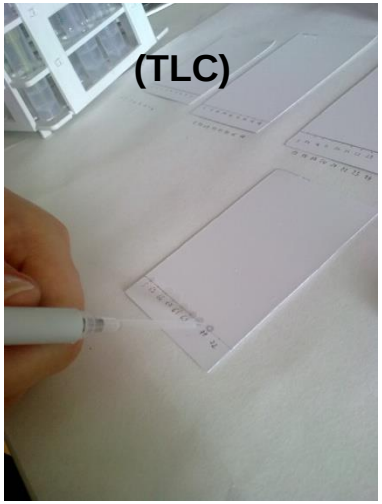


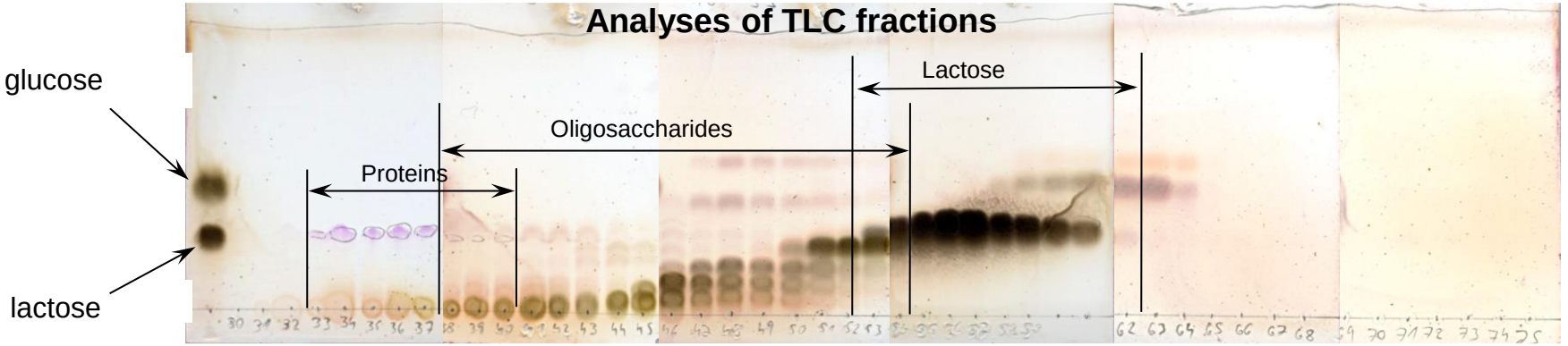
Selective detection of *B.bifidum*, *B. longum*



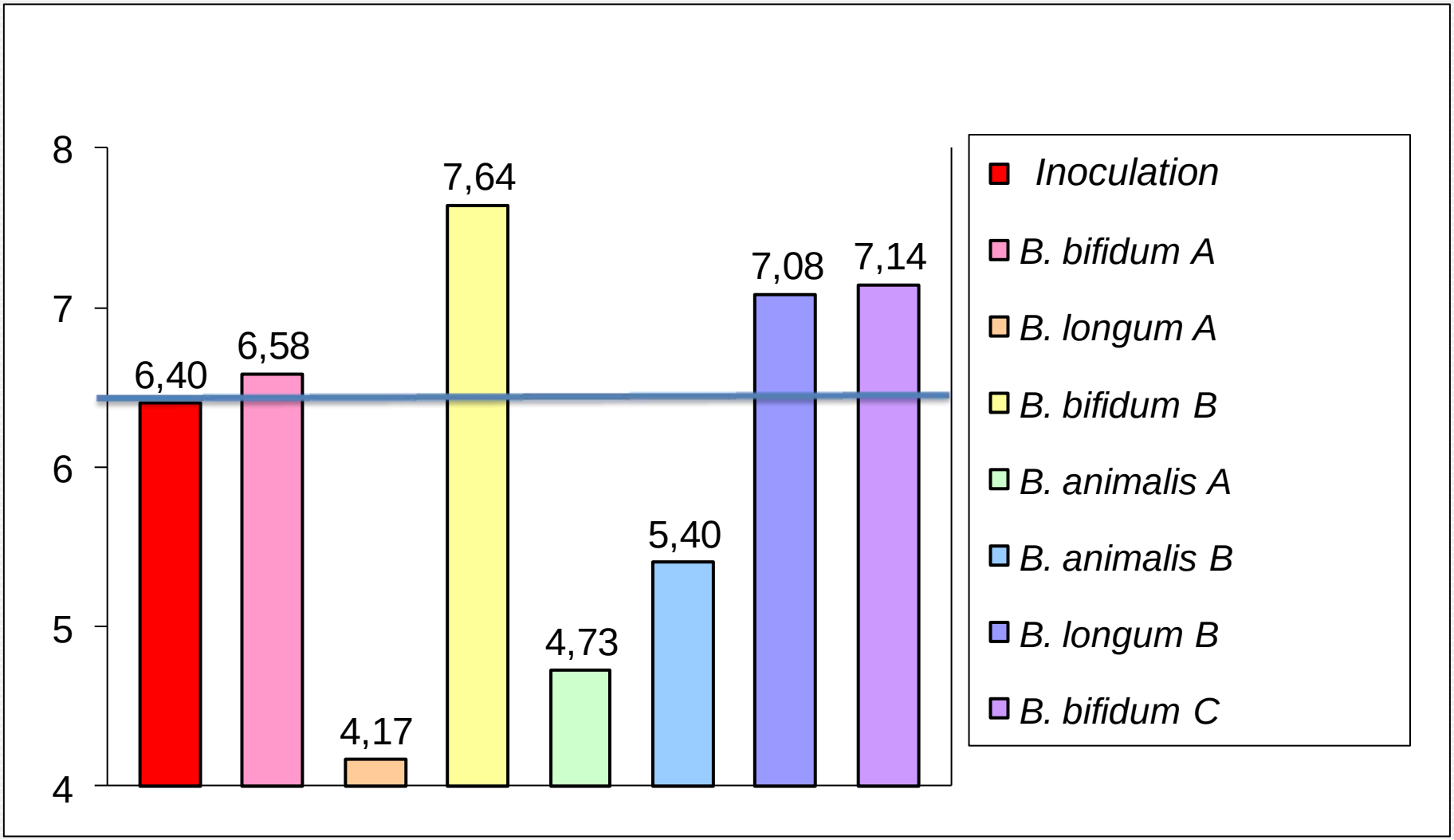
Isolation of oligosaccharides from human milk

- Removal of fat – by centrifugation
- Removal of proteins - (precipitation – by ethanol, by dichloromethane)
- Separation of fractions by gel chromatography
- Analysis of the fractions (TLC - TLC)
- Oligosaccharides were added to the media as the sole source of sugars for bifidobacteria

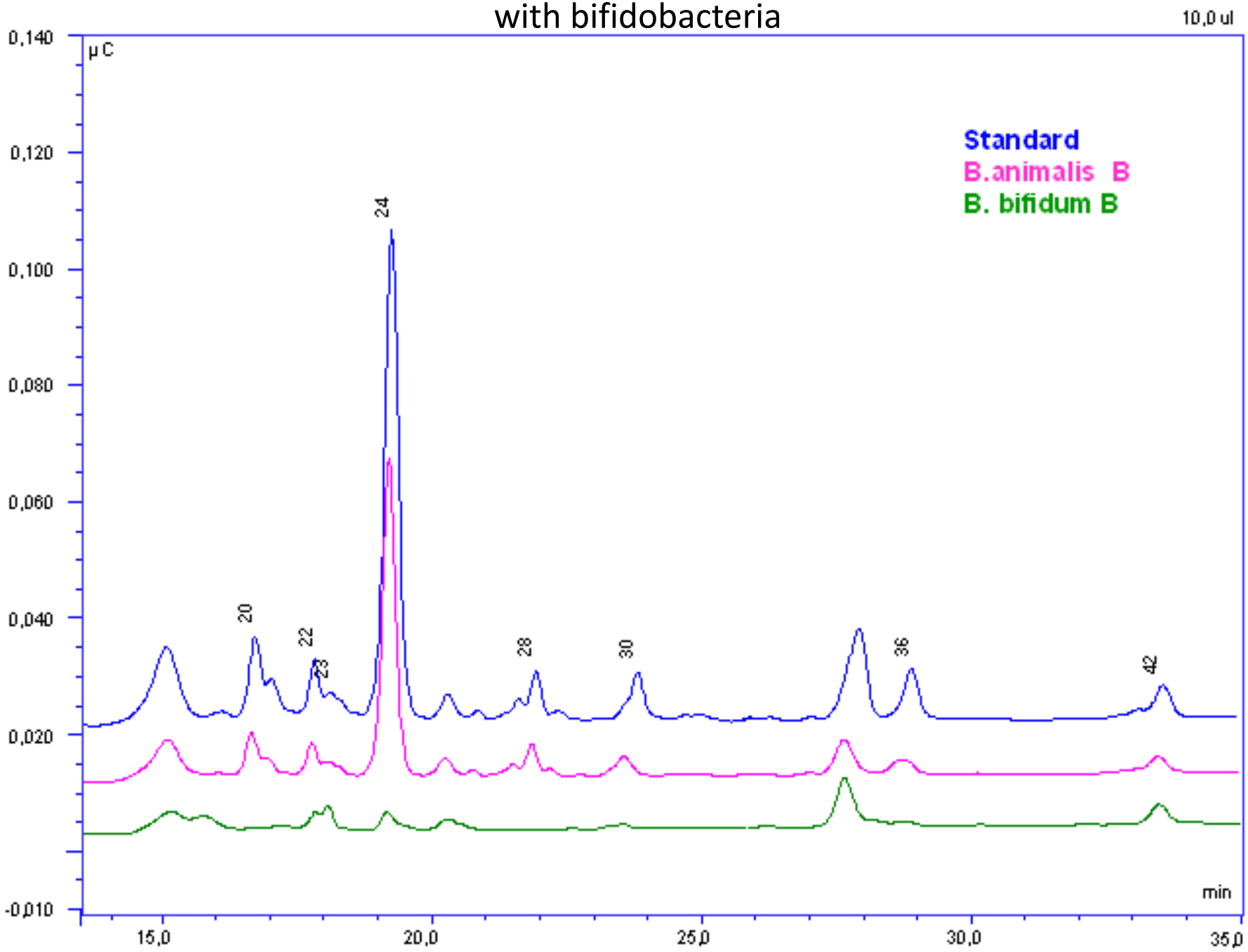




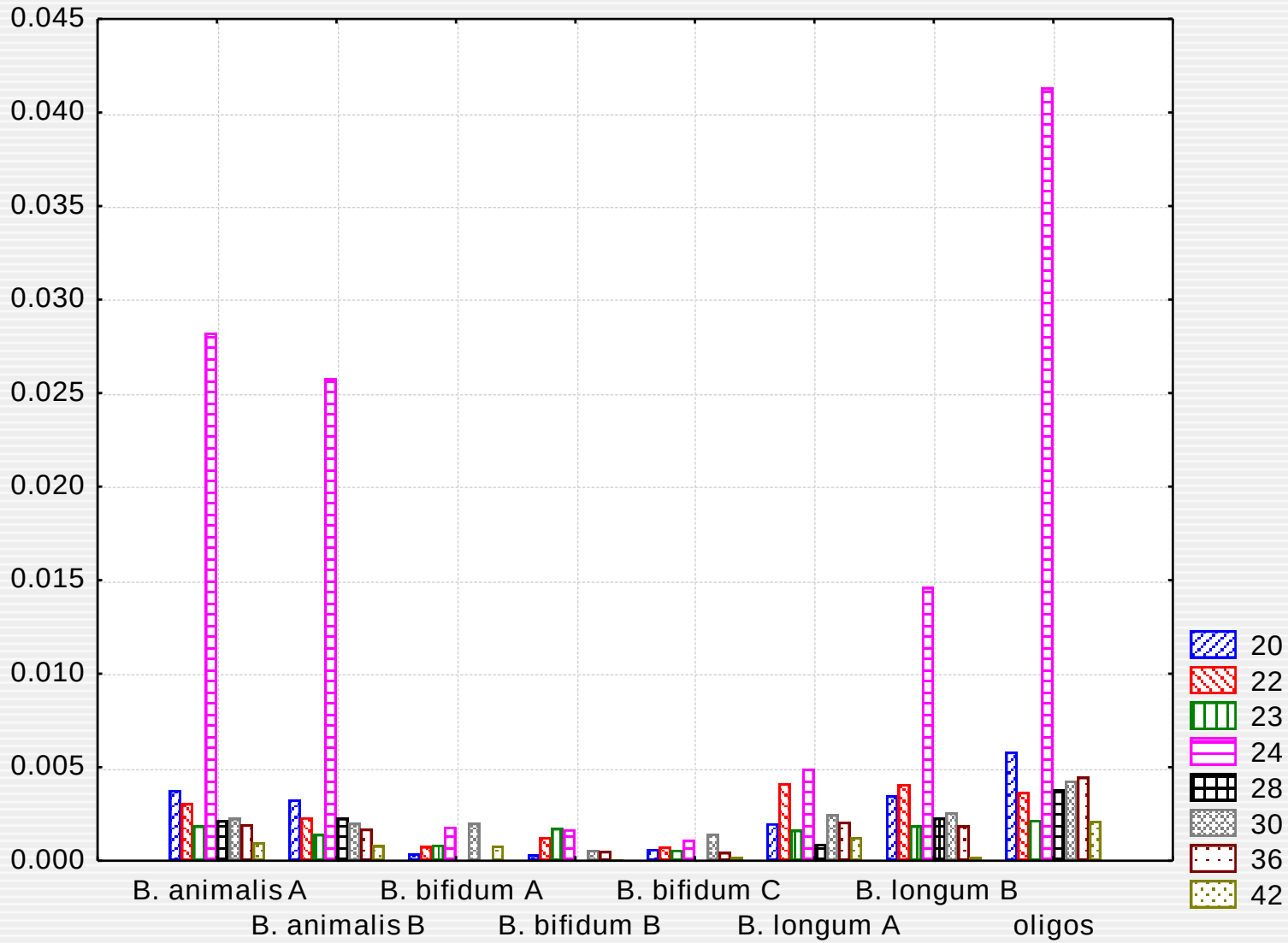
Growth of bifidobacteria in human milk (log CFU/l)



HMO profiles in cultivation media before (standard) and after incubation with bifidobacteria



Peak areas of selected HMO before (oligos) and after incubation with bifidobacteria [µC*min]



Conclusion

- There is a reliable ISO/IDF method for the enumeration of bifidobacteria in fermented milk product
- Other methods for the enumeration of probiotic bacteria in food need to be developed
- Polyphasic approach is the best way for the identification of probiotic bacteria

Thank you for your attention!