LABORATORY SAFETY

- Do not drink, eat and smoke
- Protective clothing
- Aseptic technique
- Bacteriological loop, needle
- Burner
- Bacteriological stains



Quantification of bacteria in yogurt



Starter Cultures

Yoghurt cultures:

- Lactobacillus delbrueckii ssp. bulgaricus
- Streptococcus thermophilus

≻ (45°C, 4h)







Bifidobacterium lactis BB12 and *Lactobacillus acidophilus* LA 5

Demands fof Bacterial counts in Fermented Milk Products

- Yoghurt bacteria (*Lactobacillus delbrueckii* subsp. *bulgaricus, Streptococcus thermophilus*) 10⁷ CFU/ml
- Probiotic bacteria (bifidobacteria, Lactobacillus casei, L. rhamnosus) 10⁶ CFU/ml

Enumeration of bacteria in yoghurt using cultivation on selective media



Calculation of Colony Forming Unit (CFU/g)

- $P = [(P1 + P2)/11] \times F (CFU/ml)$
- P1,P2 number of colonies in the the first and second countable dilution, respectively
- F inversed value of dilution factor



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- Probiotic bacteria (bifidobacteria, Lactobacillus casei, L. rhamnosus) 10⁶ CFU/ml

Bifidobacterium animalis subsp. *lactis*

Yoghurt bacteria: *Lactobacillus delbrueckii* subsp. *bulgaricus, Streptococcus thermophilus*





Observation of Bacteria in Fermented Milk Products

- Spread one loopful of sample over the slide.
- Allow the slide to air-dry.
- Fix with ether-alcohole for 1 min.
- Add a drop of 1%NaOH for 10 sec.
- Wash with water.
- Stain with methylene blue for 2 min.
- Wash with water.
- Allow the slide to air-dry and examine with an oil immersion objective.

Simple Staining

- Smear preparation:
 - A drop of water is placed in the centre of a slide
 - One loopfuls of organisms is transferred to the centre of slide
 - Spread the organisms over the slide
 - The smear is allowed to dry
 - Slide is passed through flame several times to heat-kill and fix organisms
- A bacterial stain is stained with crystal violet (fuchsin, methylene blue) 1 min
- Stain is briefly washed off slide with water Allow the slide to air-dry and examine with an oil immersion objective

Identification using API® (50 CHL)



Identification using API® (50 CHL)

API 50 CHL

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The above examples are not intended to replace the reading table in the package insert.

Identification using API® (50 CHL)







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API 50 CHL V5.2 Reset Instructions Color check 4 0 2 3 5 6 7 8 9 1 4 + + API 20 C AUX 12 15 17 18 10 13 16 11) 14 19 API 20 STREP API 50 CHB ÷ ÷ ÷ + ÷ API 50 CHE API 50 CHL 20 21 22 23 24 25 26 27 28 29 API CAMPY API CANDIDA API CORYNE 4 + ---. API LISTERIA 30 31) 32) 33) 34 35 36 37 38 39 RAPID 20 E ÷ 40 41 42 43 44 45 46 47 48 49 Confirm

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Gapiweb



API NH

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API API 10S

API 20 A

API 20 E

API 20 NE

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API 50 CHL V5.2	Printout	Export	New test	<u>Modify</u>
REFERENCE	DATE	15		
COMMENT	//21/	15		

GOOD IDENTIFICATION	
Strip	API 50 CHL V5.2
Profile	+++++-+++++++++++++++++++++++
Note	

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Significant taxa	% ID	Т	Tests against			
Lactobacillus acidophilus 1	97.4	0.71	MNE 96% CEL 96%			

Next taxon	% ID	Т	Tests against			
Lactobacillus acidophilus 3	0.9	0.53	AMY 1%	GEN 4%		