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Detection and Quantification of *Mycobacterium avium* Complex Organisms in Drinking Water

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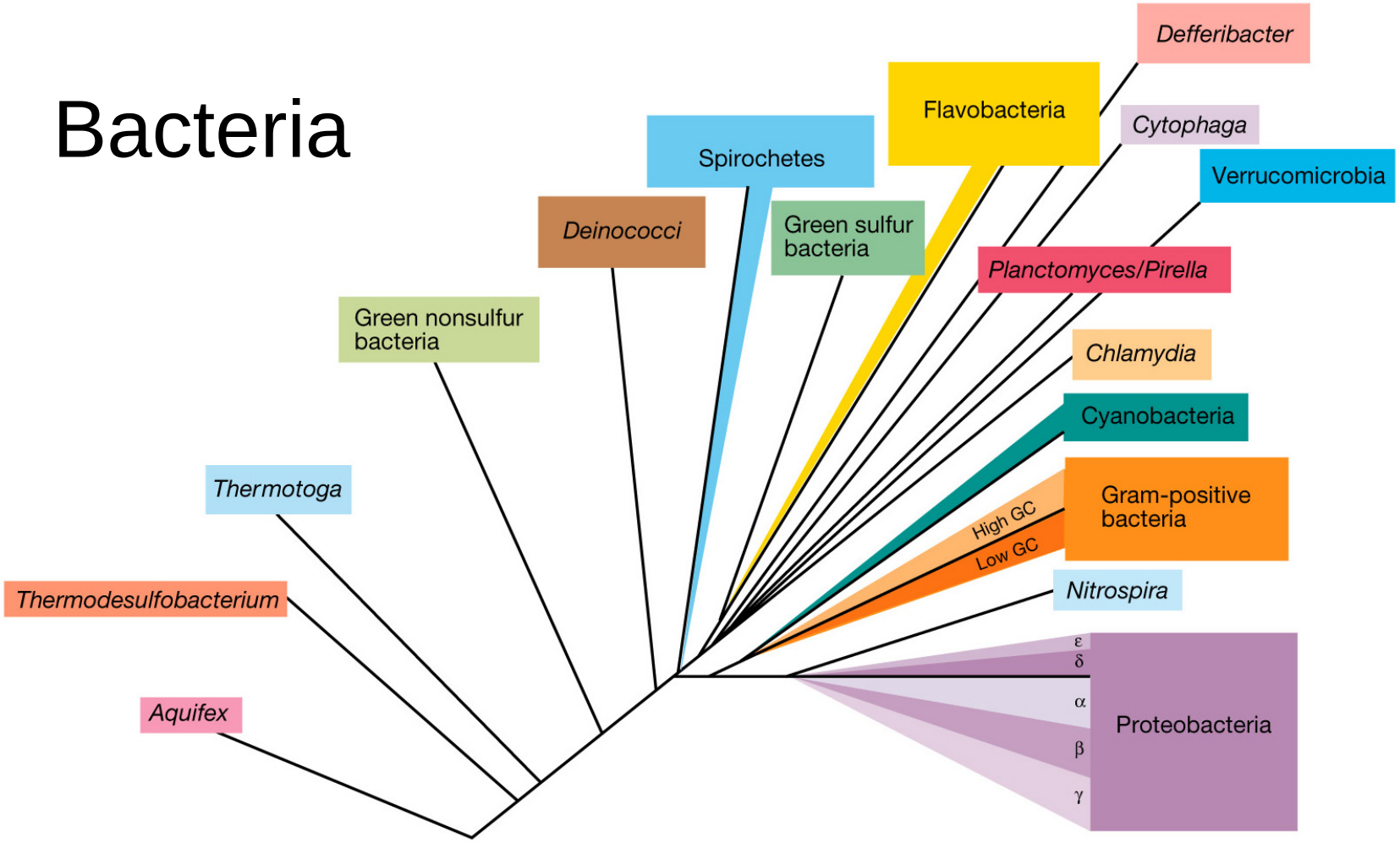
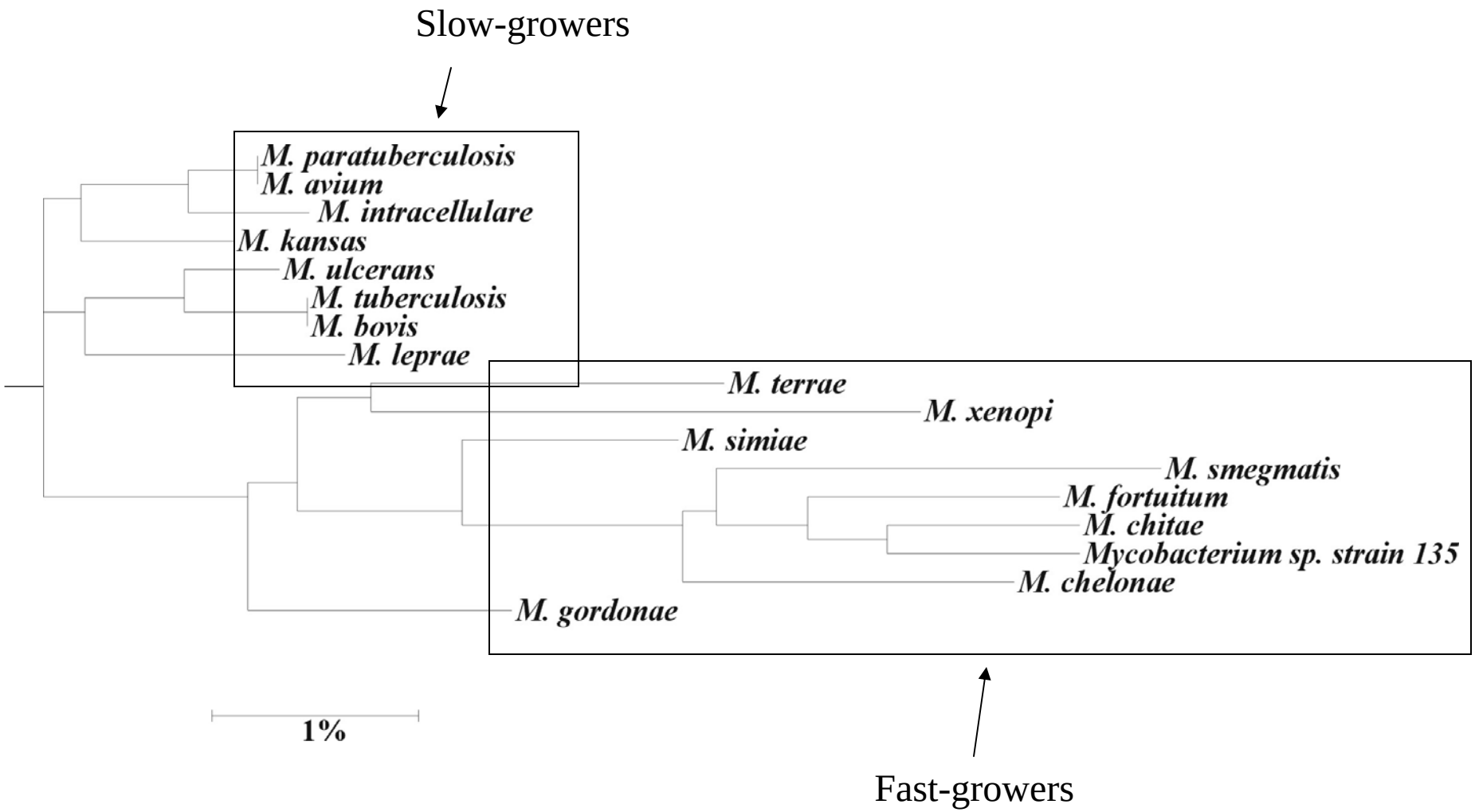
Bacteria



Mycobacterium Phylogeny



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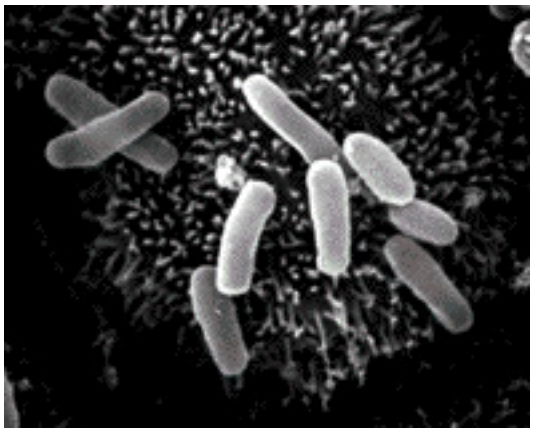
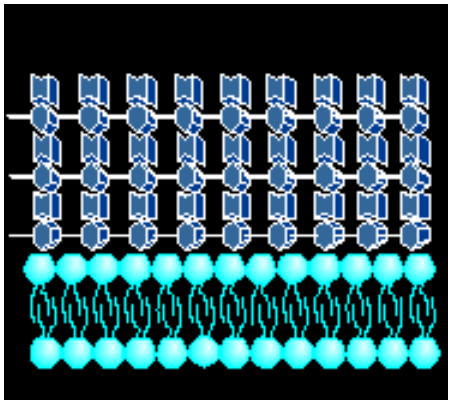
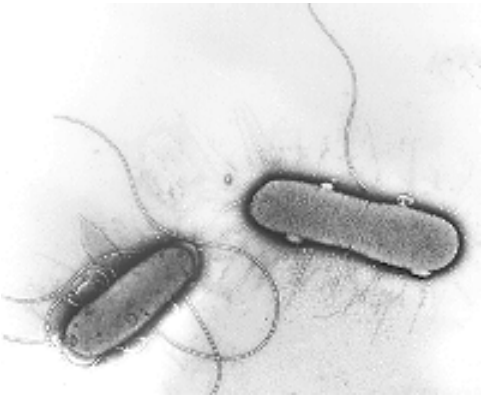
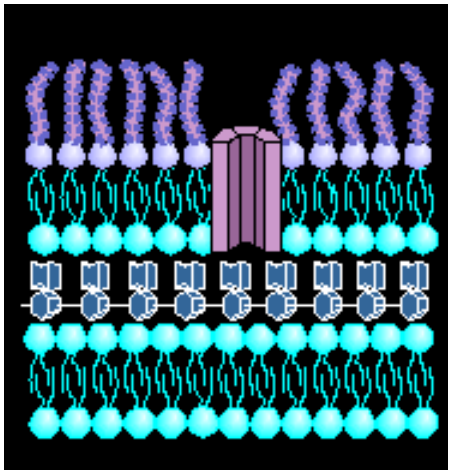
Gram positive



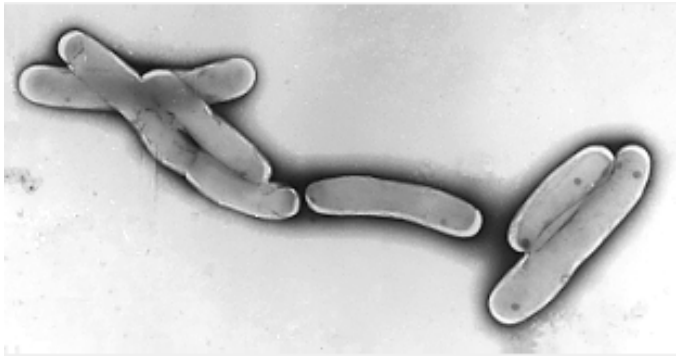


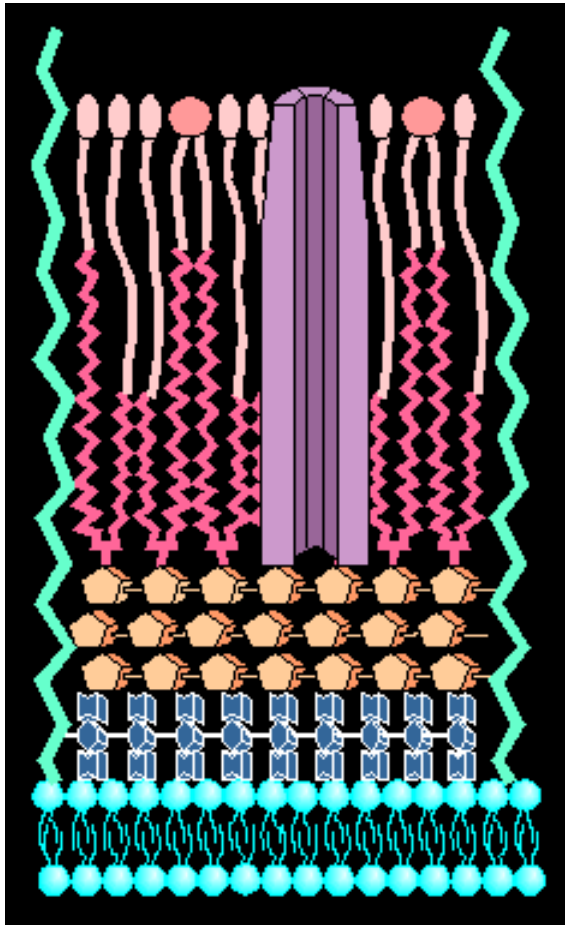
Gram negative





Mycobacterium





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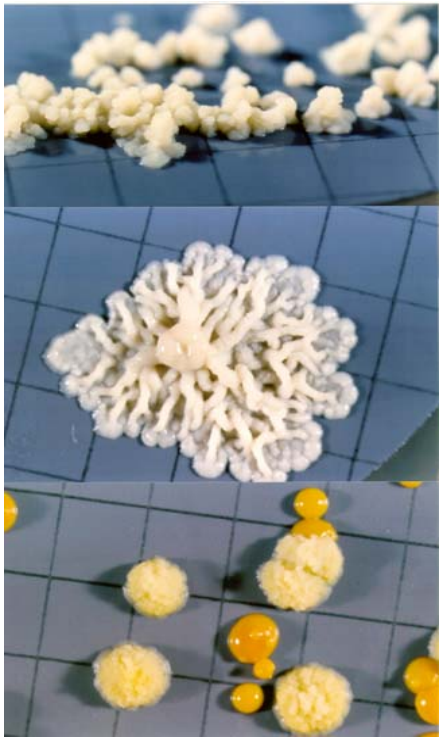
The *Mycobacterium avium* Complex (MAC)

- *Mycobacterium avium* **(MA)**
  - subspecies *avium*
  - subspecies *silvaticum*
  - subspecies *hominissuis* **(MAH)**
  - subspecies *paratuberculosis* **(MAP)**
- *Mycobacterium intracellulare* **(MI)**
- *Mycobacterium chimaera* **(MC)**
- possibly others

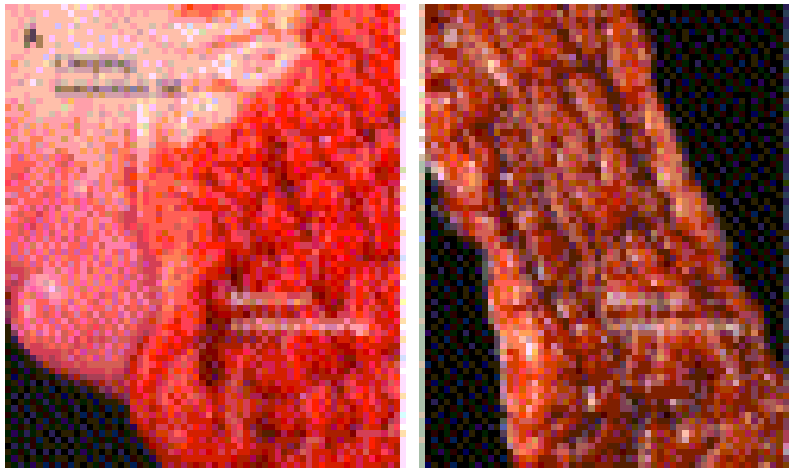


Clinical Significance of MAC infections in humans

Immuno-compromised	Disseminated disease
Children	Cervical lymphadenitis
Persons with pre-existing lung disease	Pulmonary disease
Persons heterozygous for CTFR gene (cystic fibrosis trans-membrane conductance regulator) α-1-antitrypsin gene	Pulmonary disease
Slender elderly women	Lady Windemere's disease

Mycobacterium avium subspecies *paratuberculosis* (MAP)

- **Etiologic agent of Johne's disease in cattle**
- **Associated with Crohn's disease in humans**

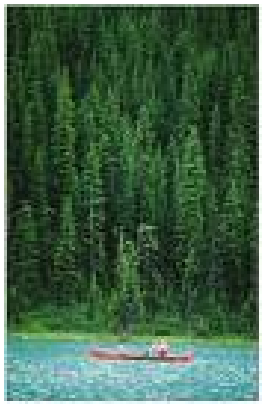


Mucosal cobblestoning in the intestine

Photos courtesy AJ Greenstein, Mount Sinai School of Medicine
AJ Cooley, University of Wisconsin

Ecology of mycobacteria

- Natural environments
  - Water
  - Aerosols
  - Boreal forest soils and peats
  - Acidic brown water swamps



- Man-made environments
  - Drinking water distribution systems (water and biofilm)
  - Building, hospital, household plumbing and aerosols
  - Hot tubs and spas
  - Potting soils
  - Metal working fluids

See JO Falkinham, III (2009) Surrounded by mycobacteria: nontuberculous mycobacteria in the human environment. J Appl Microbiol.

Culture: the traditional method for measuring occurrence of MAC in water

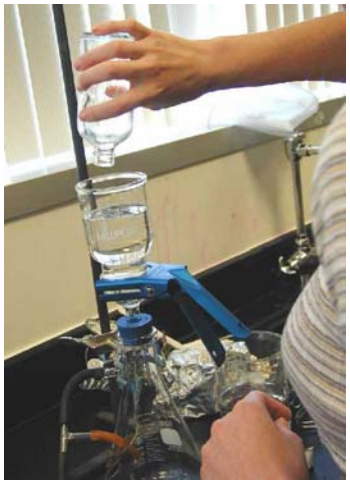
Covert et al., 1999, Appl Environ Microbiol, 65:2492-96.

1 L

500 ml **500 ml**

+ 0.04% CPC

30 min room temp



Membrane filtration

Middlebrook 7H10/ mycobactinJ
Incubate 37°C, 10% CO_2
Minimum 8 weeks

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Tap water (500 ml)

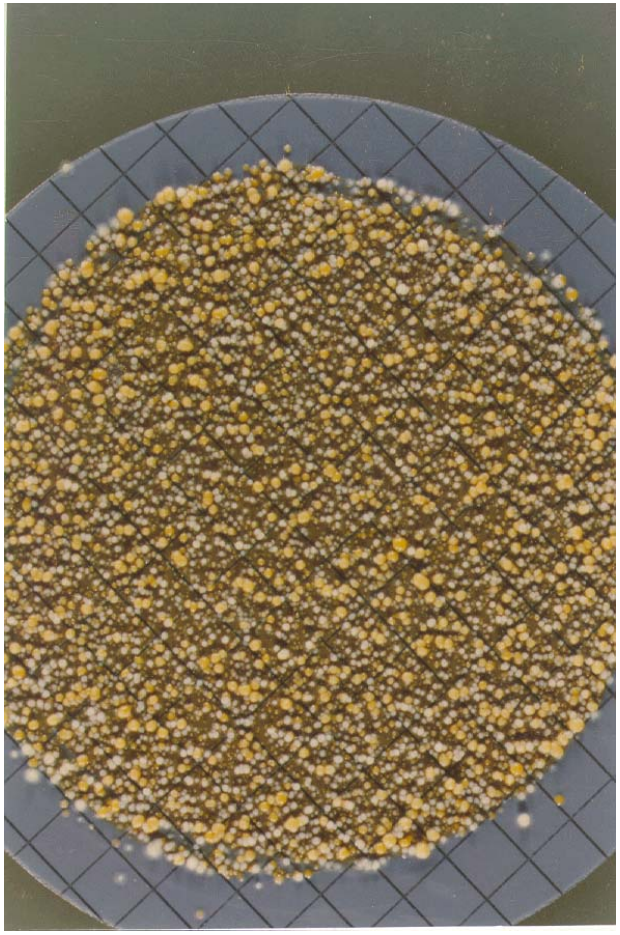

photo courtesy Tim Aronson

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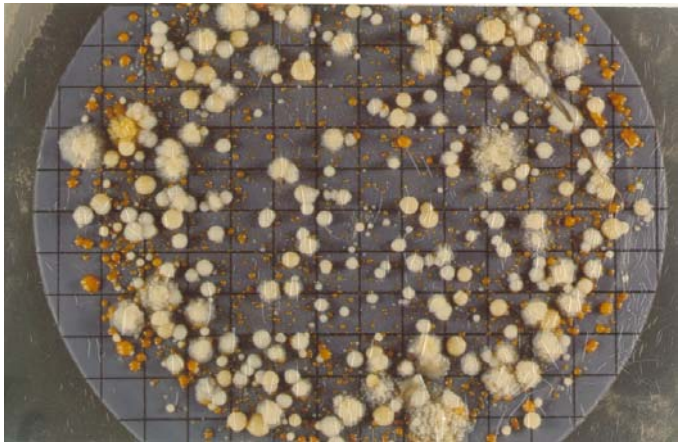
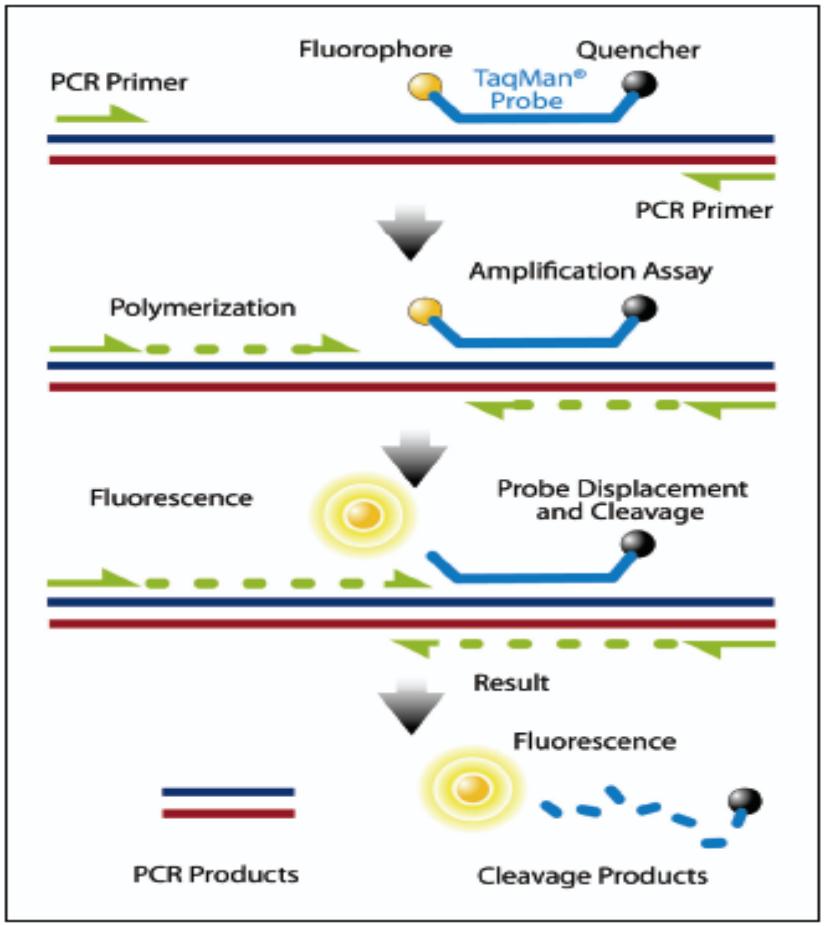
Advantages and limitations of culture



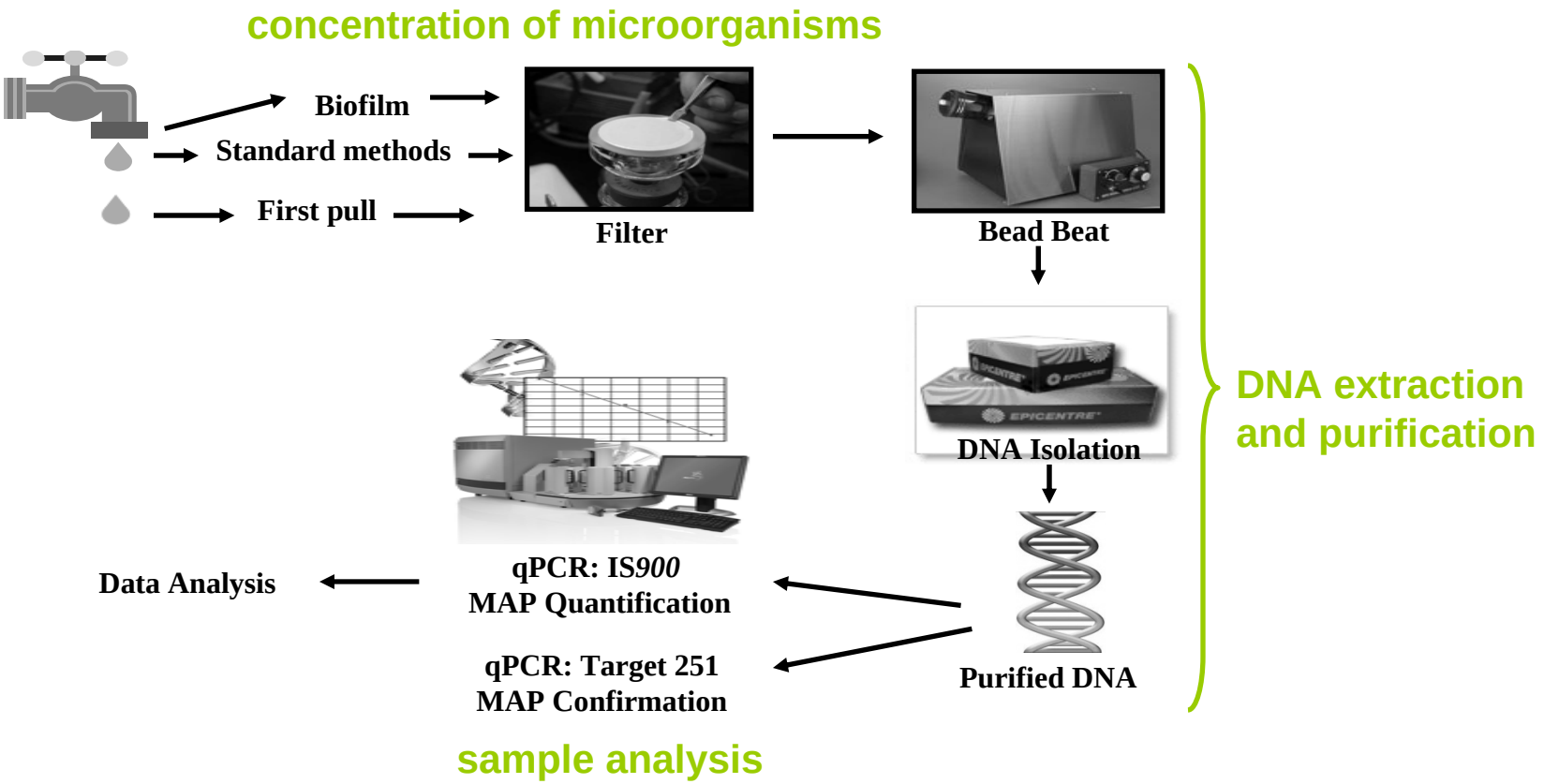
- Live-only detection
- Obtain isolates for further study
- Get a picture of the microbiological water quality of the sample
- Very long time to results
  - 8 weeks minimum to culture MA and MI
  - 16 weeks to years to culture MAP (only one isolate exists from DW in the US)
- Culture medium non-selective, isolates need further identification
- Loss of samples due to overgrowth
- Inability to recover target due to non-culturable MAC
- Inability to recover target due to CPC treatment (estimated 30 – 40% recovery of MAH)

Quantitative PCR (qPCR) for detection/quantification of microorganisms

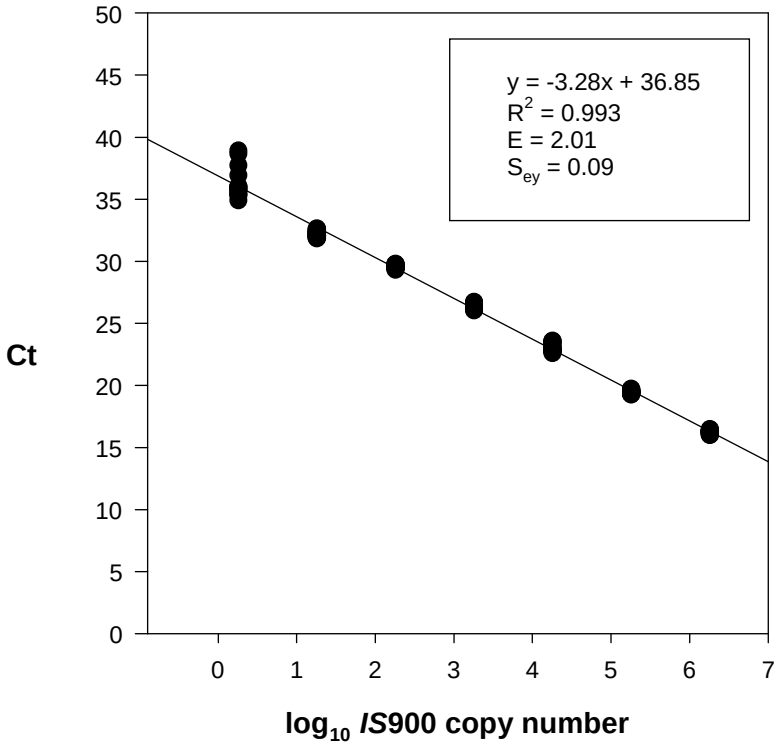
TaqMan® chemistry: fluorescence detection



Method for detection/quantification of MAP in water and biofilm samples

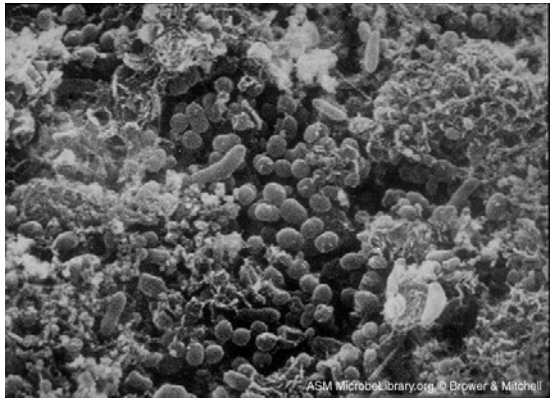


Master standard curve
(MAP genomic DNA)



MAP qPCR assay characteristics and features

- Two targets (IS*900* and Target 251)
- Commercial reagents (Applied BioSystems)
  - Environmental Master Mix 2.0
  - Exogenous internal positive control



Organism	Gene target	LOD‡	LOQ†	Sensitivity§
MAP	IS*900*	1.8 copies/assay	1.8 copies/assay	100 copies/L
MAP	Target 251	18 copies/assay	18 copies/assay	ND

‡ Lowest copy number giving CT < 40.0 in 6/6 independent assays.
† Lowest copy number/assay yielding a coefficient of variation < 25%.
§ Lowest copy number detected 100% of the time when spiking serial dilutions of known cell quantities into 1L sterile drinking water.

DW and biofilm sample collection study design

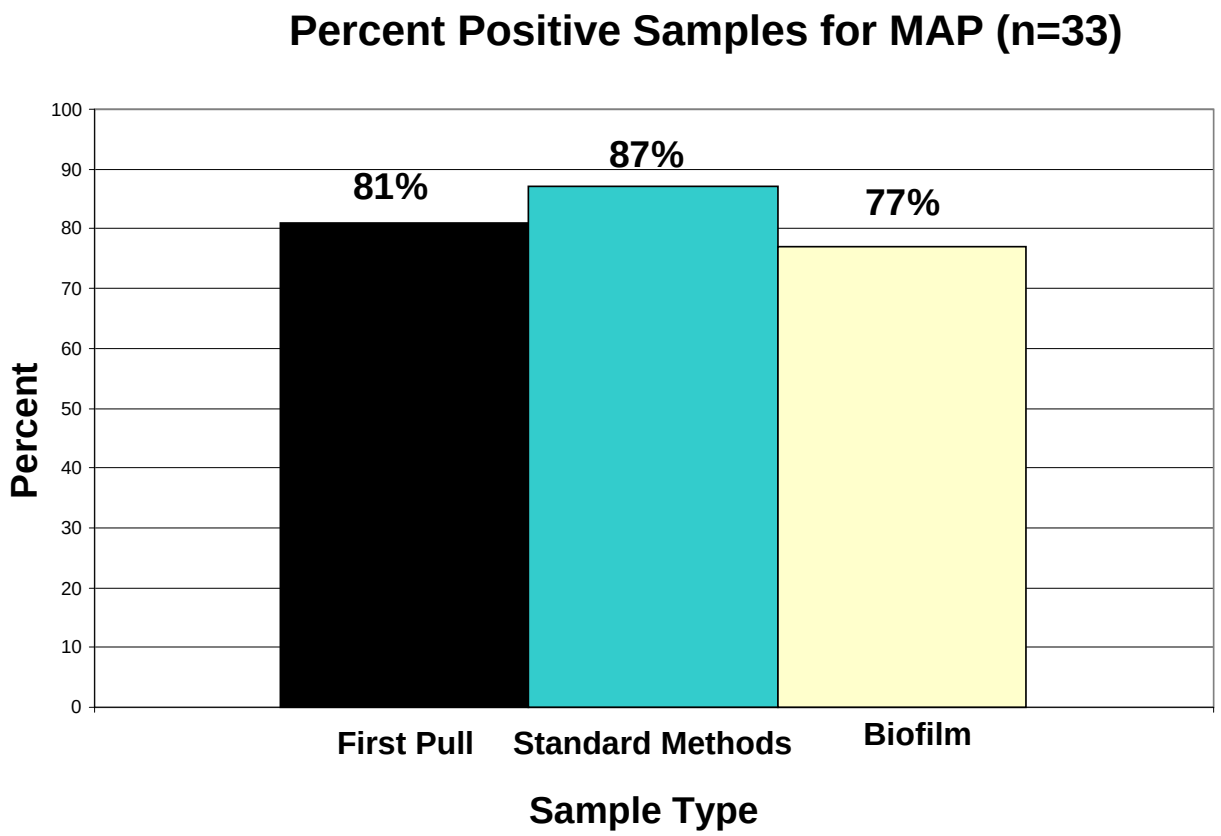
MIDWEST STUDY (two utilities)

- 33 DW and biofilm samples spring-summer, 2007
- Temporal= two homes sampled once a month for 4 months, Jan-April, 2007
- 10 water and biofilm samples spring, 2009

NATIONAL STUDY (34 utilities)

- 179 DW samples collected from geographically dispersed sites winter-summer, 2009

IS*900* detection
DW and biofilm samples
Midwest, 2007

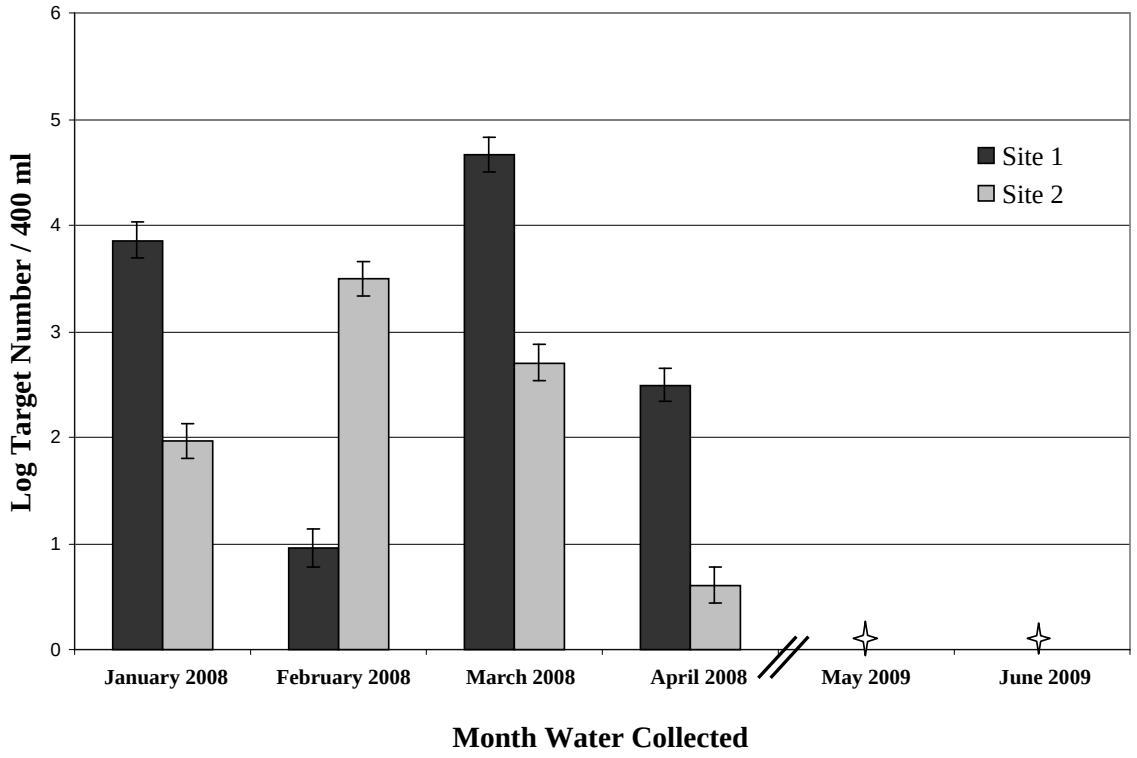


IS*900* detection
DW and biofilm samples
Midwest, 2007

IS*900* target copy number per 400 ml drinking water or biofilm

	1st Pull n = 33	"Standard Methods" n = 33	Biofilm n = 33
Range	0-262 +/- 16	0-446 +/- 25	0-3790 +/- 18
Mean	32 +/- 2	54 +/- 3	140 +/- 8
Standard Deviation	67 +/- 4	95 +/- 5	701 +/- 40

Results: 4-month temporal study Midwest, 2007



IS*900* detection
National study
winter – summer, 2009

- 0/179 DW samples positive for MAP! (IS*900* and Target 251)

- 4/179 DW samples positive for IS*900* but negative for Target 251

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IS*900* detection DW and biofilm samples Midwest, spring 2009

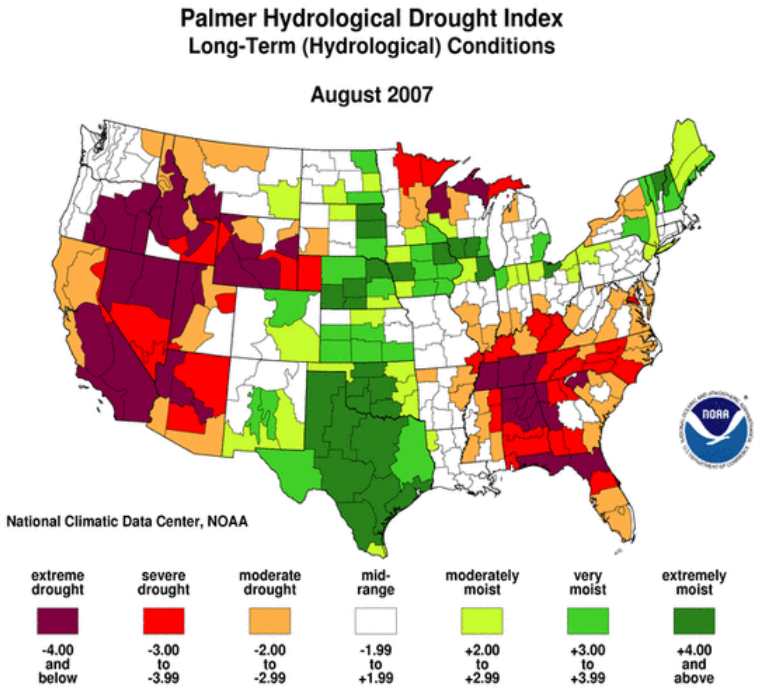
- 0/10 DW or biofilm samples positive for MAP (both assays)
- 5/10 sites were sampled previously in 2007 and were positive for MAP

Conclusions

- 1st report of detection of MAP in DW or biofilm in the US using non-culture methods
- Majority of samples from Midwest positive in 2007 (>80%), negative in 2009, all National samples negative in 2009
- Similar data obtained from localized vs. national survey for MAC organisms using culture method
- Temporal study suggests occurrence at same site is variable over time
- Cause of variability in MAP occurrence is unknown

Conclusions, continued

- Midwest experienced moderate to severe drought in 2007 but not in 2009
- 3/4 National sites positive for IS*900* in 2009 were also experiencing severe drought



http://www.ncdc.noaa.gov/sotc/index.php?report=drought&year=2007&month=ann#regdrot

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Advantages and limitations of qPCR

- Rapid, sensitive, specific
- First report of occurrence of MAP in DW and biofilm
- Do not know if MAP is alive
- Do not know if MAP is infections (or infectious dose)
- No isolates for further characterization

*USE BOTH CULTURE AND qPCR

The two together are greater than the sum of their parts

Next steps …

- Determine factors affecting geographical and temporal differences in MAP occurrence
- Evaluate the use of liquid culture for isolation of MAP from drinking water and biofilm samples
- Develop genotyping methods that do not rely on culture and provide specific information about MAP in an environmental sample (sheep type vs. cow type)
- Epidemiologic investigations into clusters of Crohn's and human health characteristics (genetic susceptibility , behaviors) that put individuals at greater risk of infection

Acknowledgments

- Amy Beumer
- Dawn King
- Terry Covert
- Jatin Mistry
- Maura Donohue