The Biology of Anaerobic Bacteria and Predominant Propagation Practices

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November 3, 2016
About ATCC

- Founded in 1925, ATCC is a non-profit organization with headquarters in Manassas, VA

- World’s premiere biological materials resource and standards development organization

- ATCC collaborates with and supports the scientific community with industry-standard biological products and innovative solutions

- Strong team of 400+ employees; over one third with advanced degrees
Outline

1. ATCC’s anaerobe collection
2. Classification of anaerobes and specific examples
3. Nutritional and atmospheric considerations
4. Propagation methods
ATCC’s anaerobe collection

942 active anaerobe holdings
- 465 are type strains

VPI Anaerobe Laboratory
- W.E.C. Moore, Lillian V. Holdeman-Moore, Tracy Wilkins, Elizabeth P. Cato
- Anaerobe Laboratory Manual
Top 5 anaerobes at ATCC

- *Clostridium sporogenes* (ATCC® 11437™)
- *Clostridium sporogenes* (ATCC® 19404™)
- *Clostridium perfringens* (ATCC® 13124™)
- *Bacteroides fragilis* (ATCC® 25285™)
- *Porphyromonas gingivalis* (ATCC® 33277™)
Classification of anaerobes

- Facultative
- Aerotolerant
- Strict
  - Environmental
  - Methanogens
Facultative anaerobes

- Can grow with or without the presence of oxygen
- Can metabolize energy aerobically (respiration) or anaerobically (fermentation)
- Ex: *Propionibacterium acnes* (ATCC® 6919™)
  - Isolated from facial acne
  - Has variable aerotolerance
  - Nutritional requirements:
    - All strains require Vitamin B5
    - Thiamine, biotin, and nicotinamide are stimulatory

Image of *Propionibacterium acnes* courtesy of the CDC
Aerotolerant anaerobes

- Not inhibited by O₂, but does not use O₂ to generate ATP
- Uses fermentation to generate ATP

- Ex: *Clostridium tertium* (ATCC® 14573™)
  - Originally isolated in 1917 by Captain Henry from war wounds
  - Type strain
  - Will grow slightly on an aerobic blood plate

Images of *Clostridium tertium* courtesy of the CDC
Strict anaerobes

- Grows only in the absence of O$_2$ and may be inhibited or killed by O$_2$
- Generates ATP through anaerobic respiration or fermentation
- Ex: *Fusobacterium nucleatum* subsp. *nucleatum* (ATCC® 25586™)
  - Isolated from a Cervico-facial lesion
  - Obligate anaerobic, Gram-negative rods with pointed ends
  - Most strains produce H$_2$S
  - All *Fusobacterium* species produce butyric acid
  - Require a rich medium for growth
- Ex: *Clostridium sporogenes* (ATCC® 19404™)
  - Isolated from gas gangrene
  - Genetically similar to *C. botulinum*
Environmental anaerobes

- Includes extremophiles
- Selective media are often specific to genus or even species
- May require a gas mixture free of CO₂
- Ex: *Thermoanaerobacterium thermosaccharolyticum* (ATCC® 7956™)
  - Requires a fermentable carbohydrate to grow
  - Type strain
  - Obligate anaerobe
  - Grows at 45°C - 60°C
Methanogens

- Oregon Collection of Methanogens (OCM)
- Very strictly anaerobic with methane always being the product of catabolic metabolism
- Ex: *Methanobrevibacter wolinnii* (ATCC® BAA-1170™)
  - Isolated from sheep feces
  - Atmospheric requirements:
    - Media has a redox potential below -110 mV
    - Large amount of headspace and pressure
  - Nutritional requirements:
    - Nitrogen source (ammonia or N₂)
    - One or more B vitamins
    - Requires acetate and/or trypticase or yeast extract
    - Growth inhibited by bile salts
Media

- PRAS commercial media is superior
  - Boiled free of molecular oxygen
  - Autoclaved, dispensed, and packaged anaerobically
  - Light-proof packaging
- Indicator - Rezazurin
  - Monitors redox potential of media
  - Non-toxic to bacteria and effective at low concentrations
  - Becomes colorless at a redox potential below -110 mV, remains pink above -51 mV
Common agar media

- Bases for blood agar media:
  - Brucella
  - Columbia-based blood agar
  - Tryptic Soy-based blood agar
  - Brain heart infusion w/ 0.5% yeast extract

- Supplements to enhance growth:
  - 5% sheep, horse, or rabbit blood
  - Vitamin K1 (1 µg/mL)
  - Hemin (5 µg/mL)

- Selective agars are commercially available
  - Bacteroides Bile Esculin agar (BBE)
Common broth media

- Chopped meat
- Reinforced clostridial
- Peptone yeast extract broth with glucose (PYG)
- Supplemented tryptic soy
  - ATCC Medium 2722
    - Additions of Yeast extract, hemin, and vitamin K1
- Broth media vessels
  - Hungate tubes
  - Balch tubes
Inhibitors

- Inhibitors that affect the quality of media
  - Oxygen
  - Light
  - Moisture/dehydration

- Inhibitors that affect cell growth
  - Bile
  - Reducing agents
  - Oxygen/incorrect gas mixture
  - Failure to add supplements
Reducing agents

- Reduces the redox potential of growth media
  - The reducing agent is oxidized by the oxygen in the media and therefore the media is reduced of the oxygen

- Ex: Coenzyme, cysteine, sodium sulfide
  - Select the reducing agent that is already used in the media formulation
  - Typically use 2 mL reducing agent per 100 mL media
  - Incubation at 37°C may speed up the process
  - Allow reducing agent to react for at least 1 hour, preferably overnight
Oxygen toxicity

Defenses
- Hemoglobin
- Superoxide dismutase
- Catalase
- Peroxidase
- Vitamin C
- Vitamin E
- Uric Acid

Damage
- Oxidation of membrane lipids
- Inactivation of enzymes
- Genetic damage
Why is oxygen toxic?

\[ \text{O}_2 + \text{Energy} \rightarrow \text{O}_2 \text{ Singlet State} \]

\[ \text{O}_2 + e^- \rightarrow \text{O}_2^- \text{ Superoxide Anion} \]
Why is oxygen toxic?

\[
\begin{align*}
\text{O}_2^- + e^- + 2H^+ & \rightarrow \text{H}_2\text{O}_2 \\
& \text{Superoxide Anion} \quad \text{Hydrogen peroxide} \\
\text{H}_2\text{O}_2 + e^- + H^+ & \rightarrow \text{H}_2\text{O} + \text{OH}^- \\
& \text{Hydrogen peroxide} \quad \text{Hydroperoxy radical} \\
\text{OH}^- + e^- + H^+ & \rightarrow \text{H}_2\text{O} \\
& \text{Hydroperoxy radical} \quad \text{Water}
\end{align*}
\]
Gas mixtures

- 100% Nitrogen
- 97% $\text{N}_2$ - 3% $\text{H}_2$
- 80% $\text{N}_2$ - 10% $\text{H}_2$ - 10% $\text{CO}_2$
- 80% $\text{N}_2$ - 20% $\text{CO}_2$
- 80% $\text{H}_2$ - 20% $\text{CO}_2$
- 100% Methane
Propagation methods: The roll tube

Image of anaerobic roll tubes courtesy of CEBTech Services. Please note that proper personal protective equipment is recommended.
Propagation methods: The roll tube

Image of *Clostridium perfringens* on a roll tube courtesy of LeBeau, MicrobeWorld
Propagation methods: The cannula system
Propagation methods: Syringe, needle, and oxygen-free gas
Propagation methods: Jars and catalysts
Propagation methods: Jars and catalysts
Propagation methods: Anoxomat™
Propagation methods: Anaerobe chambers
Propagation methods: Anaerobe chambers
2. Cox M. How to Isolate and Maintain Anaerobic Bacteria? ESCMID eLibrary, available online: https://www.escmid.org/escmid_publications/escmid_elibrary/?q=+mike+cox&id=2173&L=0&x=0&y=0
17. Miller TL, Lin C. Description of Methanobrevibacter gottschalkii sp. nov., Methanobrevibacter thaueri sp. nov., Methanobrevibacter woesei sp. nov. and Methanobrevibacter wolinii sp. nov. IJSEM 52: 819-822, 2002.
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Whitley MG500 Anaerobe Chamber is a product of Don Whitley Scientific. Bugbox Plus Anaerobe Chamber is a product of Baker. AS-580 Anaerobic Chamber and PRAS media are products of Anaerobe Systems.
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View our listing of anaerobic bacteria at [www.atcc.org/A Anaerobes](http://www.atcc.org/A Anaerobes)

Browse our related nucleic acid products at [www.atcc.org/GenuineNucleics](http://www.atcc.org/GenuineNucleics)

Please email additional questions to: tech@atcc.org