ATYPICAL MYCOBACTERIA
Y Mycobacteria other than tubercle and leprosy bacilli that exists as saprophytes of soil & water.
Y cause opportunistic disease in man, called atypical, environmental, opportunistic, tuberculoid Mycobacteria (MOTT).
Y acid-fast & alcohol fast.
Y morphology - long & even filamentous.
Y can grow at 27°C & 37°C.
Y M. xenopi, M. phlei, M. smegmatis grow at 44°C.
Y some are rapid growers, produce visible growth on LJ within one week.
✓ Some produce bright yellow or orange pigment.
✓ Resistant to antitubercular drugs, like streptomycin, INH, PAS
✓ Sensitive to rifampicin.
✓ Niacin - negative
✓ Neutral red - negative.
✓ Produce enzyme arylsulphatase.
✓ Non pathogenic for guinea-pig but pathogenic for mouse.
**CLASSIFICATION (Runyon, 1959)**

on the basis of pigment and rate of growth.

<table>
<thead>
<tr>
<th>Runyon group</th>
<th>Name</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Photochromogens</td>
<td><em>M. kansasii</em>, <em>M. marinum</em>,</td>
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<td></td>
<td><em>M. scrofulaceum</em></td>
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<td>Non-chromogens</td>
<td><em>M. avium</em>, <em>M. intra-cellulare</em>,</td>
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<td><em>M. xenopi</em></td>
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<tr>
<td>IV</td>
<td>Rapid growers</td>
<td><em>M. cheloni</em>, <em>M. fortuitum</em></td>
</tr>
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</table>
### CLASSIFICATION (Runyon, 1959)

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<tr>
<th>Gp</th>
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<td>- Colonies develop pigment following exposure to light</td>
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<td>- Growth in more than 7 days on solid media</td>
</tr>
<tr>
<td>II</td>
<td>Scotochromogens</td>
<td>- Colonies develop pigment in the dark or light</td>
</tr>
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<td></td>
<td></td>
<td>- Growth in more than 7 days on solid media</td>
</tr>
<tr>
<td>III</td>
<td>Non- chromogens</td>
<td>- Colonies are non pigmented irrespective of exposure to dark or light</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Growth in more than 7 days on solid media</td>
</tr>
<tr>
<td>IV</td>
<td>Rapid growers</td>
<td>- Colonies appear in less than 7 days on solid media</td>
</tr>
</tbody>
</table>
Photochromogens

• form colorless colonies when incubated in dark
• but when young colonies are exposed to air for 1 hr. and re-incubated for 24-48 hrs., development of bright yellow or orange colour.
• 3 important species – M. kansasii, M. marinum, M. simiae.
Photochromogens (cont'd)

✓ M. kansasii
- cause chronic pulmonary disease resembling tuberculosis.
- commonly isolated from pts. with pre-existing lung disease.
- grows well at 37 °C on LJ medium
- reduces nitrate to nitrite.
- bacilli are elongated & have a beaded appearance.
- usually sensitive to rifampicin & other anti-tuberculous drugs.
Photochromogenic
*Mycobacterium kansasii* on
*Middlebrook Agar*
Photochromogens (cont'd)

✓ *M. marinum*
  • grows poorly at 37 °C, but grows better at 33 °C.
  • causes superficial granulomatous skin disease (swimming pool granuloma).
  • failure to reduce nitrate to nitrite
  • failure to produce catalase.
✓ *M. simiae* – causes pulmonary diseases.
M. marinum
Scotochromogens

- form pigment in cultures incubated in the dark, though the intensity of colour may increase on exposure to light.

- *M. scrofulaceum* - causes scrofula (cervical lymphadenitis) in children.
  - Bacilli may be short, long or filamentous.
  - resistant to INH & sensitive to cycloserine & ethionamide
Scotochromogens (cont'd)

✓ M. gordonae often found in water
  ▪ common contaminant of clinical samples.
  ▪ rare cause of pulmonary disease.

✓ M. szulgai
  ▪ it is a scotochromogen when incubated at 37°C & photochromogen at 25°C.
  ▪ it occasionally cause pulmonary disease and bursitis.
Non-chromogens

✓ don't produce pigment even on exposure to light.
✓ *M. intracellulare* ; Battey bacillus & *M. avium* causes TB, can grow at 45° C
✓ Grouped together as MAC ( *M. avium* complex) or *M. avium* - *intracellulare* ( MAI).
✓ MAC-commonest opportunistic infections
✓ colonies are smooth, non-pigmented, easily emulsifiable.
✓ TB, lymphadenitis & disseminated disease in man.
Mycobacterium avium-intracellulare in Tissue Specimens

Low Magnification  High Magnification
M. avium-intracellulare Complex (MAC) Progression vs. CD4 Count in AIDS Patients
Non-chromogens (cont'd)

✓ *M. xenopi*: is a thermopile, grows well at $45^0\text{C}$,
  ▪ may cause pulmonary lesions
  ▪ most cases reported from South London.

✓ *M. ulcerans*: causative agent of Buruli ulcer.
  ▪ grows at $31-34^0\text{C}$ & not at $37^0\text{C}$.
  ▪ produces a powerful exotoxin.
Rapid Growers

✓ may be photo-, scoto- or non-chromogens.
✓ produce visible growth on LJ medium within 2-3 days.
✓ M. smegmatis - saprophytes
  - rough, white to buff coloured colonies.
  - since normally present in smegma, so frequent contaminant of urine sample.
  - are acid fast & not alcohol fast.
  - not seen in ZN stain if acid – alcohol is used as decolorizer.
✓ rarely causes skin, pulmonary, soft tissue, bone infections.
Rapid Growers (cont’d)

✓ M. chelonae & M. fortuitum.
- Both these rapid growers are human pathogens.
- Both are coccoid to filamentous in shape.
- M. chelonae grows better at 25 °C than at 37 °C.
- M. fortuitum reduces nitrate & assimilates iron from ferric ammonium citrate.
- Cause chronic abscess, pulmonary or disseminated disease.
DIAGNOSIS OF NTM

- Clinical signs and symptoms, Chest X-ray and Smear cannot differentiate NTM and TB infection
- Culture is the method to confirm diagnosis
- Disseminated diseases are common in immunocompromised host especially in HIV patients
- Blood culture for mycobacteria is useful for disseminated disease

- As NTM are present in the environment, so clinical significance of isolates is considered when:
  - Recovered from multiple specimens or sites
  - Recovered in large quantities
  - Recovered from a sterile site such as blood
SPECIMENS

- Should be directly from the lesion or organ concerned
- Avoid potential sources of contamination especially tap water
- Submit specimens without fixatives
- Collect samples in sterile, leak proof containers
- Limit antibiotic use during diagnostic evaluation of NTM disease
SPECIMEN PROCESSING

- DIGESTION AND DECONTAMINATION: of specimens from non sterile sites.
- SMEAR MICROSCOPY

Fluorochrome stain

Ziehl Neelsen staining
Most of NTM can grow on ordinary media for mycobacteria

<table>
<thead>
<tr>
<th>SOLID</th>
<th>LIQUID</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>• EGG BASED:</strong> Lowenstein Jensen L-J with iron</td>
<td>BACTEC 12B medium Middlebrook 7H9 broth Septi-check AFB Mycobacteria Growth Indicator Tube</td>
</tr>
<tr>
<td><strong>• AGAR BASED:</strong> Middlebrook 7H10</td>
<td></td>
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<tr>
<td></td>
<td>Middlebrook 7H11</td>
</tr>
<tr>
<td></td>
<td>Middlebrook biplate</td>
</tr>
</tbody>
</table>
• **Few NTM have special growth requirements:**
  - *M. haemophilum* - requires media enriched with iron containing compounds like ferric ammonium citrate, hemin or hemoglobin
  - *M. genavense* and *M. paratuberculosis* - need media enriched with mycobactin J
  - *M. ulcerans* - needs egg yolk supplementation

• **Incubation:**
  - Optimal temperature - between 28°C & 37°C
  - Exceptions:
    - *M. haemophilum* - 28°C to 30°C
    - *M. ulcerans* - 25°C to 33°C
    - *M. xenopi, MAC* - 45°C
IDENTIFICATION OF ISOLATES

- Phenotypic Characteristics:
  - Growth Rate
  - Pigment production
  - Biochemical tests
- HPLC
- Molecular methods
- Animal pathogenicity- mice
PIGMENT PRODUCTION

- Three LJ slants inoculated with organism
- Two slants completely shielded from light with cardboard tube or aluminum foil
- When growth detected in unshielded tube, growth examined in one shielded tube
- If colonies not pigmented tube exposed to light (100-W tungsten bulb for 2 hrs) with cap loosened (maximal oxygenation required for pigmented production)
- Tube is rewrapped and returned to the incubator. Examine after 24-48 hrs.
- Colonies compared for pigmentation with light-exposed tube to shielded tube not exposed to light
PHENOTYPIC CHARACTERISTICS

PHOTOCHROMOGENS

*M. marinum*  
*M. kansasii*
SCOTOCHROMOGENS

M. szulgai  M. gordonae  M. scrofulaceum
<table>
<thead>
<tr>
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<tbody>
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<td>intracellulare</td>
</tr>
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</table>

![Image of intracellular M. avium](image1)

![Image of M. avium](image2)

![Image of M. xenopi](image3)
RAPID GROWERS

M. abscessus

M. fortuitum