ATYPICAL MYCOBACTERIA

- ✓ Mycobacteria other than tubercle and leprosy bacilli that exists as saprophytes of soil & water .
- ✓ cause opportunist disease in man , called atypical , environmental ,opportunist, tuberculoid Mycobacteria (MOTT).
- ✓ acid – fast & alcohol fast.
- ✓ morphology – long & even filamentous.
- ✓ can grow at 27° C & 37 ° C.
- ✓ M. xenopi, M. phlei, M. smegmatis grow at 44 ° C.
- ✓ 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.

Runyon group	Name	Species
I	Photochromogens	*M. kansasii*, *M. marinum*,
II	Scotochromogens	*M. scrofulaceum* *M. gordonae*
III	Non- chromogens	*M. avium* *M. intra- cellulare*, *M. xenopi*
IV	Rapid growers	*M. cheloni*, *M. fortuitum*

CLASSIFICATION (Runyon, 1959)

Gp	Name	Characteristics
I	Photochromogens	- Colonies develop pigment following exposure to light - Growth in more than 7 days on solid media
II	Scotochromogens	- Colonies develop pigment in the dark or light - Growth in more than 7 days on solid media
III	Non- chromogens	- Colonies are non pigmented irrespective of exposure to dark or light - Growth in more than 7 days on solid media
IV	Rapid growers	- Colonies appear in less than 7 days on solid media

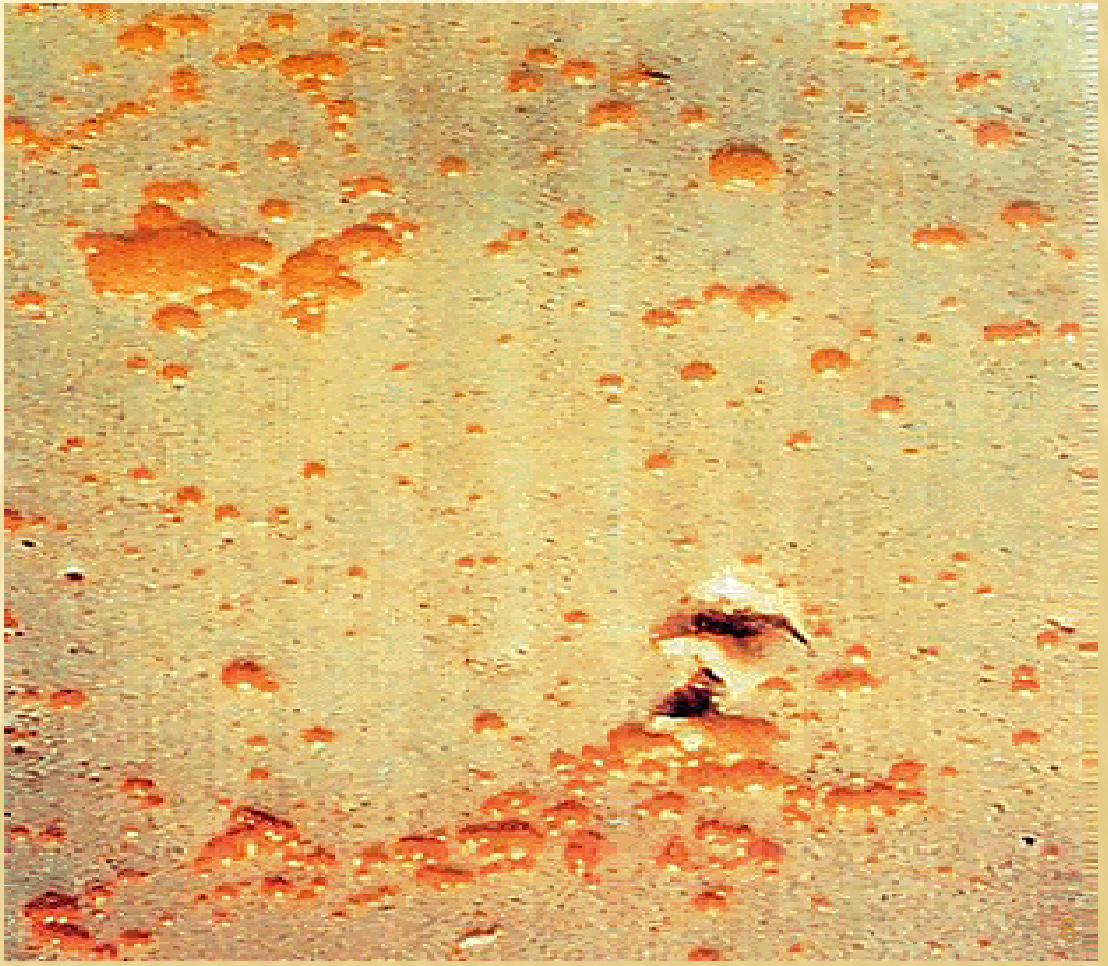
Photochromogens

- 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

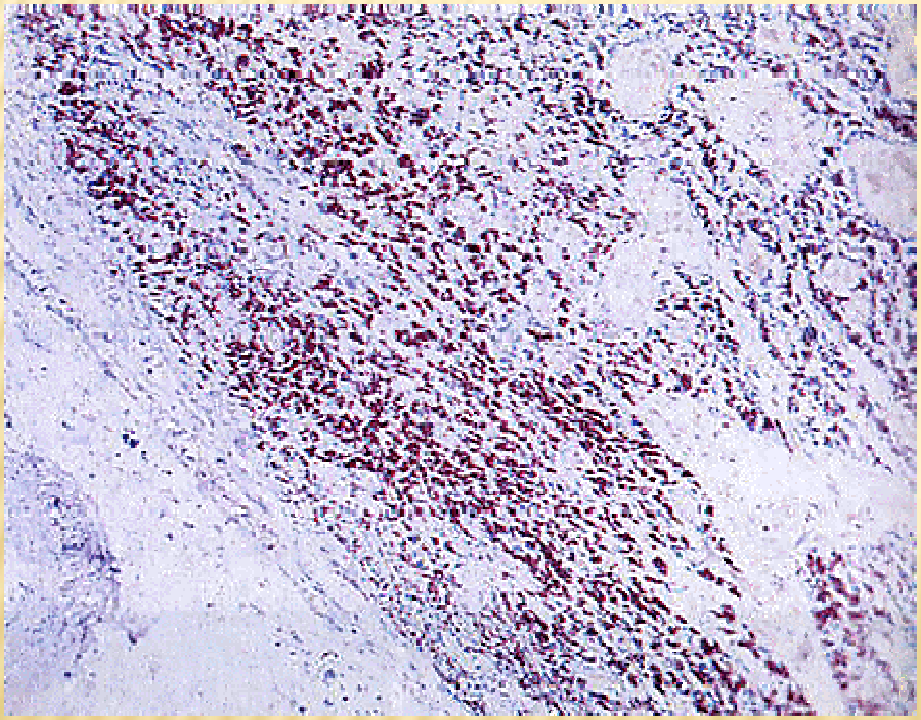
Scotochromogens(cont'd)

- ✓ 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 37^0 C & photochromogen at 25^0 C.
  - it occasionally cause pulmonary disease and bursitis.

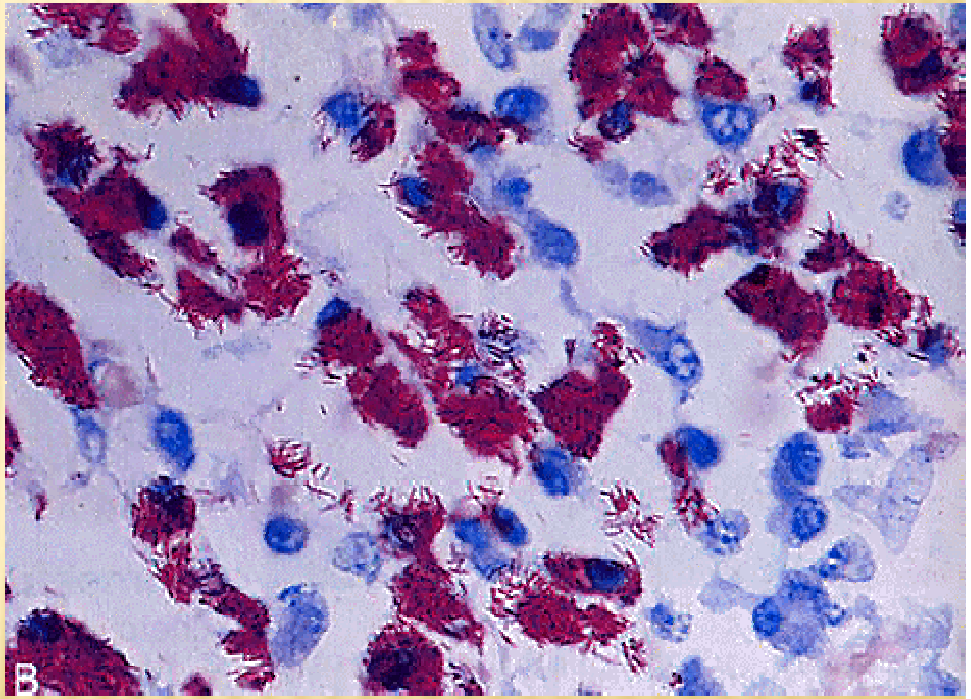
Non-chromogens

- ✓ 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

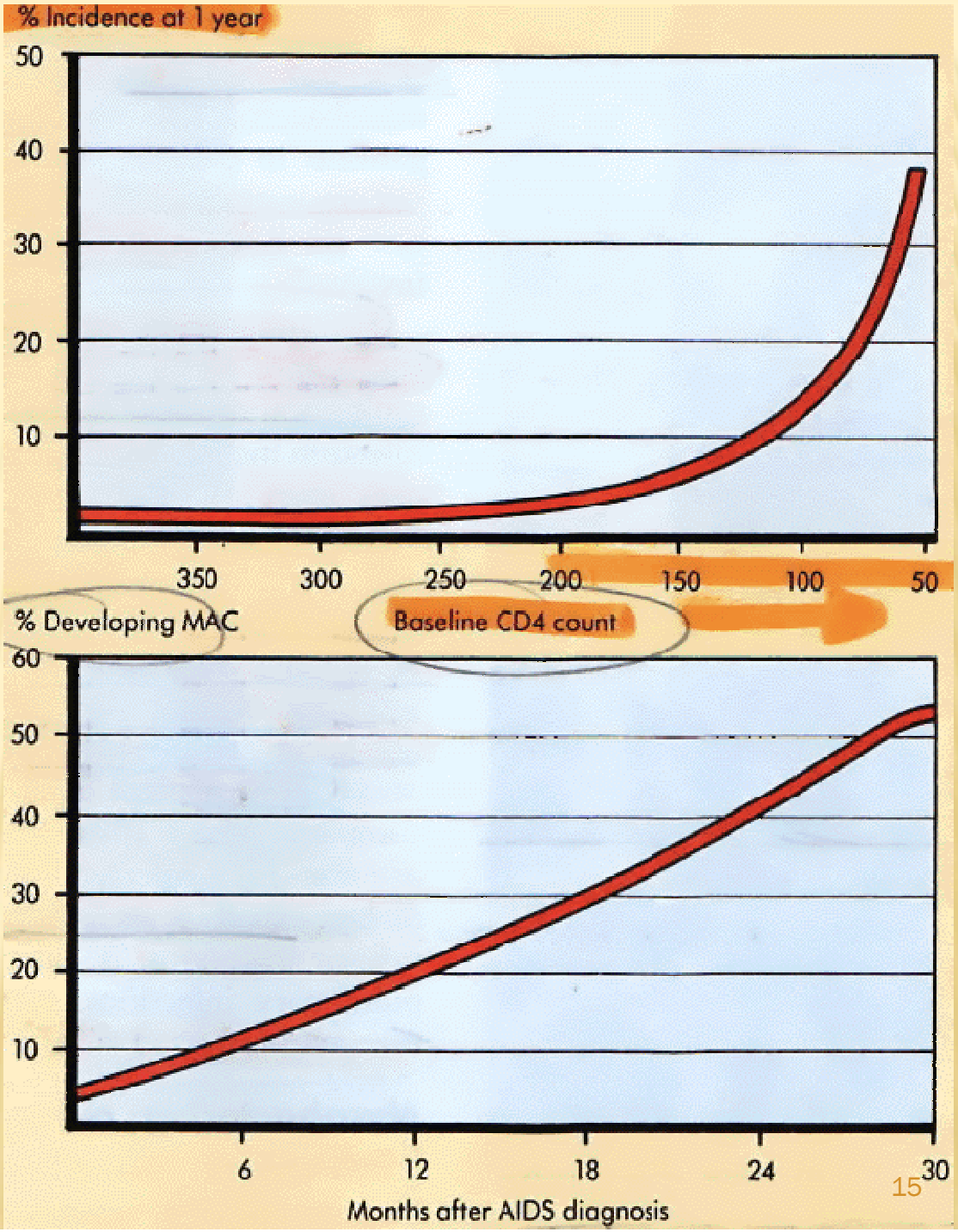


Low Magnification



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 C,
  - ▪may case pulmonary lesions
  - ▪most cases reported from South London.
- ✓ M. ulcerans: causative agent of Buruli ulcer.
- ▪ grows at 31-34 ^{0} C & not at 37 ^{0} 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 ^{0} C than at 37 ^{0} 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

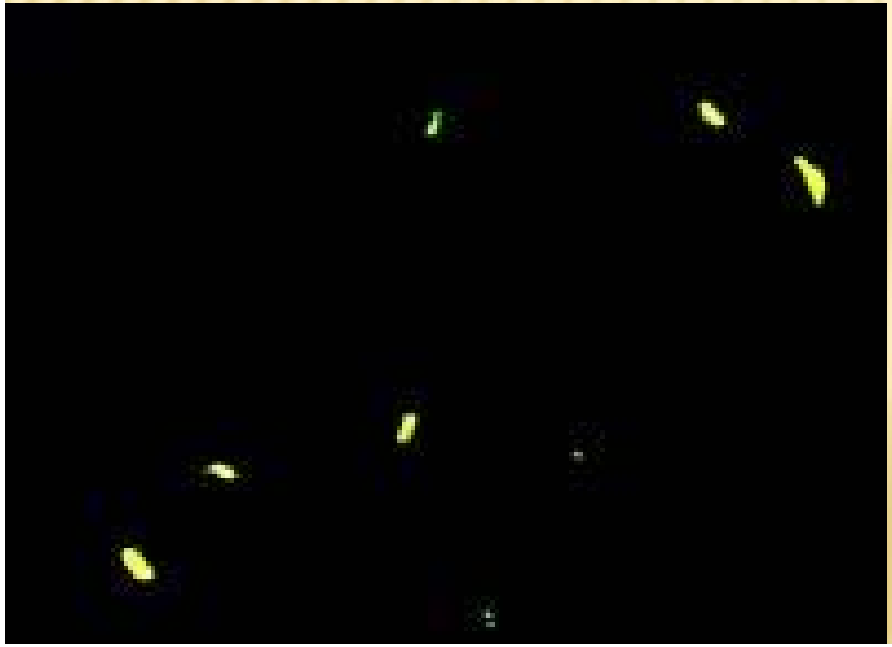
SPECIMENS

- 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

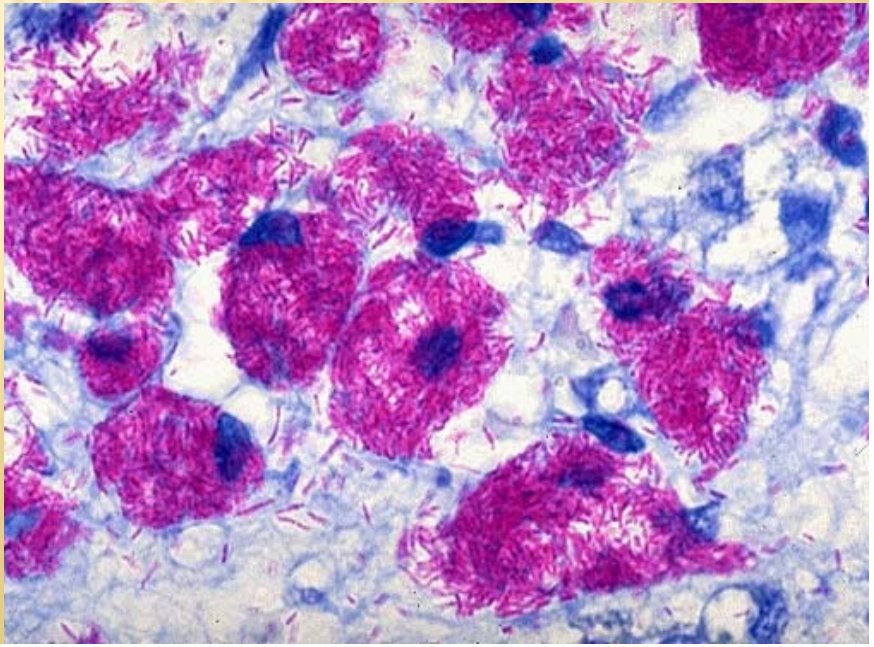
SPECIMEN PROCESSING

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

Fluorochrome stain



Ziehl Neelsen staining



CULTIVATION

- Most of NTM can grow on ordinary media for mycobacteria

SOLID	LIQUID
•EGG BASED: Lowenstein Jensen L-J with iron •AGAR BASED: Middlebrook 7H10 Middlebrook 7H11 Middlebrook biplate	BACTEC 12B medium Middlebrook 7H9 broth Septi-check AFB Mycobacteria Growth Indicator Tube

- **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

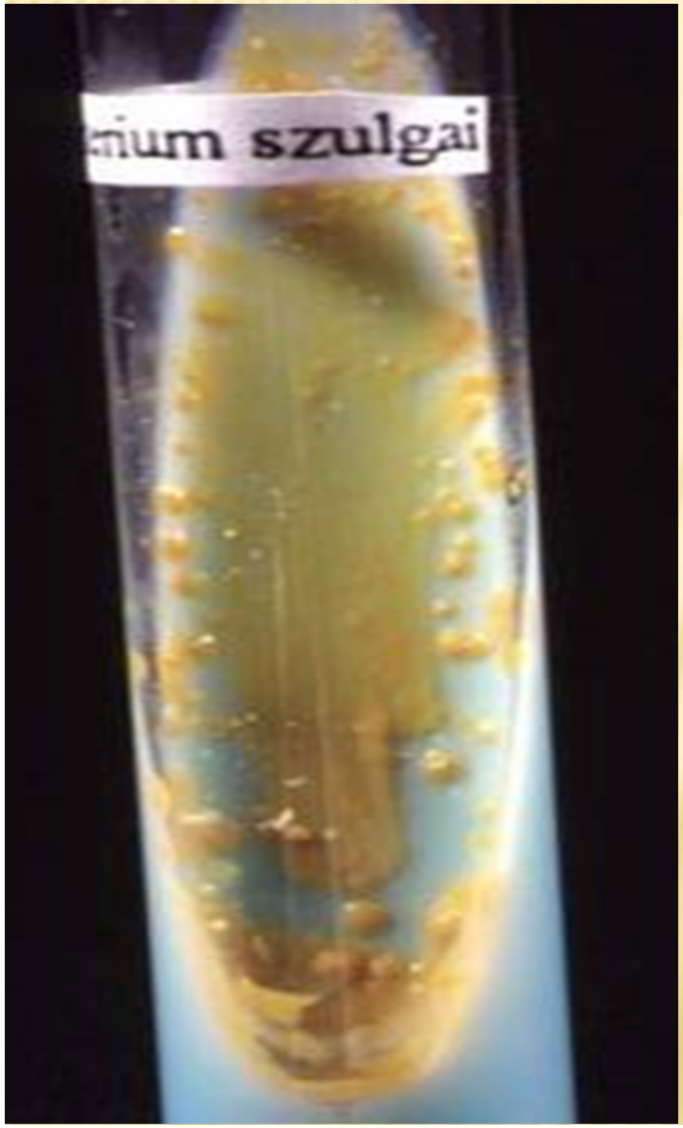
PHOTOCHROMOGENS

M. marinum

M. kansasii

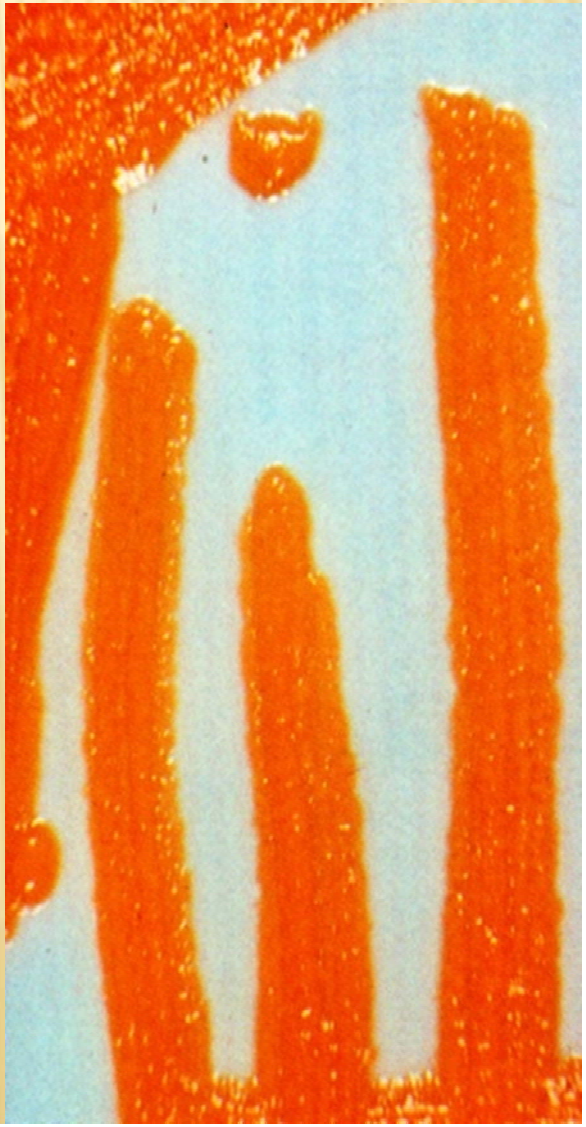
SCOTOCHROMOGENS

M. szulgai



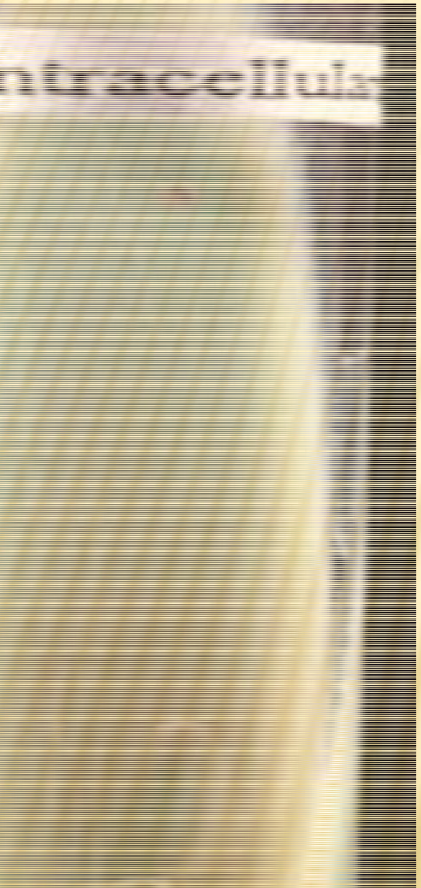
M. gordonae

M. scrofulaceum

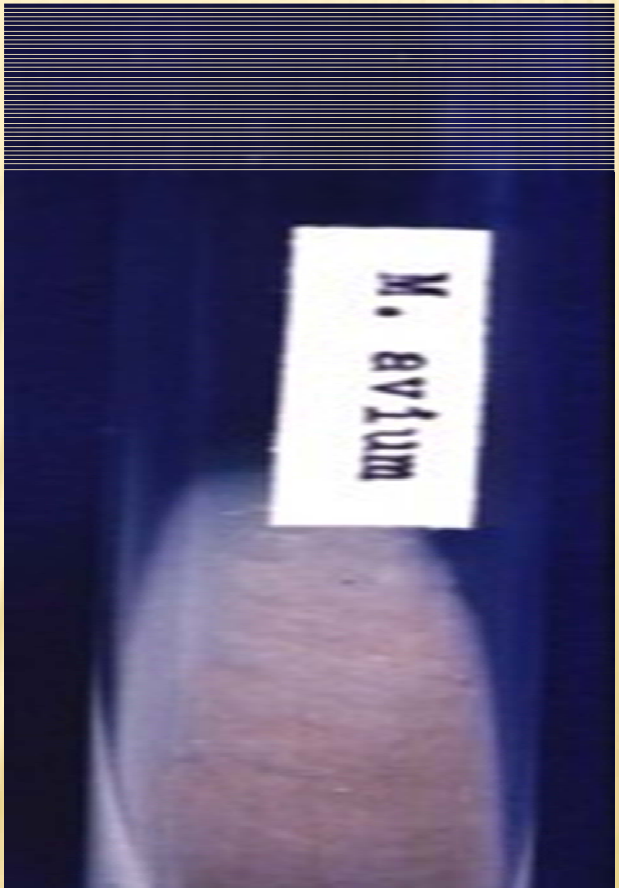


NON PHOTOCHROMOGENS

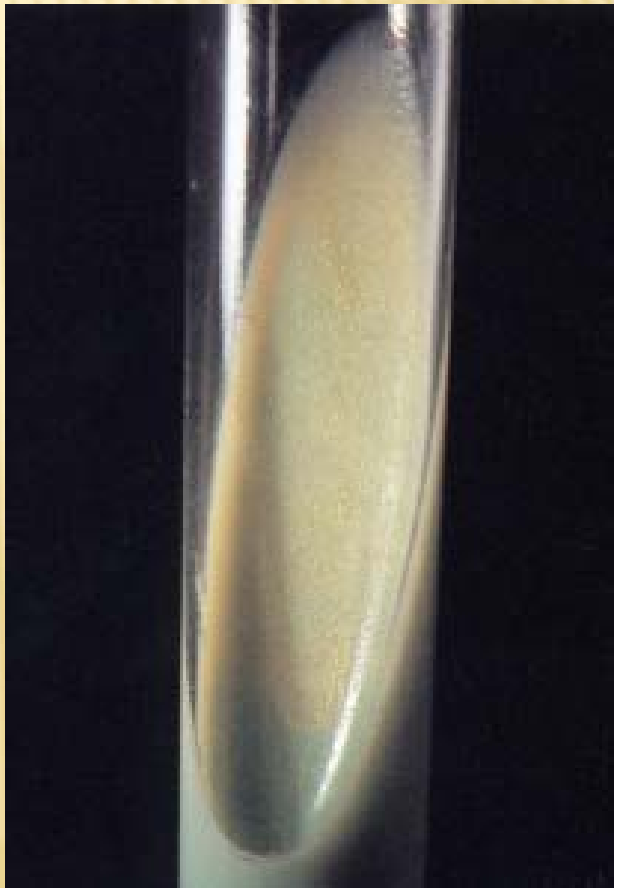
intracellulare



M. avium



M. xenopi



RAPID GROWERS

chelonae

M. abscessus

M. fortuitum