

Institute for microbiology shows:

TRACING THE CRIMINAL



Part Five:

Gram-Negative Criminals II

Survey of medically important G - rods



Story	Endo	Group
P04	grows	<i>Enterobacteriaceae</i> (GLC +, OXI -)
P04	grows	<i>Vibrionaceae</i> (GLC +, OXI +)
P04	not	<i>Campylobacter</i> and <i>Helicobacter</i>
3. + 4.	grows	G- non-fermenters (GLC -)
1. + 2.	not	<i>Pasteurellaceae</i>
P06	not	More G- rods, see next practical

Pseudomonas aeruginosa – a microbiological everGREEN 😊

textbookofbacteriology.net



Survey of topics

Clinical characteristics – *Pasteurellaceae*

Clinical characteristics – G– glucose non-fermenters

Diagnostics of *Pasteurellaceae*

Diagnostics of G– glucose non-fermenters

Pictures of *Pasteurellaceae* and glucose non-fermenters



Clinical characteristics – *Pasteurellaceae*

Story One



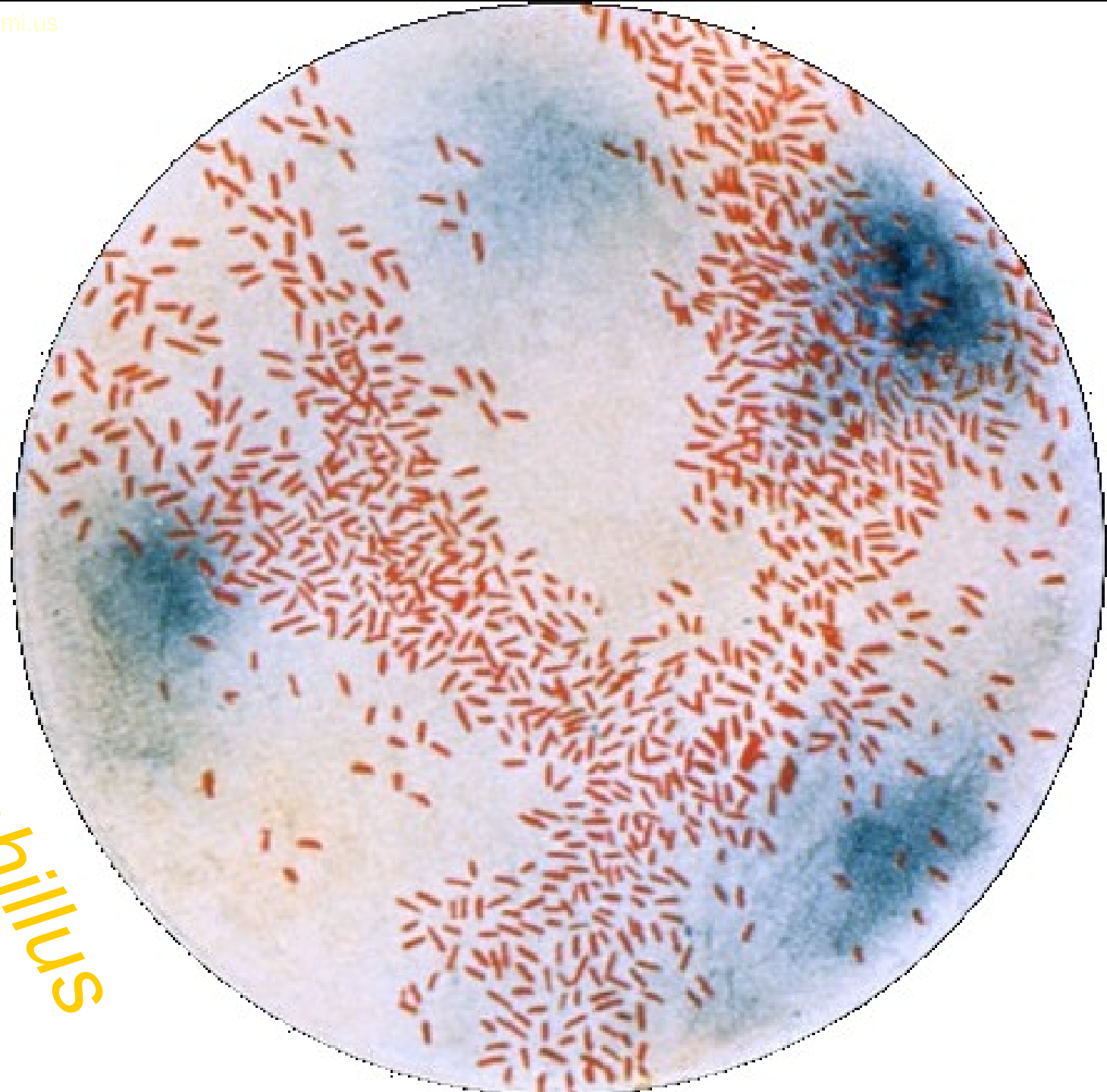
- **Jimmy** is a fine boy, but his parents are members of a strange religious society and so they **do not wish let him get vaccinated**. They would like to keep him at home, but they sent him to a kindergarden.
- After a month **Jimmy started to have a cold**, difficult breathing, gasping for air, and it became so hard that emergency had to be called. Emergency even thought about cricothyrotomy, but finally it was not necessary. **It was epiglottitis** – a disease not too common today...

Who did this to Jimmy?

- Criminal: *Haemophilus influenzae* ser. b (Hib)
- Haemophili are **short Gram negative rods**. Unlike e. g. enterobacteria, they **do not grow on Endo agar**. And even more: they even **do not grow on blood agar**, as they are unable to get inside erythrocytes to get the nutrients.
- Haemophili belong to the family *Pasteurellaceae*, together with *Pasteurella* (see later)

Classification of haemophili

- ***Haemophilus influenzae***
 - capsular type b (Hib)
 - capsular types a, c, d, e, f
 - non-encapsulated strains
- ***Haemophilus parainfluenzae*** (much more common, much less pathogenous)
- ***Haemophilus aphrophilus*** and many other species
- ***Haemophilus ducreyi***, causative agent of a sexually transmitted disease **ulcus molle**



Haemophilus

Pathogenicity of haemophili

- The most severe diseases caused by haemophili are **epiglottitis, meningitis and sepsis**. This is mostly typical for *Haemophilus influenzae*, serotype b.
- Other common diseases are **otitis media and sinusitis** (after *Streptococcus pneumoniae* and together with *Moraxella catarrhalis*)
- Their **presence in throat is very common** and their pathogenic role is very query. Especially in case of *Haemophilus parainfluenzae*, we usually do not suppose them to be pathogens.

A Haemophilus disease

<http://www.immune.org.nz>





H

I

B

מכ-הנאכ

Ulcus molle

- It is a sexually transmitted disease found mostly in sub-tropical and tropical countries



www.fmt.am.gov.br



www.fmt.am.gov.br



<http://www.sexsgdom.dk>

Ulcus molle – chancroid – caused by *Haemophilus ducreyi*

Ulcus durum – chancre – one of symptoms of syphilis, caused by *Treponema pallidum*

Story Two

- Joana went walking in gardens as usually. Unfortunately, one garden fence was too old and rotten and the dog inside too strong. The dog run out and just met Joana. So, **Joana was bitten into her leg.**
 - The owners of the dog had proven that the dog was vaccinated against rabies. Nevertheless, some **pus was found soon in the wound.** So the pus was sent to the laboratory. Ant the criminal was...
-

Pasteurella multocida



- *Pasteurella multocida*.
- It is common flora in dog mouth.
- In humans, it causes mainly pyogene wound inflammations after being bitten by a dog or another animal.
- It **smells similarly as *Haemophilus***, but unlike *Haemophilus*, it grows on blood agar (but not Endo agar).
- The morphology is something between *Streptococcus* and *Enterococcus*, but is **Vancomycin resistant** and this gives a suspicion to the microbiologist

Clinical

characteristics –

Gram– glucose

non-fermenters

Story Three



- **Mr. Phosphoros** is a pyroman. Several days ago, he burned himself. Now, his burn is **inflamated**. He is hospitalised on a specialized centre and feels very badly. Doctors knew that it has no sense to try antibiotics accidentally, so they **performed a swab**. Thanks to this, a **target therapy** was found, and Mr. Phosphoros healed. Of course, only temporarily: sooner or later, he will probably play with his matches again (*like some students of the practical*).

Who is guilty this time?

- It is *Pseudomonas aeruginosa*, the most common so named „Gram – non-fermenters“
 - On the other hand, the guilty one could be any of that group, e. g. *Acinetobacter*, *Burkholderia cepacia* or *Stenotrophomonas maltophilia*
 - Commonly: they are bacteria from outer environment, often plant pathogens, „not-brave-bacteria“, which are not able to infect a healthy person. Their target are patients with burns, clients of emergency units, transplant centers, e. t. c.
-

In disabled persons,
they can
cause even
such
problems as
a nail
inflammation



Dr. Zahradníček's autocaustics

- Friday, 13th January 2006: fall into a not-covered canalisation hole in the city of Padang, West Sumatera, Indonesia. Quite large wound, reaching tibial periost
- Some three weeks later, the wound started to smell like *Pseudomonas*, and really, this bacterium was successfully cultured from it.
- Dr. Zahradníček decided for local treatment (mixture of gentamicin and polymyxine)
- The therapy was successful.

Padang

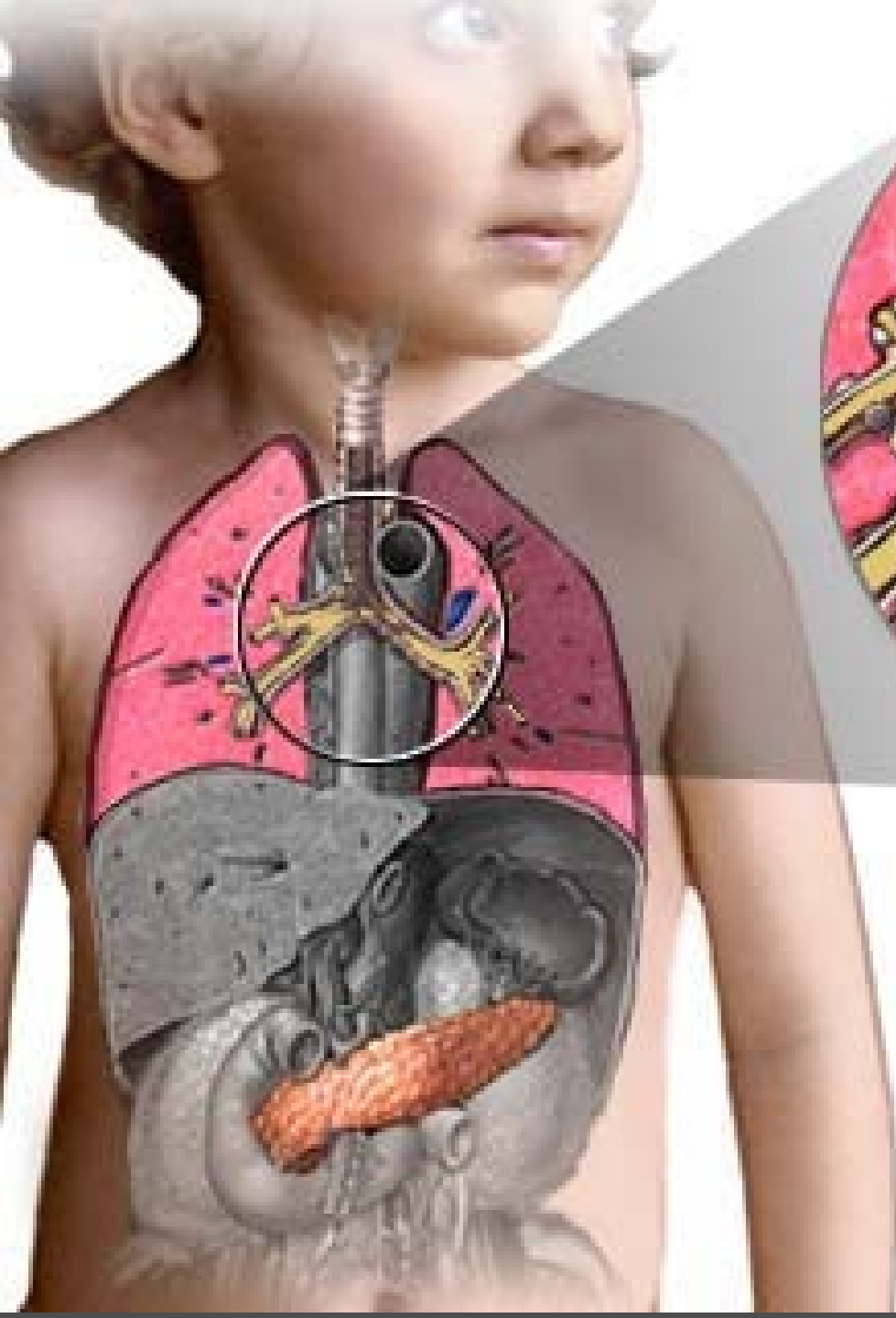


Story Four

- Linda was a poor girl: she suffered because of **an inborne disease, cystic fibrosis.**
 - Her **lung surfactant was different from surfactant of healthy people.** So, he was often infected.
 - Last time it was *Staphylococcus aureus*. This time it was different: **the causative agent was *Burkholderia cepacia***, one of G– non-fermenters.
-

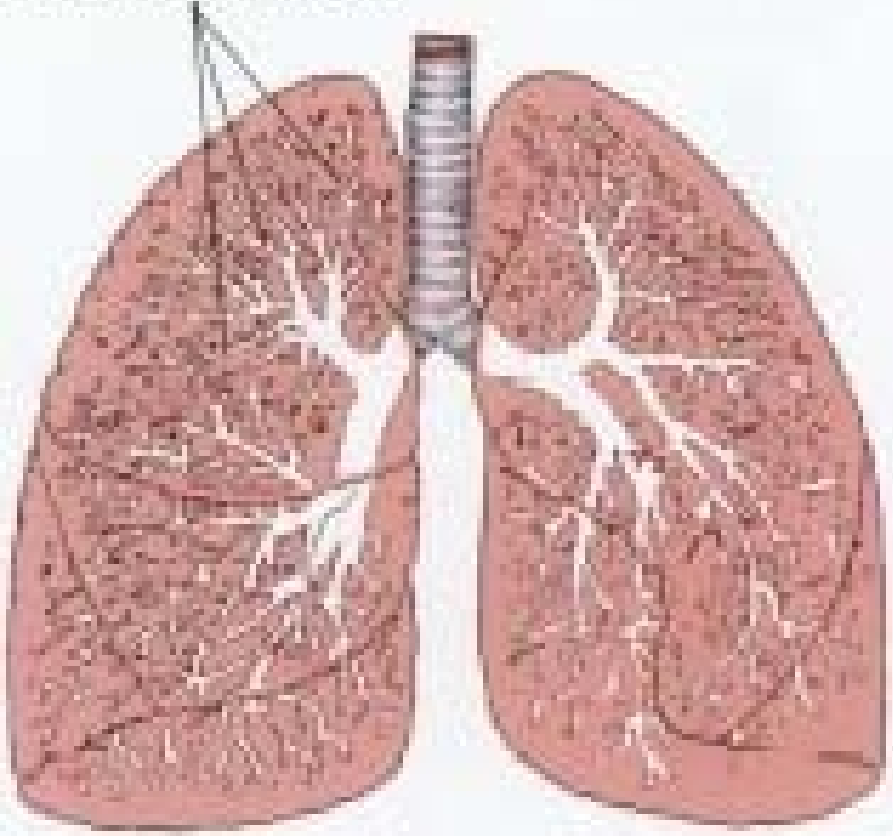
Non-fermenters and Cystic fibrosis

- Cystic fibrosis is a severe, **inborn lung disease**, with failure of production of normal lung surfactant. This leads to changed characteristics of lungs, including many times increased risk of infection
 - **Most common causative agents** are *Pseudomonas aeruginosa*, *Burkholderia cepacia* and *Staphylococcus aureus*. Strains often become **polyresistant** and many children with cystic fibrosis die very young.
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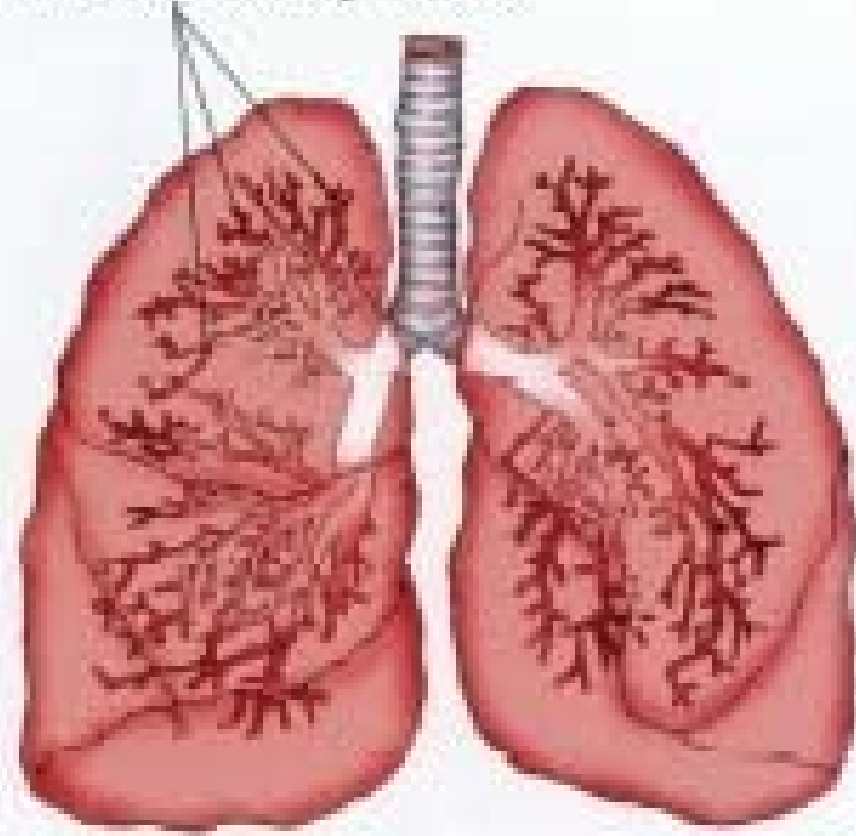
Cystic fibrosis is a hereditary disorder characterized by lung congestion and infection and malabsorption of nutrients by the pancreas

Unobstructed bronchial tubes



Healthy lungs

Bronchial tubes are blocked by mucus



Lungs with cystic fibrosis

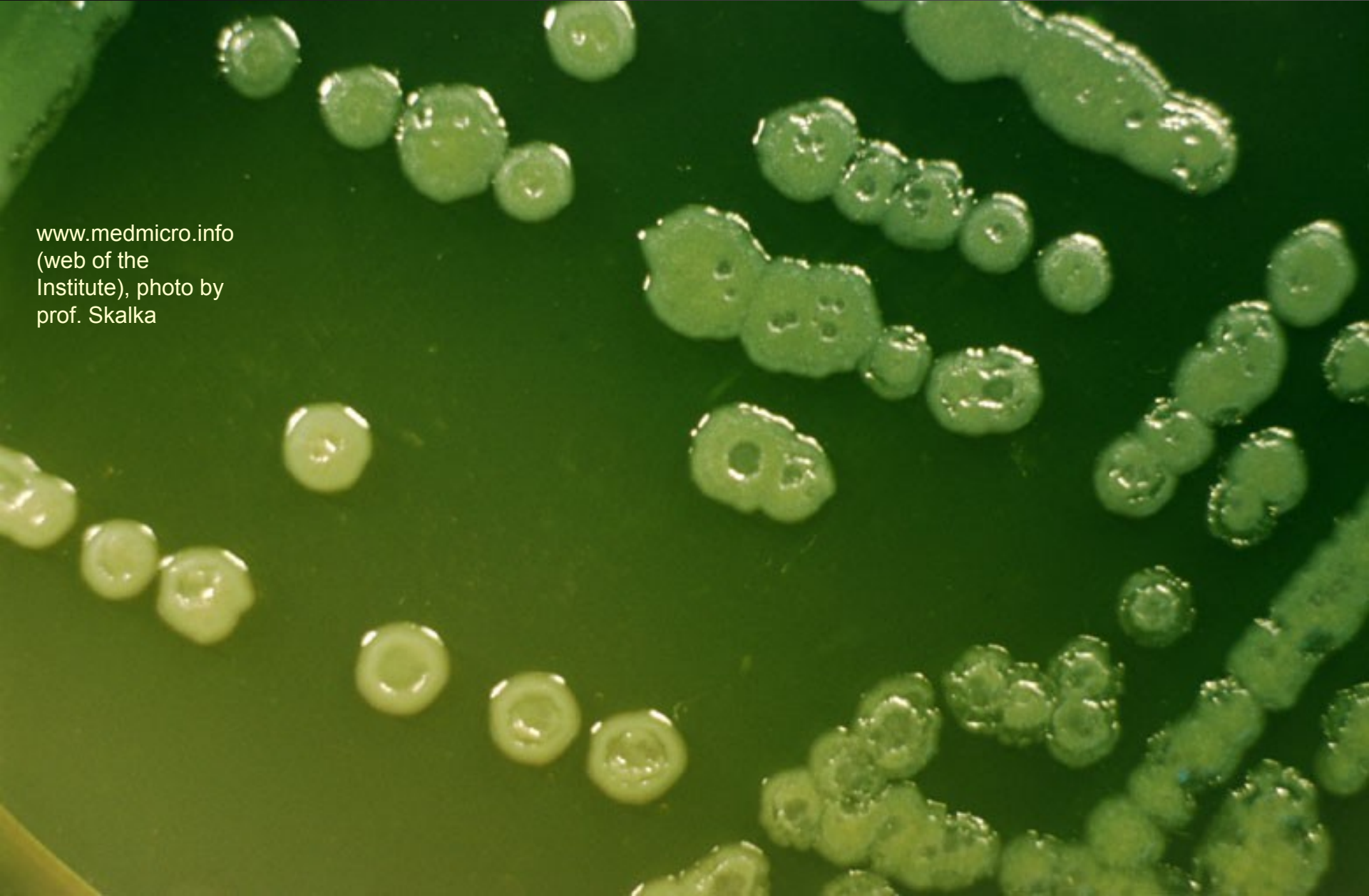


A few words concerning bacterial metabolism

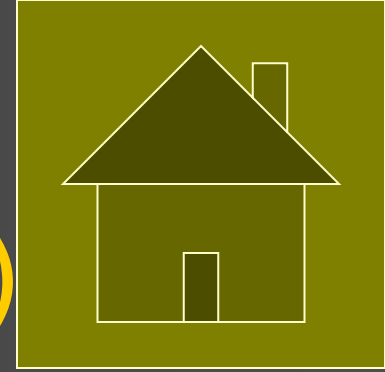
- As you know, clinically important bacteria use mostly one of two metabolism types: **fermentation and aerobic respiration**.
- *Escherichia coli*, having **enough nutrients**, but not enough **oxygen** (unlike other gases 😊) **prefers glucose (and other substrates) fermentation**.
- On the other hand, *Pseudomonas* has **oxygen enough**, but **nutrients not enough**. It uses **aerobic respiration**: enables better exploitation of nutrients.
- Adaptation to outer environment is also demonstrated by **pigment production** in non-fermenters – see next.

Pseudomonas aeruginosa on MH

www.medmicro.info
(web of the
Institute), photo by
prof. Skalka



Pseudomonas as a strict aerobe (unlike other bacteria)



- Unlike **strain I** (*Escherichia coli*) and **strain II** (*Bacterioides fragilis*, a strict anaerobe), *Pseudomonas aeruginosa* (**strain III**) is a strictly aerobic bacterium (*Bacterioides fragilis*, more in P07)

Strain	Broth	VL-broth	Result
I	growth	clear	Strictly aerobic bacterium
II	clear	growth	Strict anaerobe
III	growth	growth	Facultative anaerobe

Diagnosics of *Pasteurellaceae*

Survey of methods used for diagnostics of these bacteria

■ Direct methods

- **Microscopy** – if we need it for differentiation
- **Culture** – non-fermenters grow on majority of media, *Pasteurellaceae* are more specific
- **Biochemical identification** – in both groups; in non-fermenters it is based on aerobic respiration reactions, it requires ↓ temp. and 2 d culture
- **antigen analysis** – mostly in haemophili (Hib)

Indirect methods used rarely

More to culture of these bacteria

- **Haemophilli need factors from RBC**, but they are not able to break an RBC themselves. So they are not able to grow on blood agar alone. They need it to be broken, e. g. by heating (→chocolate agar) or by presence of another bacterium (→Satelite phenomenon)
- **Pasteurellae** grow on blood agar, but not Endo agar
- **G– non fermenters** grow on all common media (blood agar, Endo agar, Müller-Hinton agar)

Differentiation of *Pasteurellaceae* (differential diagnostics)

- **Gram staining:** Gram– rods × other bacteria
- **Endo medium:** as we now, among clinically important bacteria, only *Enterobacteriaceae*, *Vibrionaceae* and Gram – non-fermenters are able to grow. ***Pasteurellaceae* do not grow.**
- *Pasteurellaceae* are detected by typical smell, biochemical properties, typical antibiotic susceptibility etc.

To *Haemophilus* and *Pasteurella* diagnostics



<http://www.uni-ulm.de>

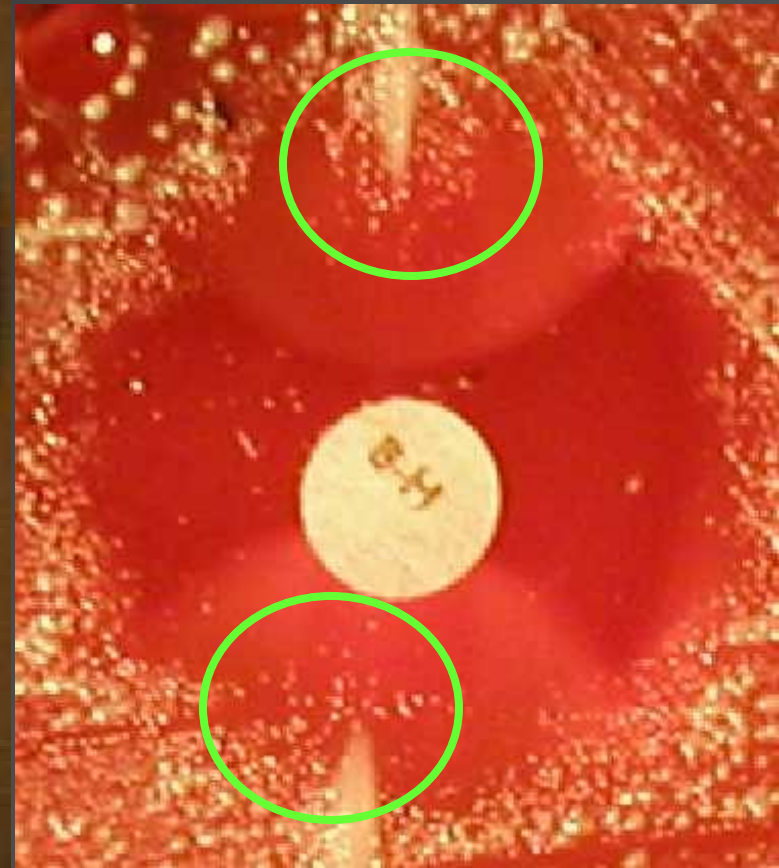
- *Pasteurella* grows on blood agar
- *Haemophili* are not able to grow on blood agar, they are not able to „open the RBC“. So, they grow on **chocolate agar**
- On BA, they are able to grow, if a bacterium that „opens the RBC“ is present (**satelite phenomenon**). Such bacterium is e. g. golden *Staphylococcus*
- They grow in **tiny colonies**, so we use a disc to disable growth of other bacteria (**bacitracin**, but in higher concentration than in bacitracin test)

Satelite phenomenon

- As we know already, haemophilli need factors from RBC, but they are not able to break an RBC themselves. They need the RBCs to be broken
 - by heating – chocolate agar
 - by presence of another microbe
 - **Satelite phenomenon** is an example of the second way how to make haemophilli be able to exploit blood factors. That means the growth of *Haemophilus* around *Staphylococcus* line only.
 - **Colonies are very tiny**, they require careful observing!
-

Detection of haemophili

Haemophili are more resistant than the bacteria of the common flora, so they grow inside the zone, but only around staphylococcus line (satelite phenomenon!)



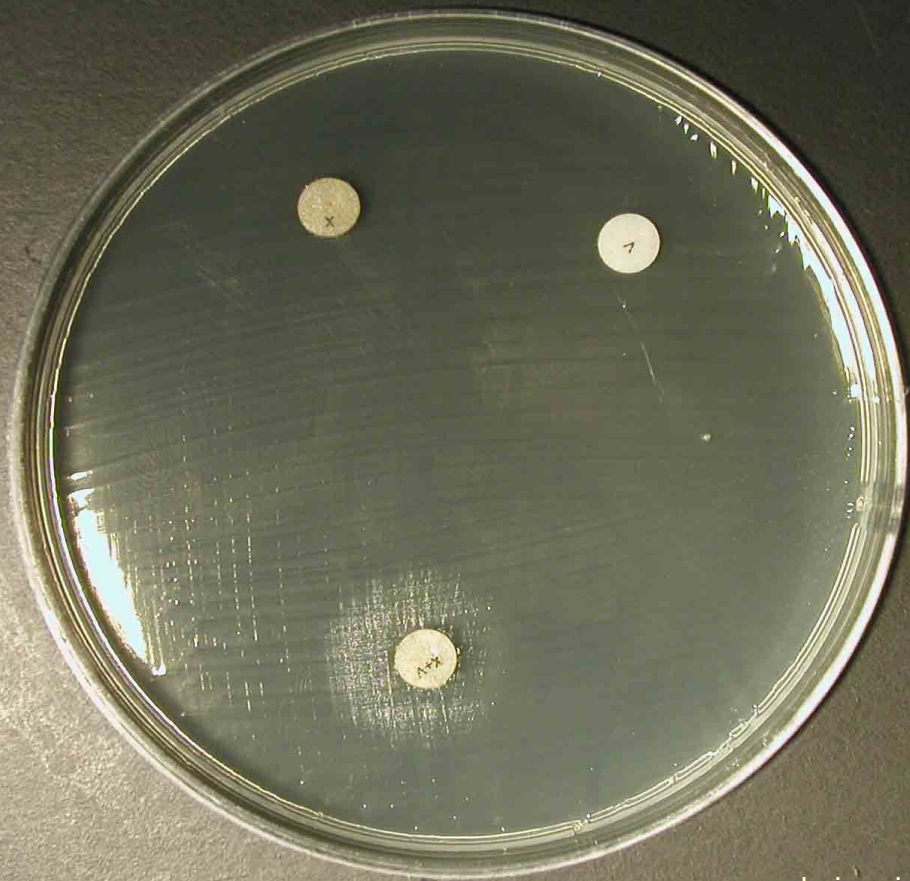
Growth factors of Hemophili

- Haemophilli need factors from blood, but the need of individual factors is species specific.
 - *H. parainfluenzae* needs factor **V** (= NAD)
 - *H. aphrophilus* needs factor **X** (= hemin)
 - *H. influenzae* needs **both factors**.
 - We use discs with these factors: one with **X**, another with **V**, and the third with a **mixture** of both of them.
-

Growth factor test of *Hemophil*

One disk is with factor X, second with factor V, third a mixture

H. influenzae (left),
H. parainfluenzae (right)

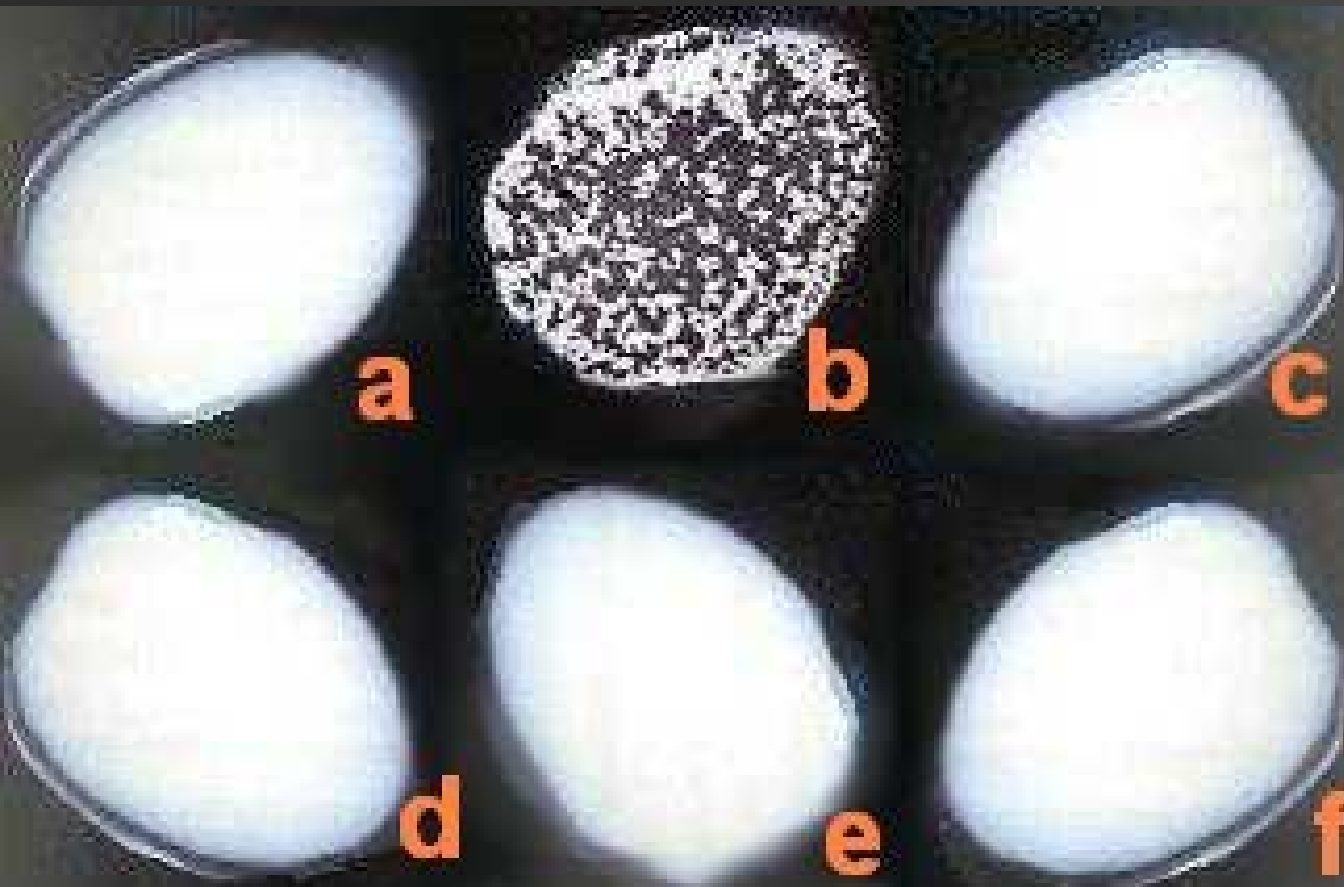


Haemophili: antigen analysis

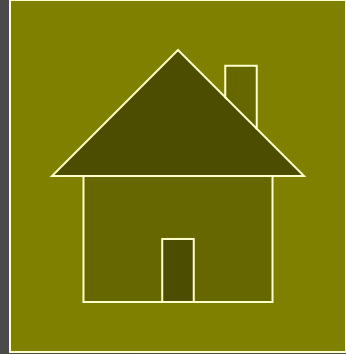
- Antigen analysis in Hemophilli is performed like in other bacteria. The main goal is diferenciation of Hib. Today, we have **comercionally available sets**, containing e. g. latex particles e. t. c.
 - Formerly, so named **co-agglutination with *Staphylococcus* strain** was used: agglutinate was more dense because of *Staphylococcus* binding the Fc-end of anti-haemophilus antibody
-

Antigen analysis of haemophili: an example of the result

- Write down the result, as you can see it on the following picture (it belongs to strain K):



Detection of *Pasteurella* using typical susceptibility pattern



- **No Gram-negative bacterium is susceptible to vancomycin.** Vancomycin can be used for Gram-positive bacteria only, but here it is very strong; all streptococci and majority of staphylococci and enterococci are susceptible
- On the other hand, **very little bacteria are susceptible to penicillin, especially among G- bacilli.**
- **So, susceptibility to penicillin and resistance to vancomycin is quite typical for *Pasteurella*.**

Diagnosics of Gram–non- fermenters

Differentiation of G– non-fermenters (differential diagnostics)

- **Gram staining:** Gram– rods × other bacteria
- **Endo medium:** as we now, among clinically important bacteria, only *Enterobacteriaceae*, *Vibrionaceae* and **Gram– non-fermenters** are able to grow. So, **G– non-fermenters do grow**
- **Non-fermenters** are differentiated from enterobacteria/vibria by non-fermenting glucose (e. g. Hajna medium remains completely red after culture, no colour change)

Tests of atb susceptibility

- Haemophilli do not grow on MH agar
 - Usually **Levinthal agar** (fibrateed chocolate agar), is used – for this purpose, it is better than classical chocolate agar
 - Our laboratory use „**Haemophilus agar**“, similar to Levinthal agar
-

An example of *Pasteurellaceae* antibiotic set

Antibiotikum	Abbrev.	Reference zone
Ampicilin (aminopeniciline)	AMP	22 mm
Co-amoxicilin (am.+inhib.)	AMC	18 mm
Chloramphenicol	C	29 mm
Doxycycline (tetracycline)	DO	29 mm
Co-trimoxazol (mixture)	SXT	16 mm
Azithromycin (macrolid)	AZM	12 mm

To non-fermenters diagnostics

- *Pseudomonas* is usually detected by:
 - Presence of typical **odour** (young cultures)
 - They form **pigments**, mostly **green**, sometimes blue or maroon. Best visible on MH, worse on BA and Endo agar
 - Positive **oxidase**
 - **Other non-fermenters**, or not-sure *Pseudomonas*, should be differentiated biochemically, e. g. by NEFERMtest 24
-

Pseudomonas on MH agar and other media

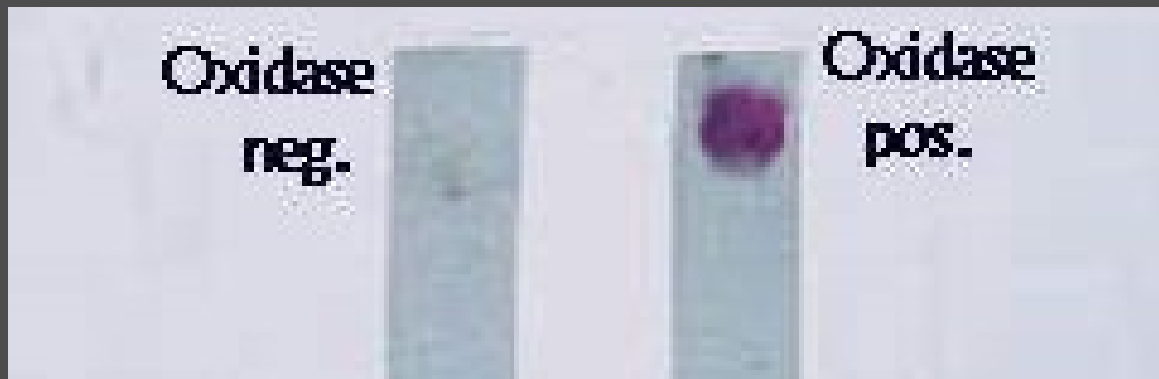
- Remember, that MH agar itself is nearly colourless (or slightly yellowish).
- All green colour you see is product of *Pseudomonas*, or more precisely, of its pigment pyoverdinin
- On BA and Endo, pigment production is not so strong, but partially visible, too. Nevertheless, something more visible on these media is the typical pearl smooth surface of the colonies

Hajna medium for non-fermenters determination

- The strains growing on Endo agar could be G– non-fermenters, *Enterobacteriaceae* or *Vibrionaceae*.
- Typical for G– non-fermenters is **absence of any change of colour of Hajna medium** (it remains red; **eventual light brown colour does not matter, it is due to presence of pigments**)

Oxidase test in non-fermenters

- Among the most common G– non-fermenters, *Pseudomonas* is oxidase positive, *Burkholderia* usually too; on the contrary, *Stenotrophomonas* is usually negative and *Acinetobacter* too.

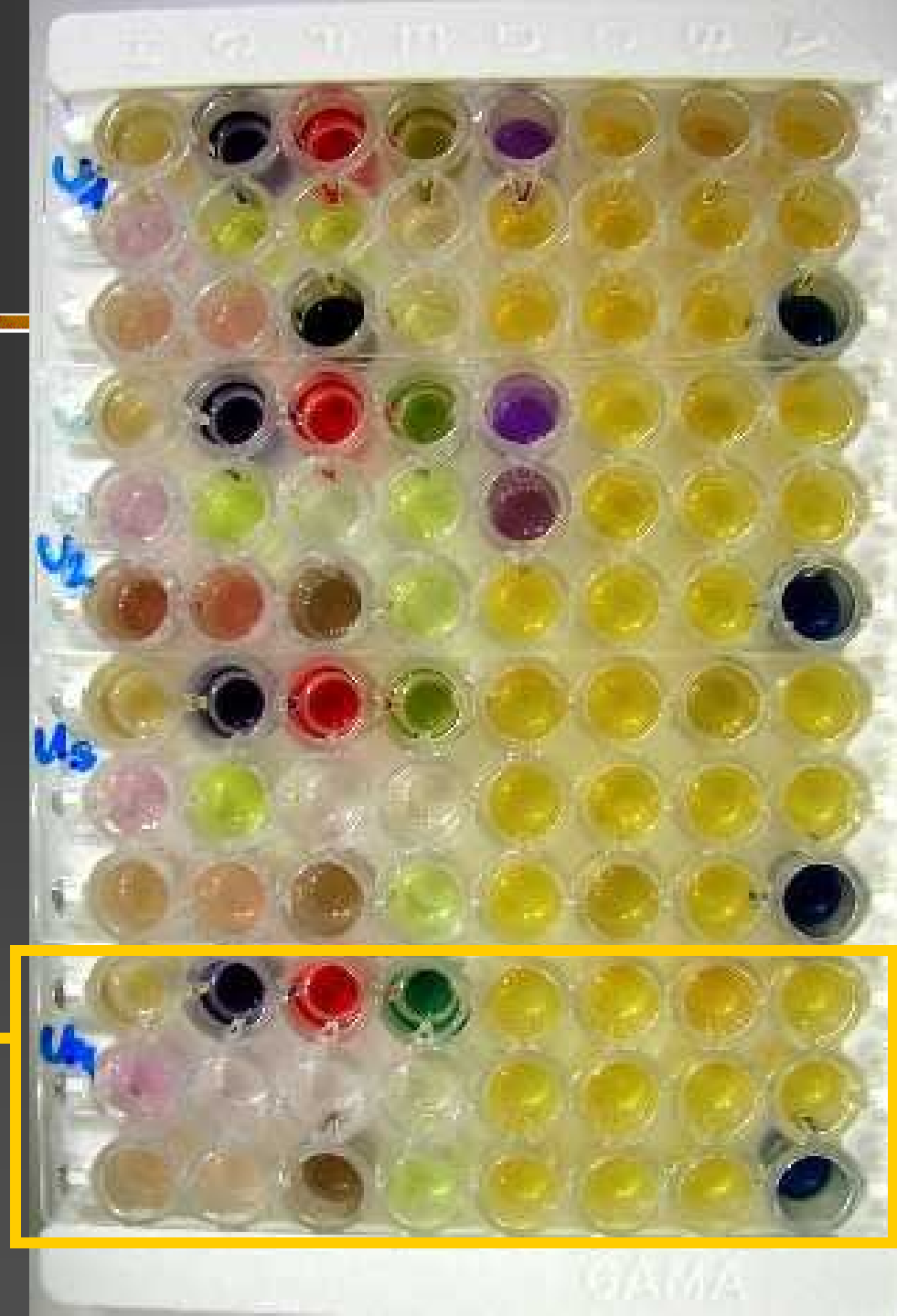


NEFERMtest 24

- For precise biochemical identification of G– non-fermenters we use mostly Nefermtest 24 (or similar test of other provenience).
- It is a triple-strip (not double as last week)
- There is a different way of code-formation
 - first number is 0 (oxidase –) or 1 (oxidase +)
 - next 6 numbers come from collumns H to C
 - collumns B and A are not counted (they are eventually used for more detailed determination)

NEFERMtest 24

- One frame is used for four triple-strips (for four strains). Each strain is detected using 24 reactions.
- Requires 30 °C, 48 h



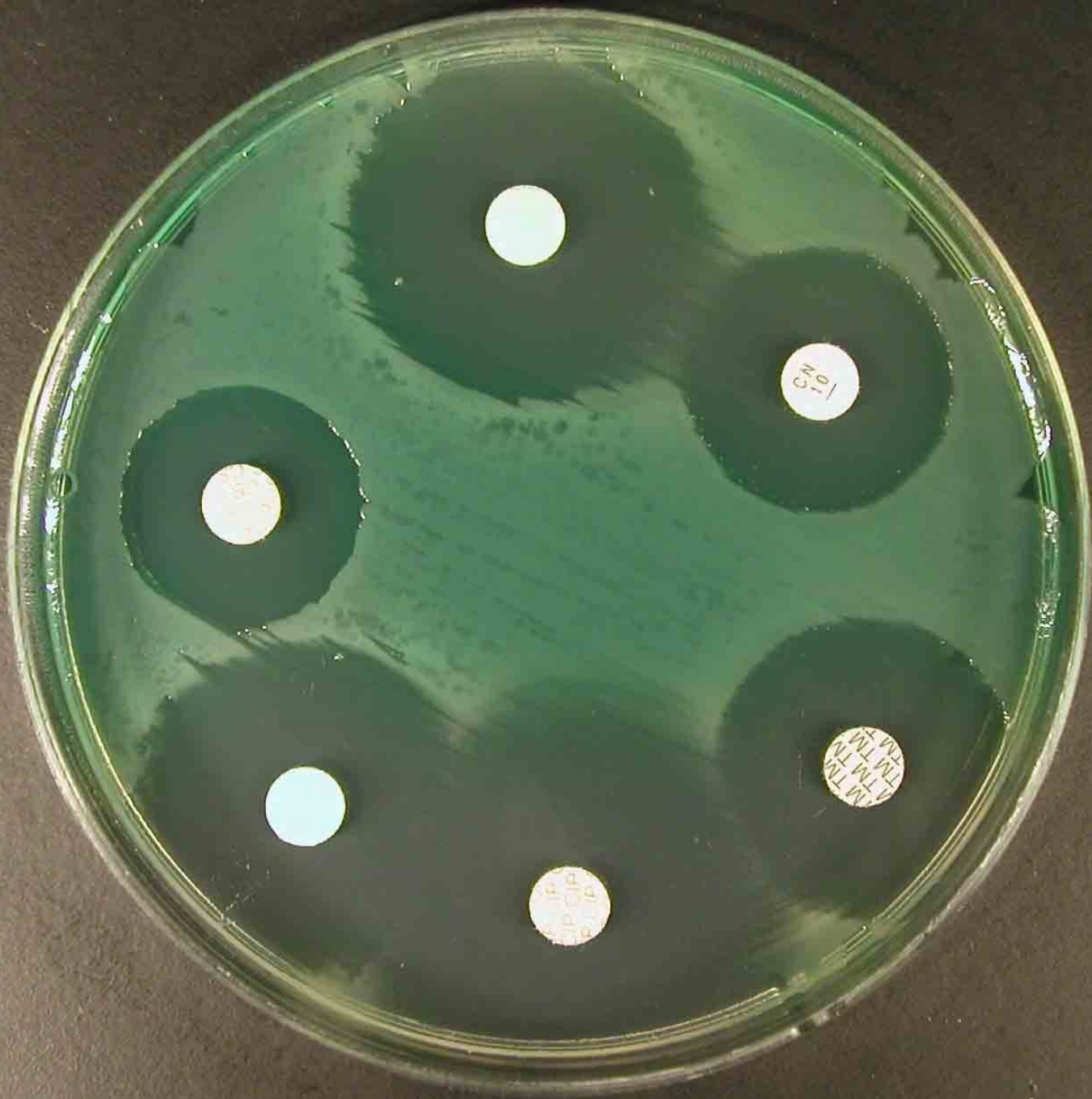
Tests of atb susceptibility

- **G– non-fermenters** may be tested on common media.
 - We use very strong antibiotics, that would be too strong for use with more common bacteria. Majority of them should be kept only for real G– non fermenters infections (but not mere colonisations by such strains!)
-

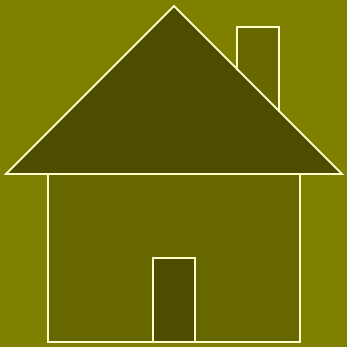
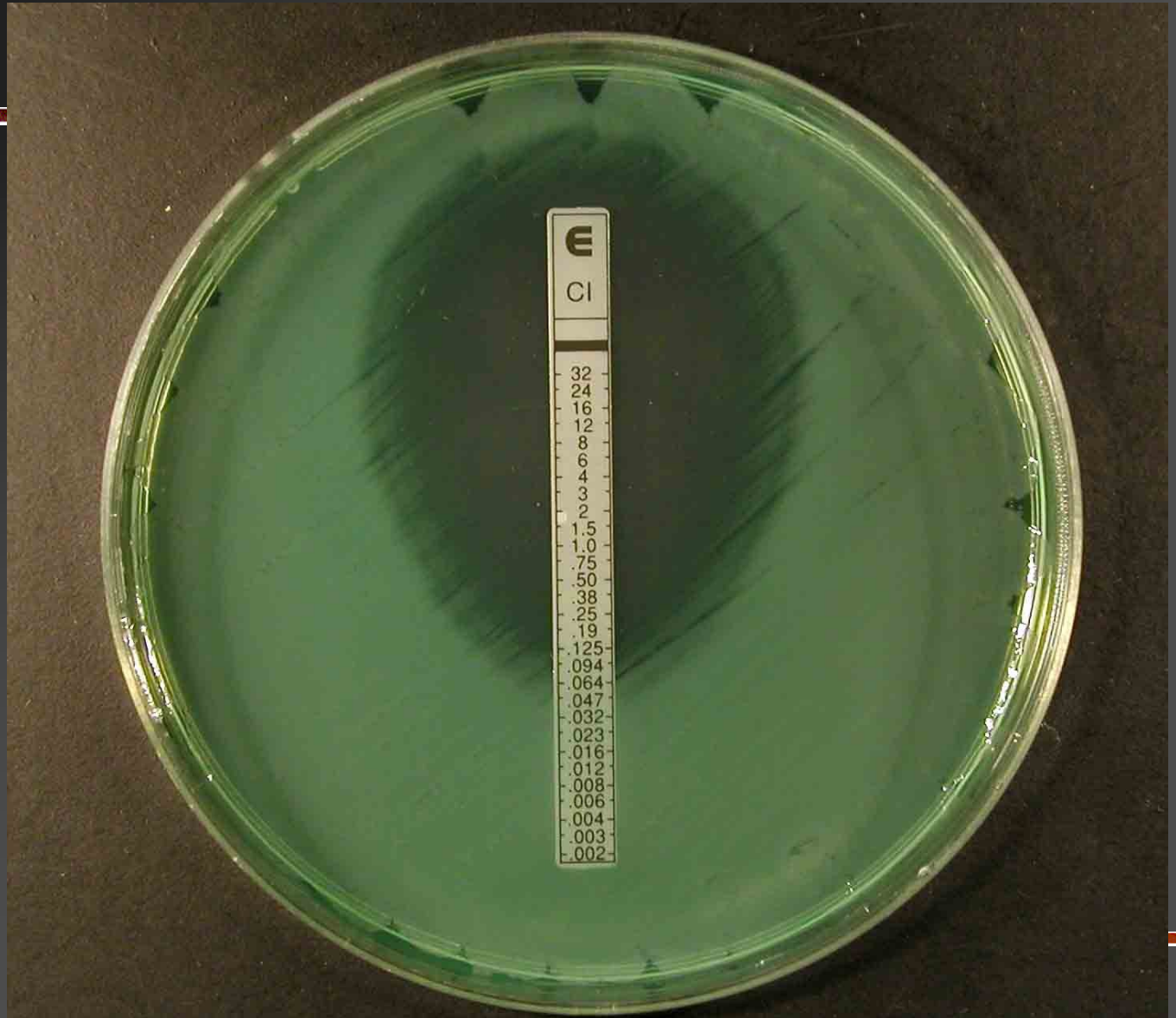
An example of NF atb set

Antibiotic	Abbrev.	Reference zone
Piperacilin + tazobactam*	TZP	22 mm
Gentamicin (aminoglykos.)	CN	18 mm
Imipenem (karbapenem)	IMP/IMI	22 mm
Ciprofloxacin (quin 3 gen)	CIP	29 mm
Ceftazidim (CS 3 gen)	CAZ	16 mm
Colistin (polypeptide)	CT	12 mm

*antipseudomon. peniciline + β -lactamase inhibitor

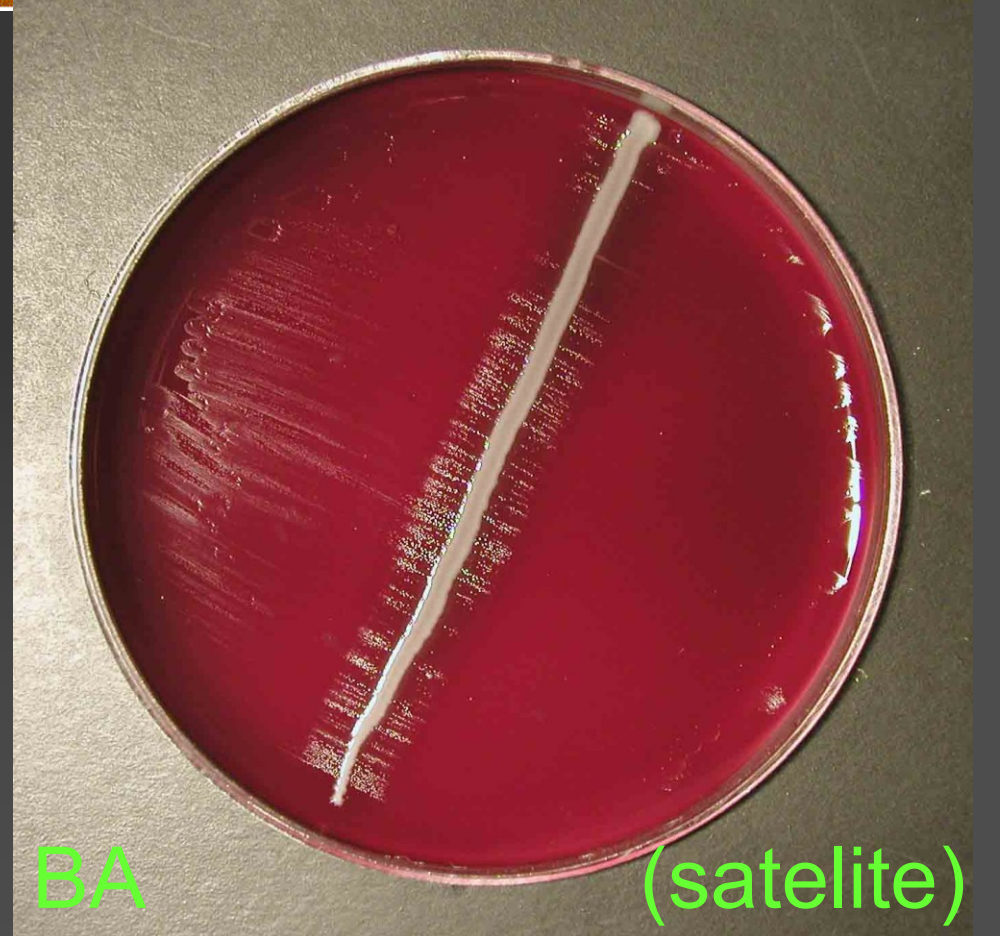


Somebody use E-test...



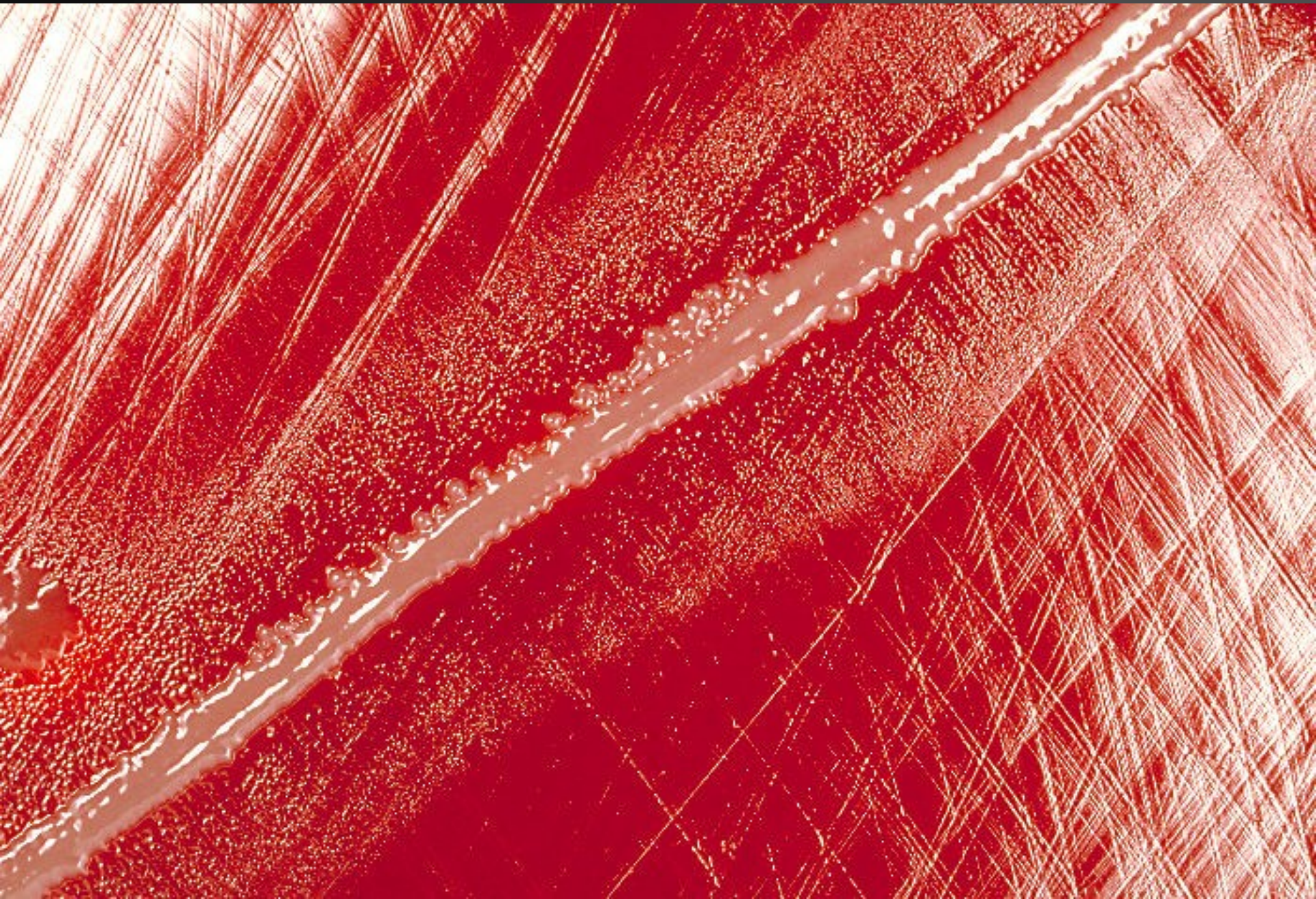
Pictures of *Pasteurellaceae* and Gram–non- fermenters

Photographs of criminal database Haemophili



Satellite once more

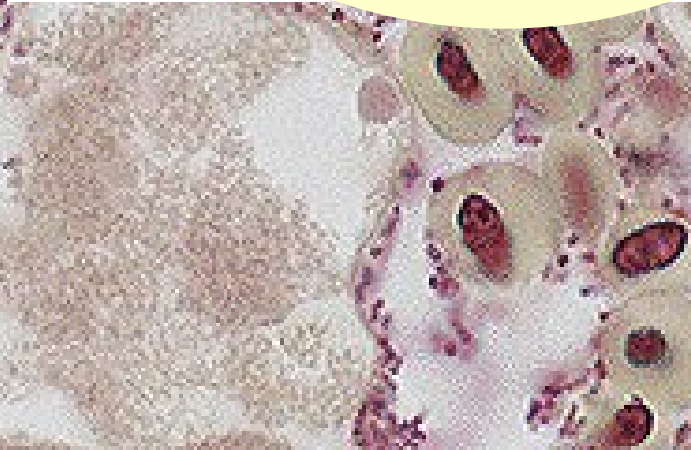
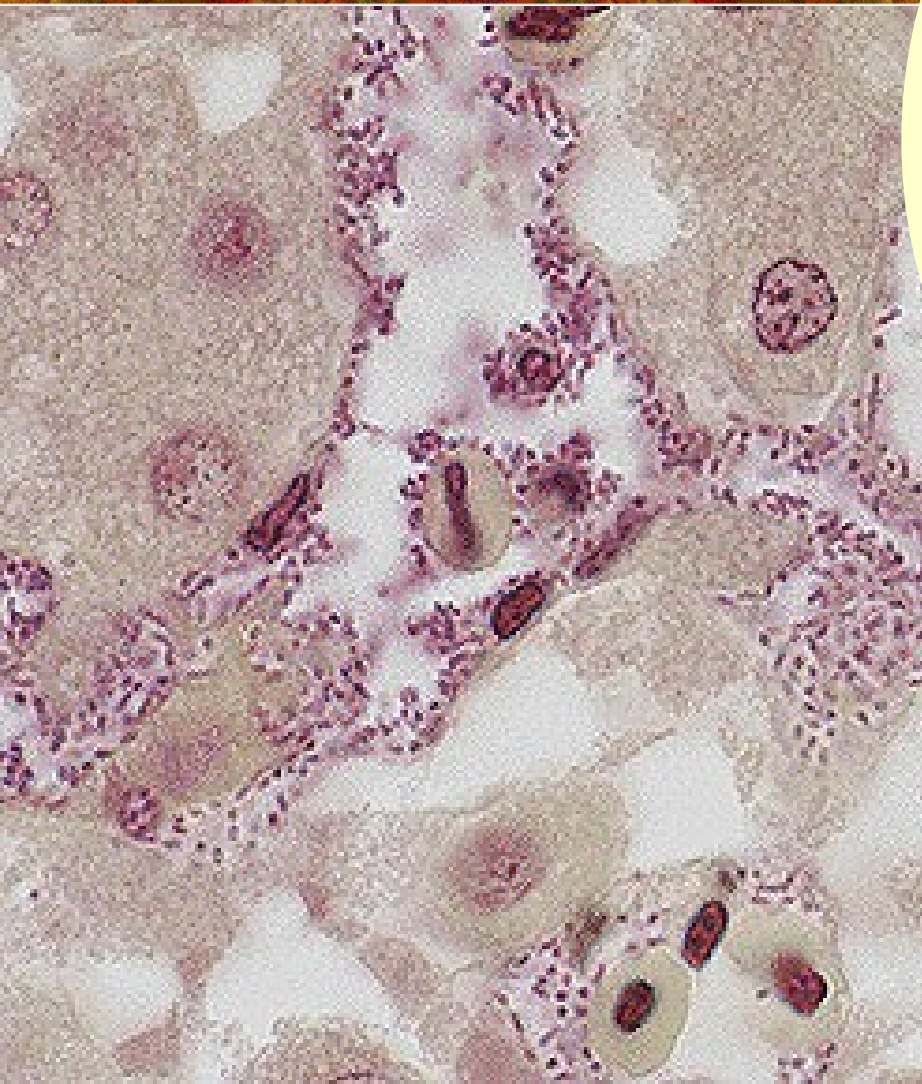
<http://phil.cdc.gov>



Pasteurella multocida

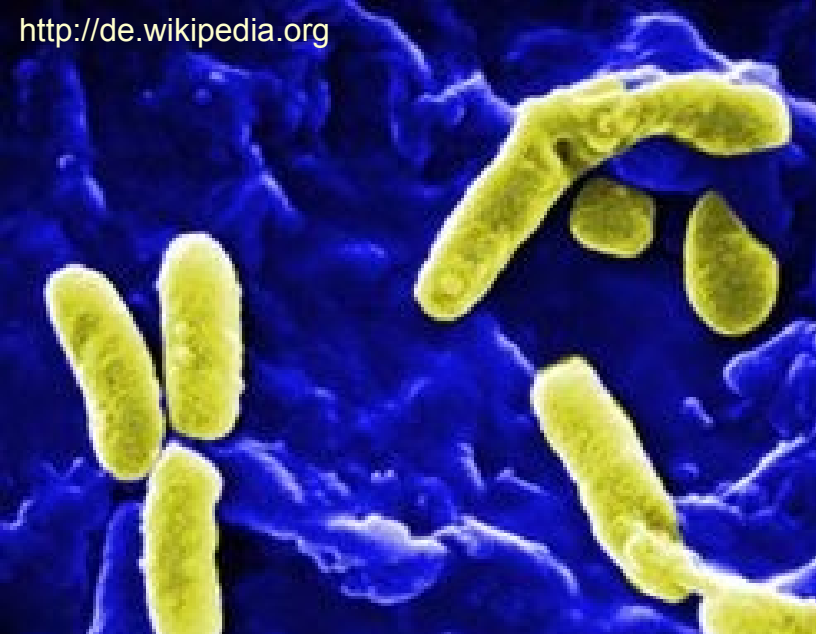
<http://library.thinkquest.org>

<http://www.biologico.sp.gov.br>



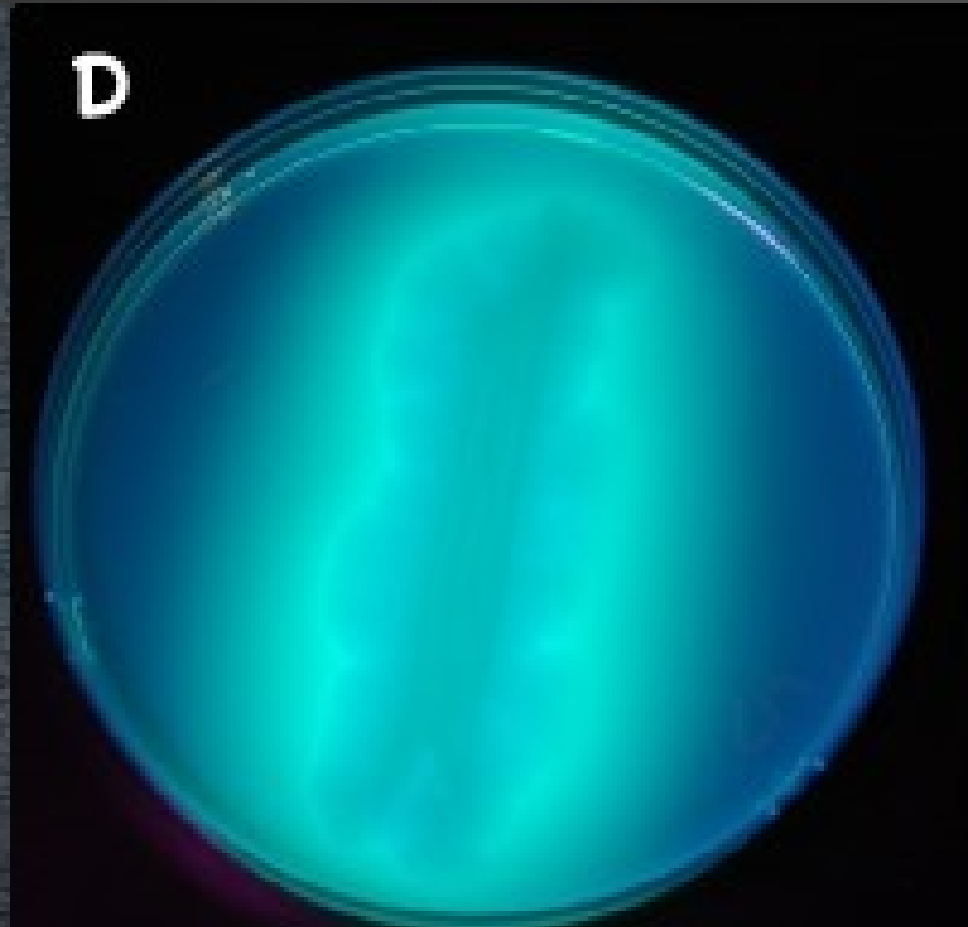
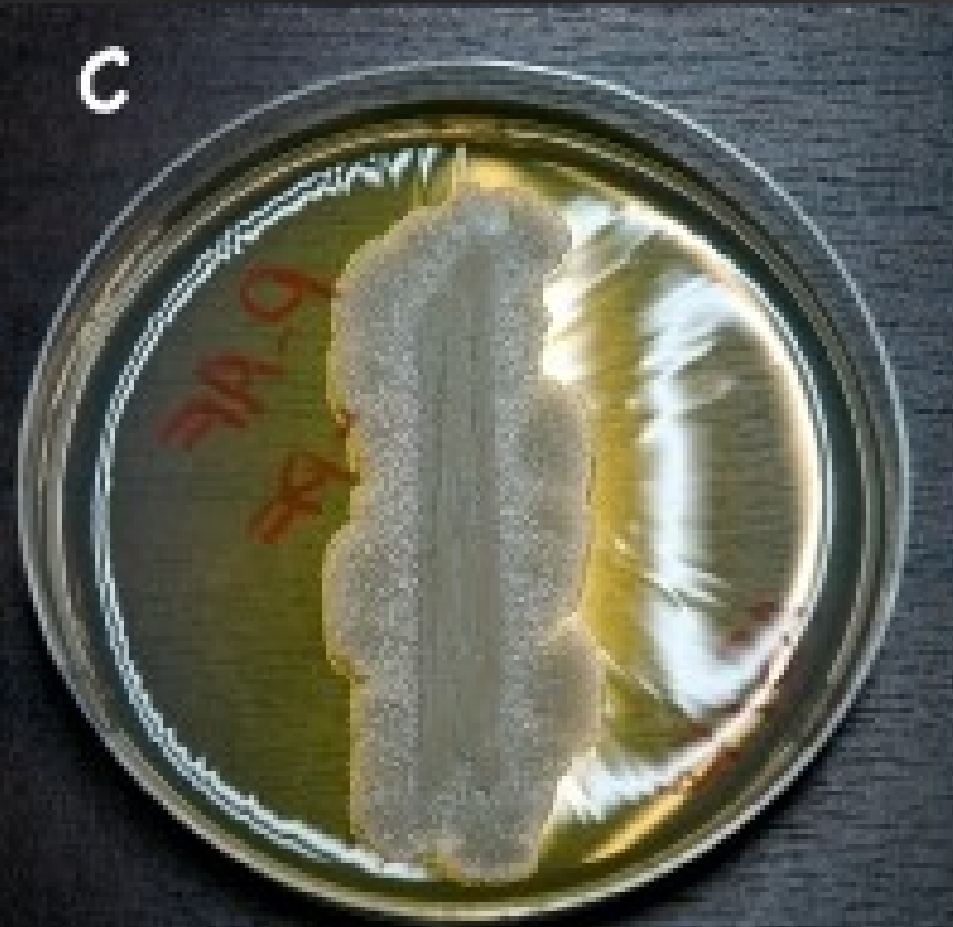
Photographs of criminal database *Pseudomonas aeruginosa*





Exceptional
Pseudomonas
strain with blue
pigmentation

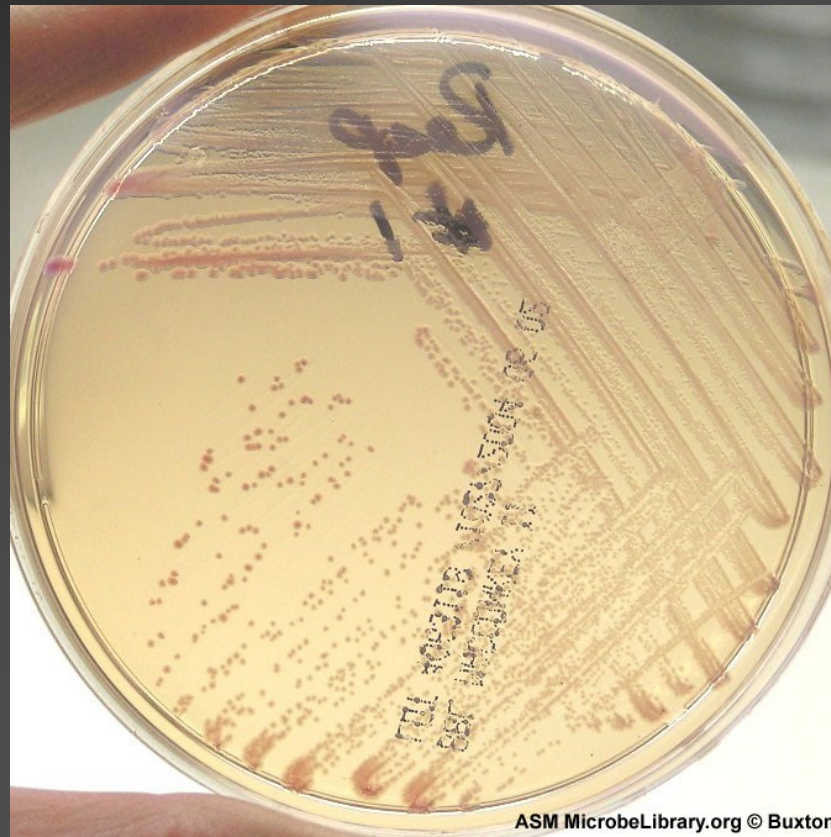
More Gram non-fermenters: *Pseudomonas fluorescens*





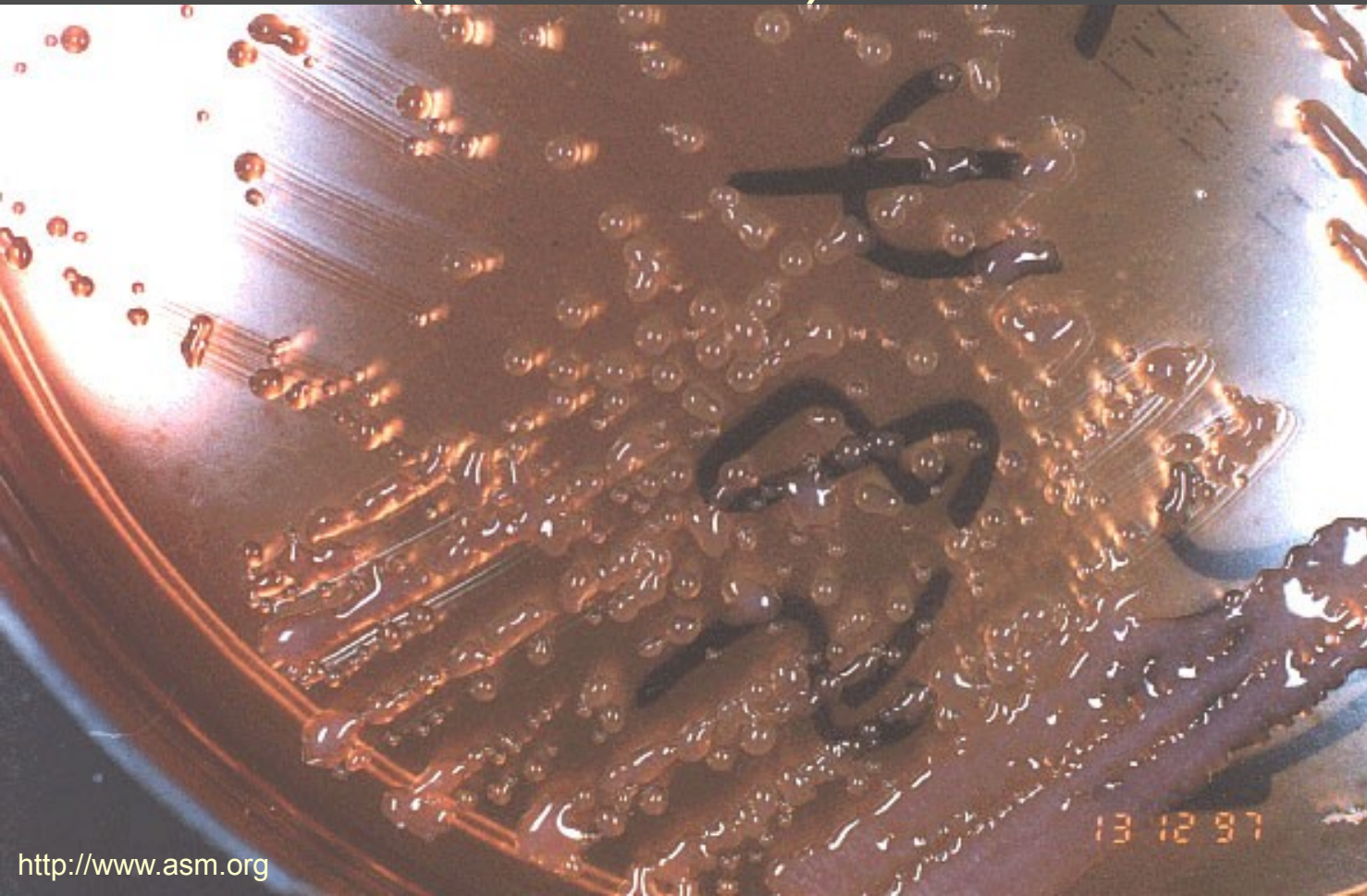
Burkholderia cepacia

Burkholderia cepacia is responsible for rotten onions (*Allium cepa*), so it is really a typical plant pathogen



Burkholderia pseudomallei

Burkholderia pseudomallei is causative agent of melioidosis. Related *B. mallei* is causative agent of malleus (a zoonosis)

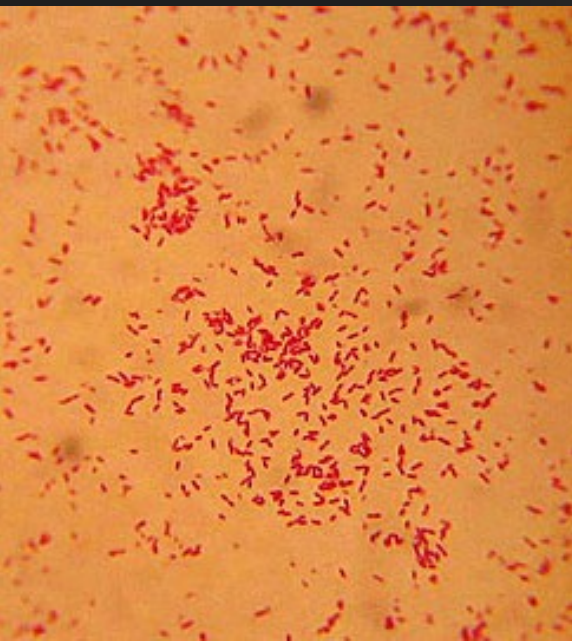


Stenotrophomonas maltophilia

<http://www.scielo.cl>

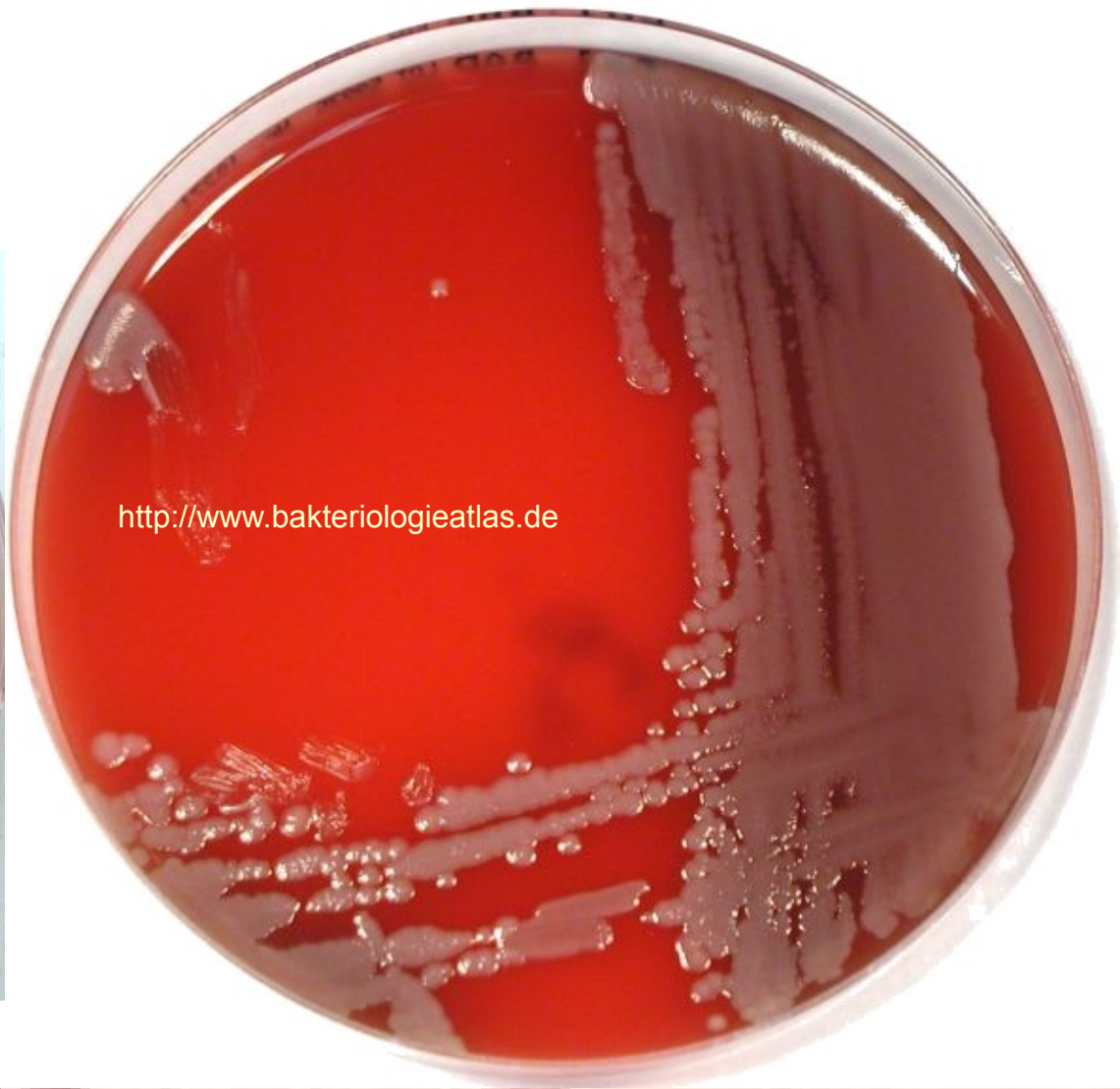
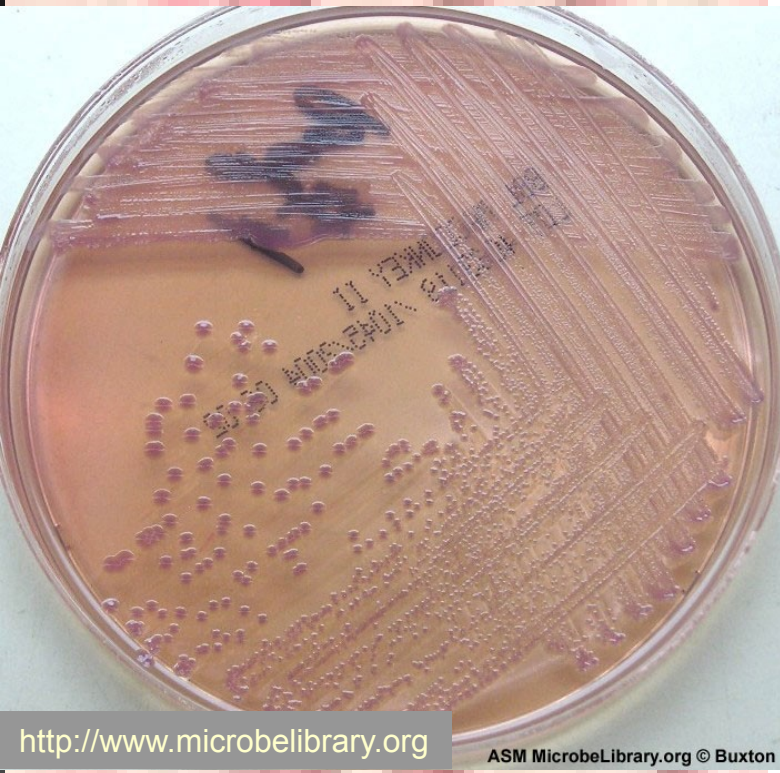
<http://clinicalmicrobiology.stanford.edu>

<http://www.microbelibrary.org>



Stenotrophomonas maltophilia is a long name, but it is possible to learn it easily: it is narrow-nutrition-unit maltose-loving, so it is a „bacterial panda“, chewing maltose instead of bamboo 😊.

Acinetobacter



<http://www.buddycom.com>

Greek: a-kineto- = „non motile“

The End

