BIOCHEMICAL TESTS
for identification of bacteria

Mgr. Tomáš Kastl
KOH test

→ prove of G⁻ bacteria
  - damage of bacterial cell wall → viscous matter
  ! can’t be used when bacteria produce mucus

PROCEDURE:
- drop 3% KOH onto a slide and add bacterial culture

E. coli pouring out its content

CATALASE test

Catalase – enzyme, which decomposes H₂O₂ ⇒ defence against oxidative agents

PROCEDURE:
- add a drop of H₂O₂ to the smeared cell culture on a slide
  • in a case of catalase positive bacteria (CAT⁺) bubbles will appear
→ most of G⁻ bacteria are CAT⁺
→ Staphylococcus & Bacillus are CAT⁺ too
OXIDATION/FERMENTATION (OF) test

- aerobic (oxidative) catabolism = GLC $\rightarrow$ CO$_2$ + H$_2$O
- anaerobic catabolism (fermentation) = GLC $\rightarrow$ organic molecule (+ gas)
  - in both aerobic and anaerobic conditions
  - some bacteria don’t utilise GLC

PROCEDURE:
1. use needle to inoculate pure bacterial culture into the two test tubes with semi-solid medium
2. overlay the culture in one of these test tubes with paraffin
3. cultivate 24h

- aerobic metabolism
- fermentation of GLC
- no utilisation of GLC
TRIPPLE-SUGAR IRON (TSI) test

- triple sugar iron medium is a differential medium that can distinguish between a number of G- enteric bacteria based on their physiological ability:
  → metabolize glucose, lactose and/or sucrose
  → conduct fermentation to produce acid (ind.: phenol red)
  → produce gas during fermentation (bubbles)
  → generate H$_2$S (black FeS)

PROCEDURE:
- needle the culture in the medium and after that inoculate the slants’ surface with zig-zag (snake) pattern
- cultivate 24h and observe it’s dyeing

source of sulphur and Fe$^{2+}$ = FeSO$_4$ in medium

A) *Psuedomonas aeruginosa*: Gluc (-), Lac/Suc (-), H$_2$S (-)

B) *Escherichia coli*: Gluc (+), Lac/Suc (+), H$_2$S (-)

C) *Salmonella typhimurium*: Gluc (+), Lac/Suc (-), H$_2$S (+)

D) *Shigella boydii*: Gluc (+), Lac/Suc (-), H$_2$S (-)
ENTEROTEST 16

- designed for routine identification of important strains of family Enterobacteriaceae
- this kit contains 16 tests in 2 series aimed on numerous metabolic pathways + 1 tube test proves β-galactosidase

PROCEDURE:
- prepare 3ml of cell solution with turbidity 1
- remove the foil and label the tablet with bacterial strain
- add 100µl properly shacked solution of cells into each well
- overlay the wells D – H with 2 drops of paraffin
- put the tablet in the bag
- cultivate 24h at 37°C
  (the same apply to tube test – NO PARAFFINE!)
### ENTEROTEST 16 - evaluation

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<td>CEL</td>
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<td>acid production from sucrose</td>
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<td>acid production from sorbitol</td>
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<td>TRE</td>
<td>acid production from trehalose</td>
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<tr>
<td>MAN</td>
<td>acid production from mannitol</td>
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</tbody>
</table>

**Legend**

- **Positive**: Positive reaction
- **Negative**: Negative reaction

**Notes**

- *Paradina oxej, 2 keyky: Paradina oxej, 2 drops*
- *Indole prec: Reagent for: IND*
- *Ornithine prec: Reagent for: ORN*
- *Aesculin prec: Reagent for: ESL*
- *Citrate prec: Reagent for: SCI*
ONPG paper test

→ proof of β-galactosidase production (Lac\(^+\)/Lac\(^-\))
→ o-nitrophenyl β-D-galactopyranoside undergo the hydrolysis on yellow o nitrophenol

PROCEDURE:
- insert the ONPG test stripe into bacterial solution
- cultivate 24h at 37°C

OXIDASE paper test

- cytochrome c oxidase transfer e\(^-\) on O\(_2\)
- oxidase reagents change their colors depending on their oxidative states
→ differentiation Pseudomonas, Alcaligenes, Flavobacterium...

PROCEDURE:
- smear the bacterial culture on a paper and submerge it into the ENTEROtest
- positive result is intensively blue max. 2min.
→ NEXT WEEK

- **short test** (5 – 6 questions/10 – 15min.) similar to the previous one
  - a.) b.) c.) d.) + counting

- **practical examination (1h)**
  - preparation of some kind of preparative
  - cells’ description + additional questions

It’s quite possible that THERE WILL BE NO SUBSTITUTIONAL TERM !!!