# Relationship between disinfectants and antibiotic resistance: A new dimension in '**Superbug**'

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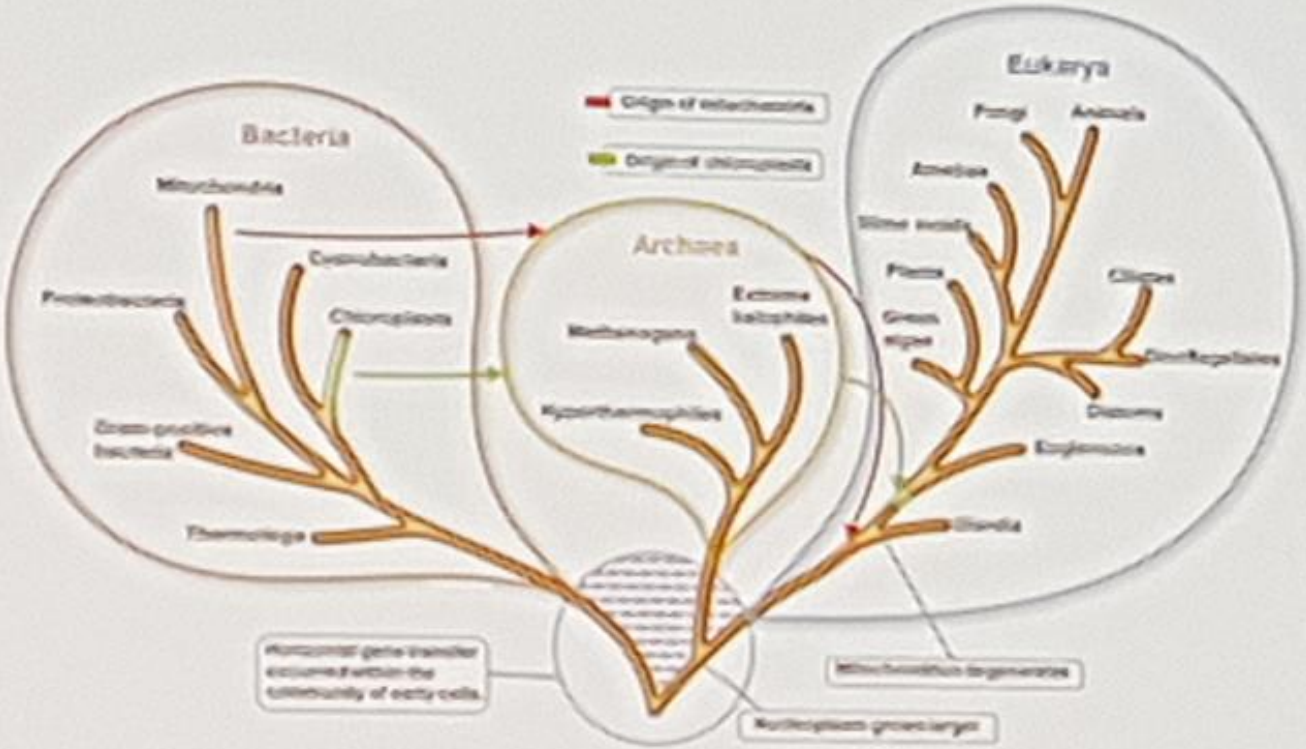
# Microbes

Tiny creatures which have enormous functionality





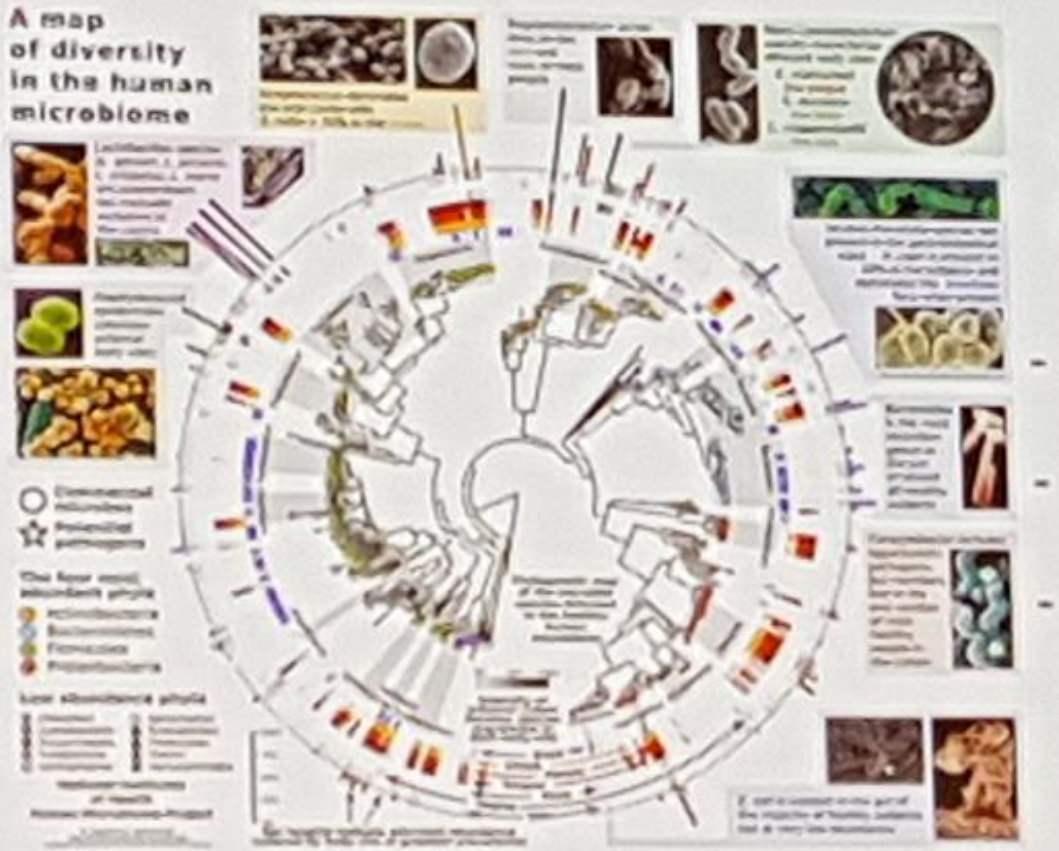
- On Earth from the beginning
- Diverse
- Evolve easily
- Exchange functionality

# Microbes

## Bad Microbes

Cause disease

*Pathogens*




## Good Microbes

Make beer, cheese etc.

- Help digestion
- Protect from chemicals
- Etc.

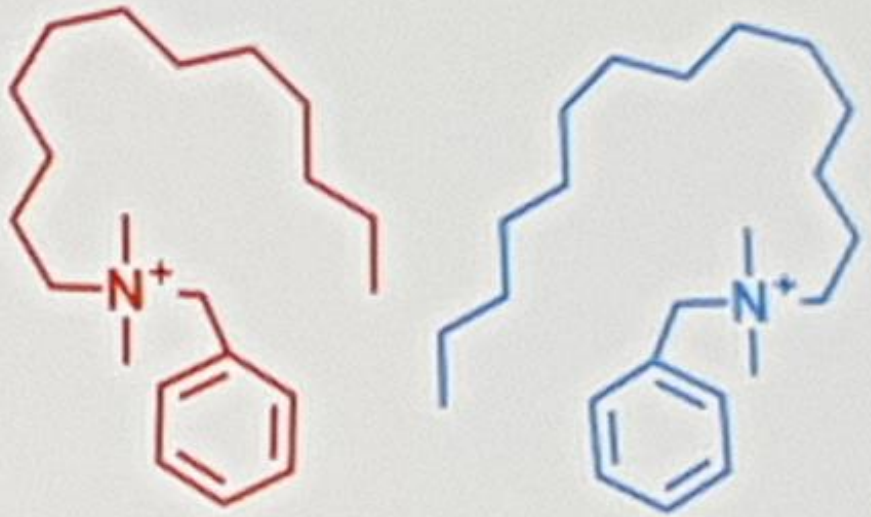
# Fight against pathogens

Starting from the World War II, **antibiotics** and **biocides** were recognized as the weapons against pathogens; *Sulfonamides, beta-lactams, quaternary ammonium compounds*

Antimicrobials save lives of millions

# QACs in general


Domestic products




Benzalkonium Chlorides

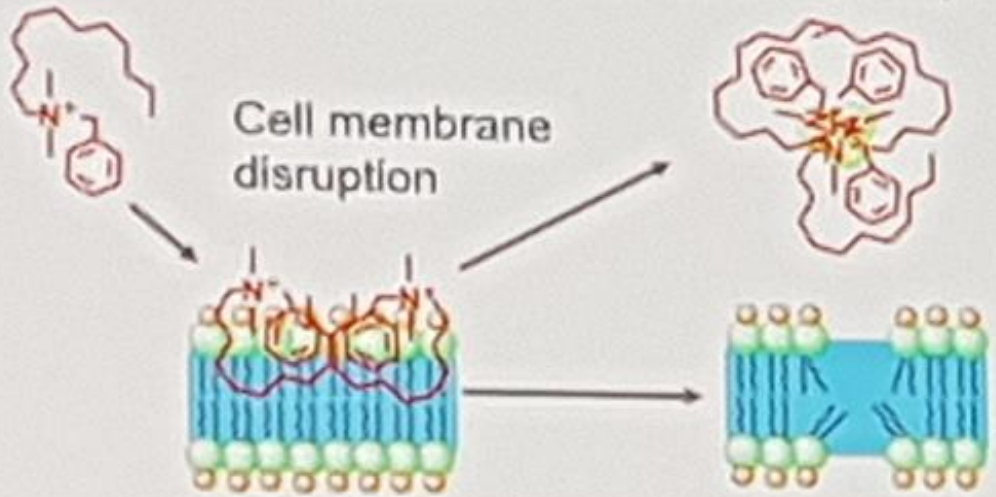
BACs are used in **HOSPITALS**

Clean floors
Clean equipments

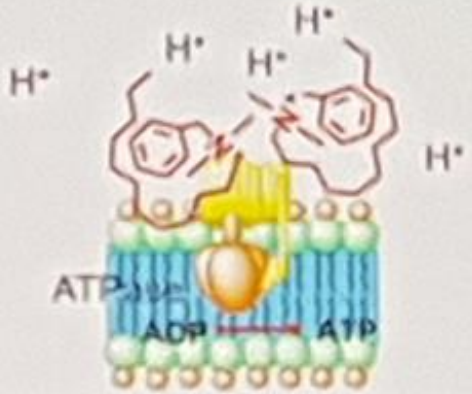
**400-1000 mg/L**

# Mode of action

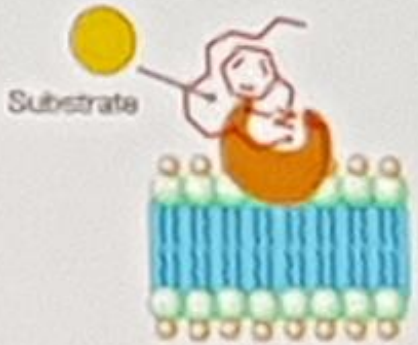
**@ high concentration (CMC)**




**@ moderate and low concentration**




Inhibits ATP synthesis
by dissipation of PMF



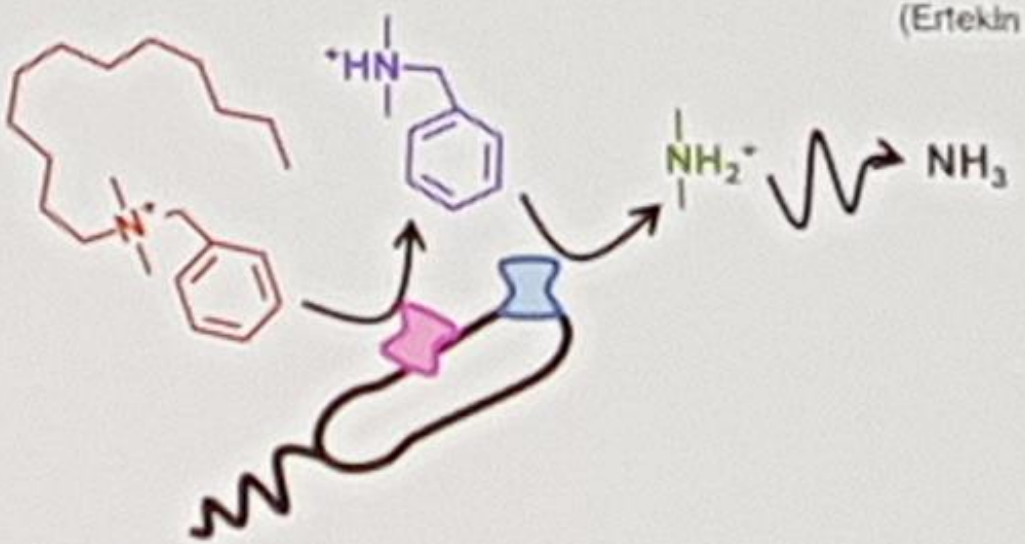

Enzyme-substrate binding

# BAC Degraders

**BUT**

We have isolated a new bacterium

(Ertekin etal., ES&T, 2016)

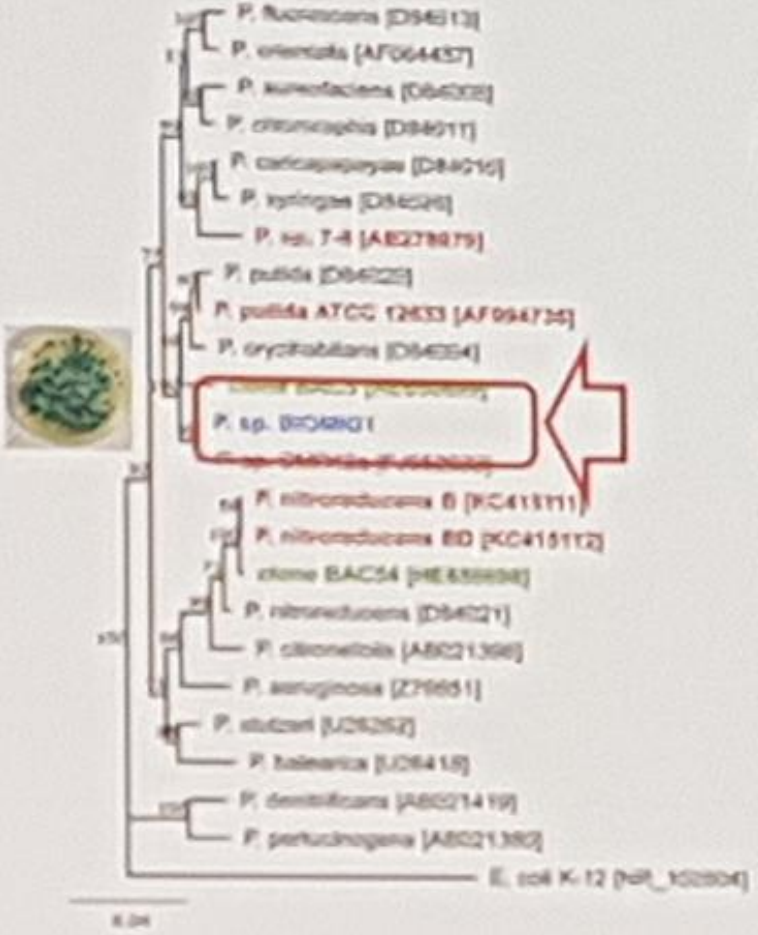



***Pseudomonas sp.* BIOMIG1**

Resistant to biocides and antibiotics
but
Not PATHOGENIC

# *Pseudomonas sp.* BIOMIG1

16S rRNA based Phylogenetic Analysis

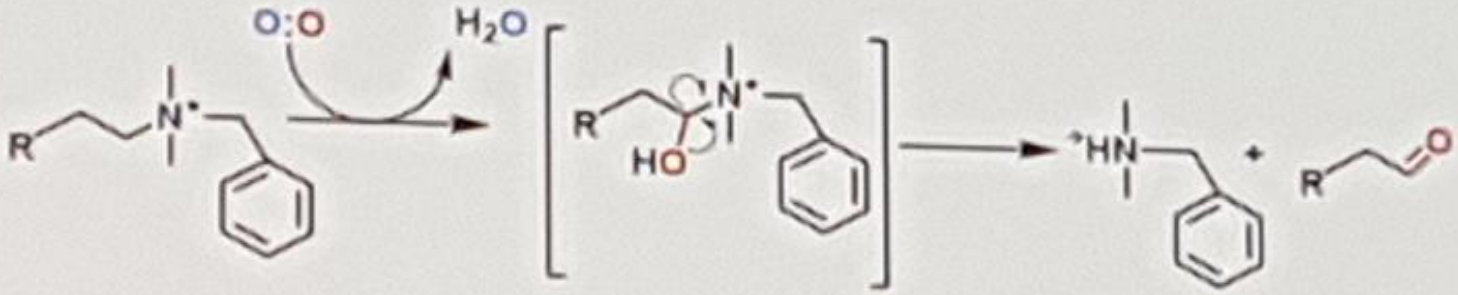



BAC degraders are RARE

BIOMIG1 mineralizes BACs to $NH_3$ and $CO_2$

# Biotransformation Pathway

## N-dealkylation Reaction




**Enzyme** catalyzed reaction

# Comparative Genomics

Ertekin et al. 2017, ES&T

BIOMIG1 Phenotypes

1 Complete BAC degrader
[BIOMIG1$^{BAC}$]

GENE is on a
mobile genetic element

2 BDMA accumulator
[BIOMIG1$^{BDMA}$]

# Comparative Genomics

Erfekin et al. 2017, ES&T

BIOMIG1 Phenotypes

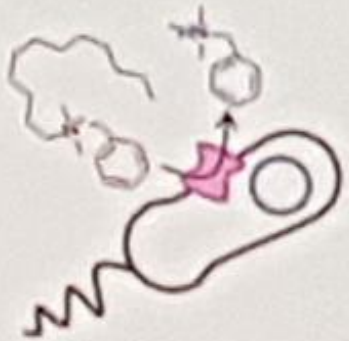
Feed with BDMA
Dilution to extinction

Can only degrade BDMA

1 Complete BAC degrader [BIOMIG1$^{BAC}$]

3 BDMA degrader [BIOMIG1$^{BD}$]

**GENE is on a mobile genetic element**



2 BDMA accumulator [BIOMIG1$^{BDMA}$]

# Comparative Genomics

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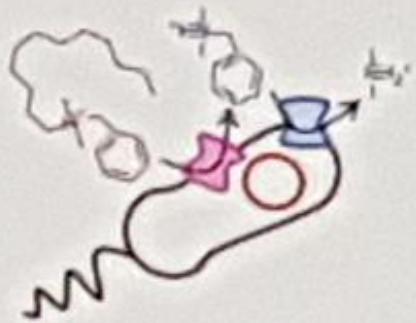
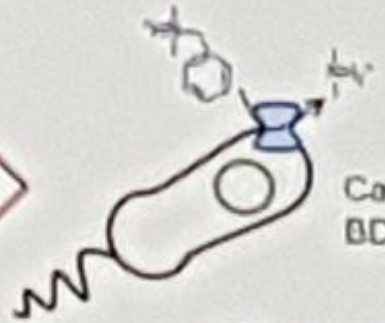


Feed with BDMA
Dilution to extinction



Can only degrade BDMA

1 Complete BAC degrader [BIOMIG1$^{BAC}$]

3 BDMA degrader [BIOMIG1$^{BC}$]

**GENE is on a mobile genetic element**

Culture in broth excluding BAC for a few generations

Loses its capability to accumulate BDMA

2 BDMA accumulator [BIOMIG1$^{BDMA}$]

4 Non-degrader [BIOMIG1$^{0}$]

# Comparative Genomics


Sequence their GENOMES

Pairwise comparison

Identify different genes

[BIOMIG1$^{ST}$]

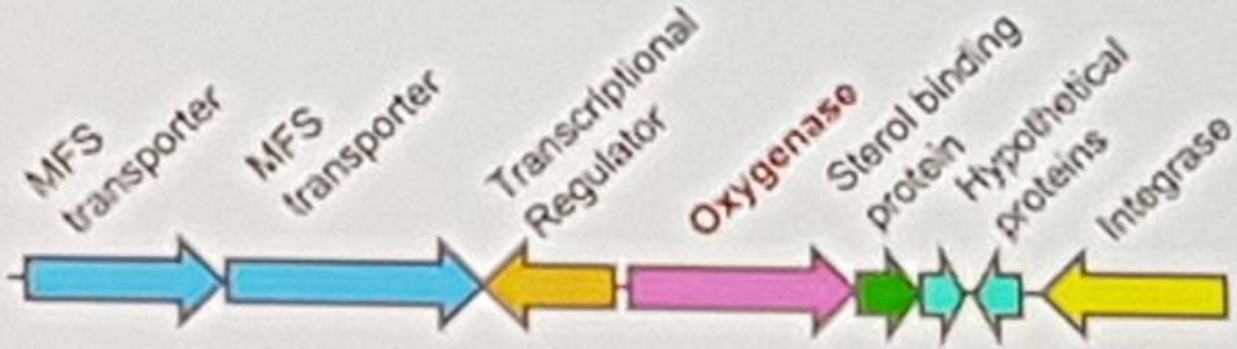
[BIOMIG1$^{BD}$]

[BIOMIG1$^{BDMA}$]

[BIOMIG1$^{BAC}$]

# Gene for BAC→BDMA

An array of 8 genes are responsible for BAC biotransformation to BDMA



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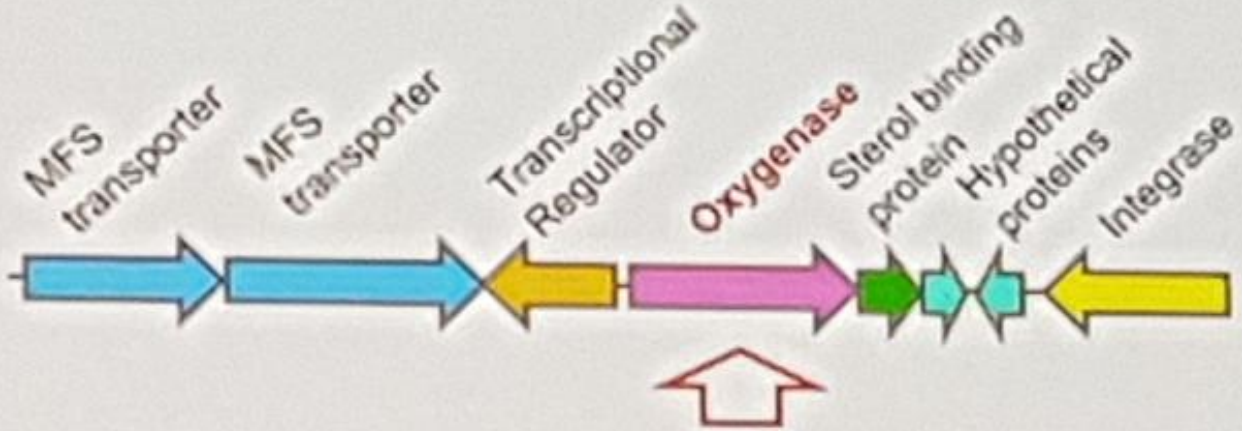
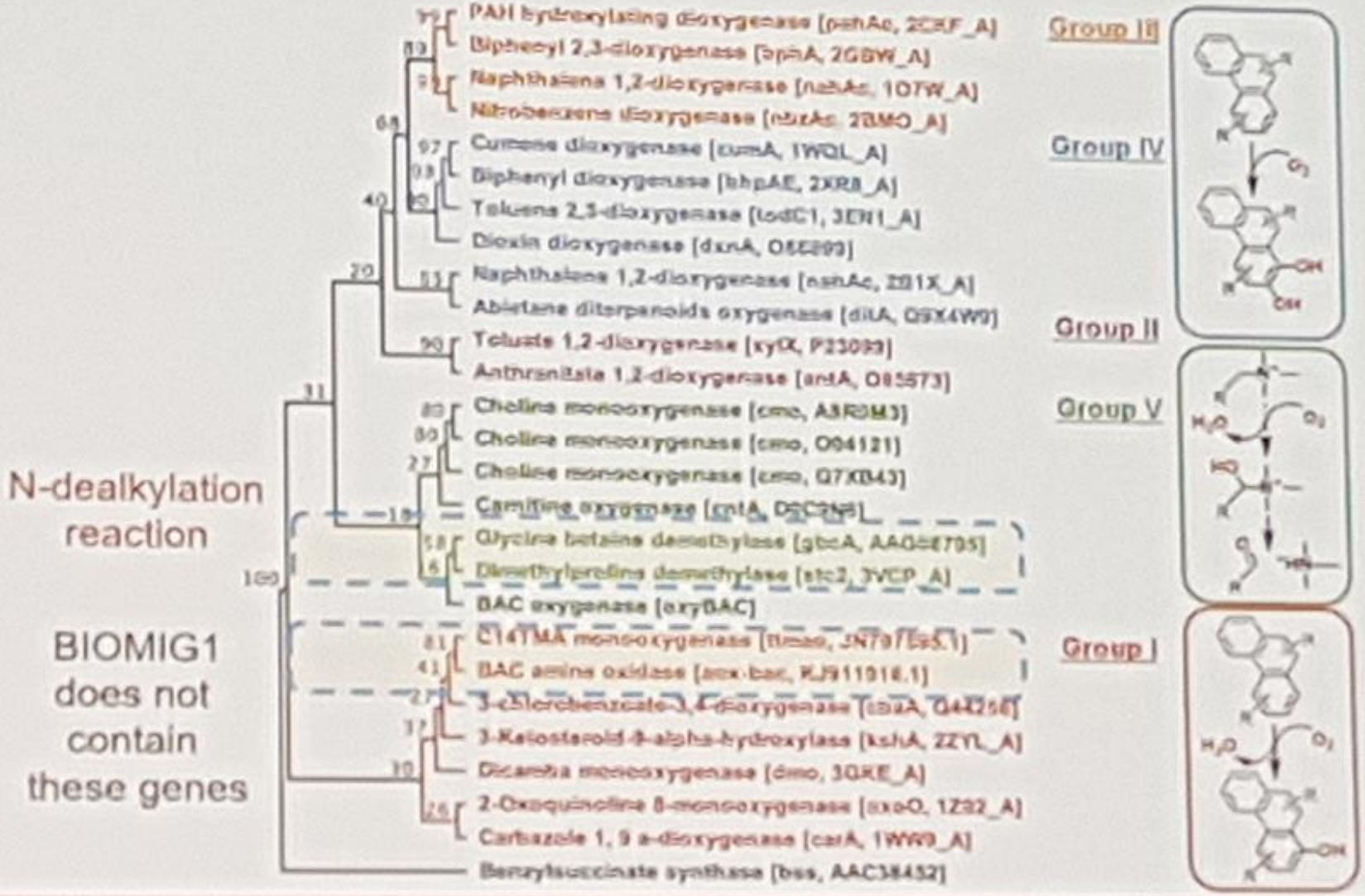



Only catabolic gene

*oxyBAC*

# Phylogenetic classification of oxyBAC

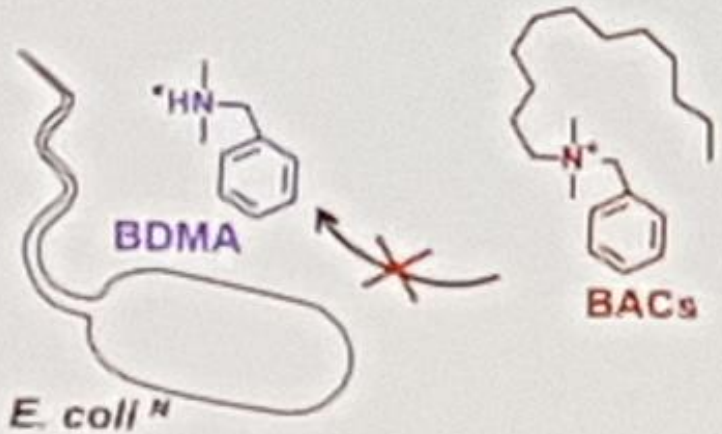
oxyBAC is a Rieske Oxygenase
Rieske Domain + Non-heme Iron center



# Heterologous expression of *oxyBAC* in *E. coli*

Last confirmation:

- *E. coli* cannot degrade BACs



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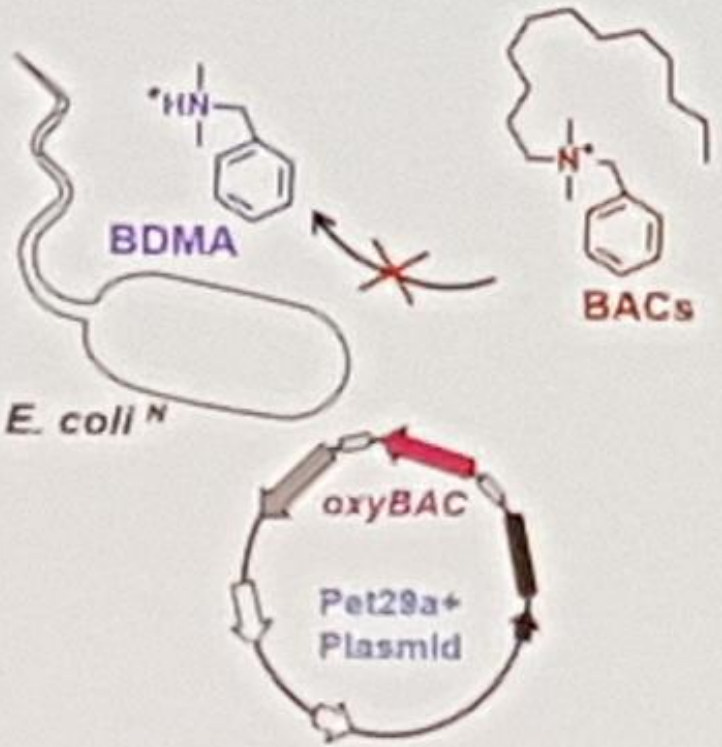
- *oxyBAC* gene was synthesized and cloned into a vector



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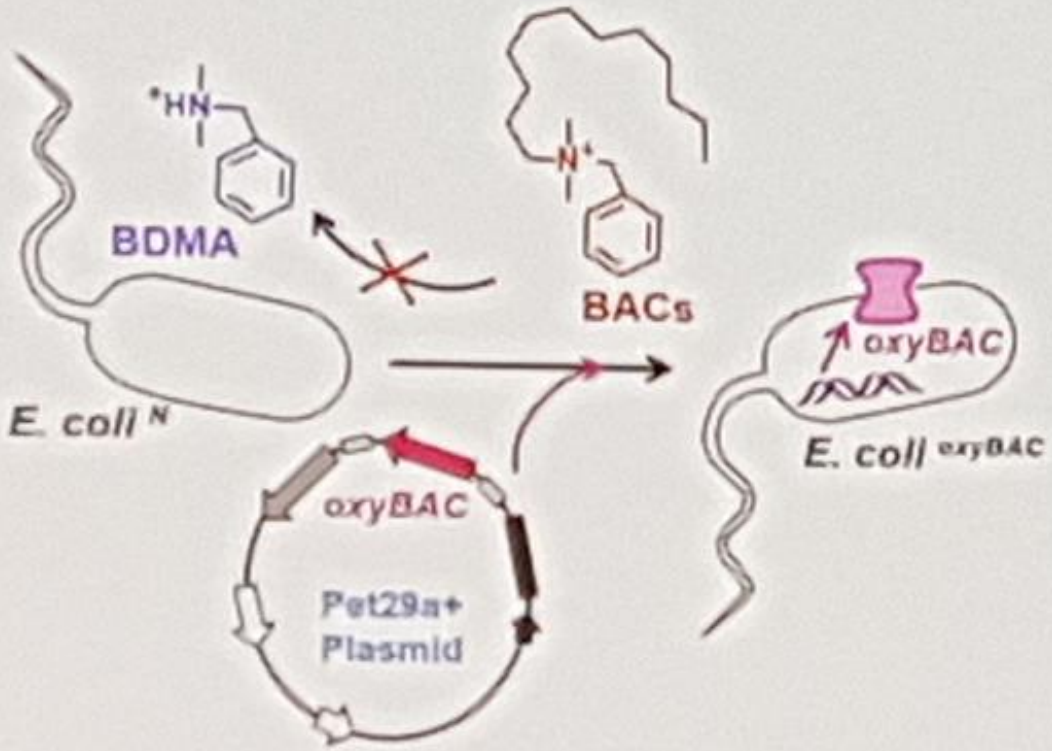

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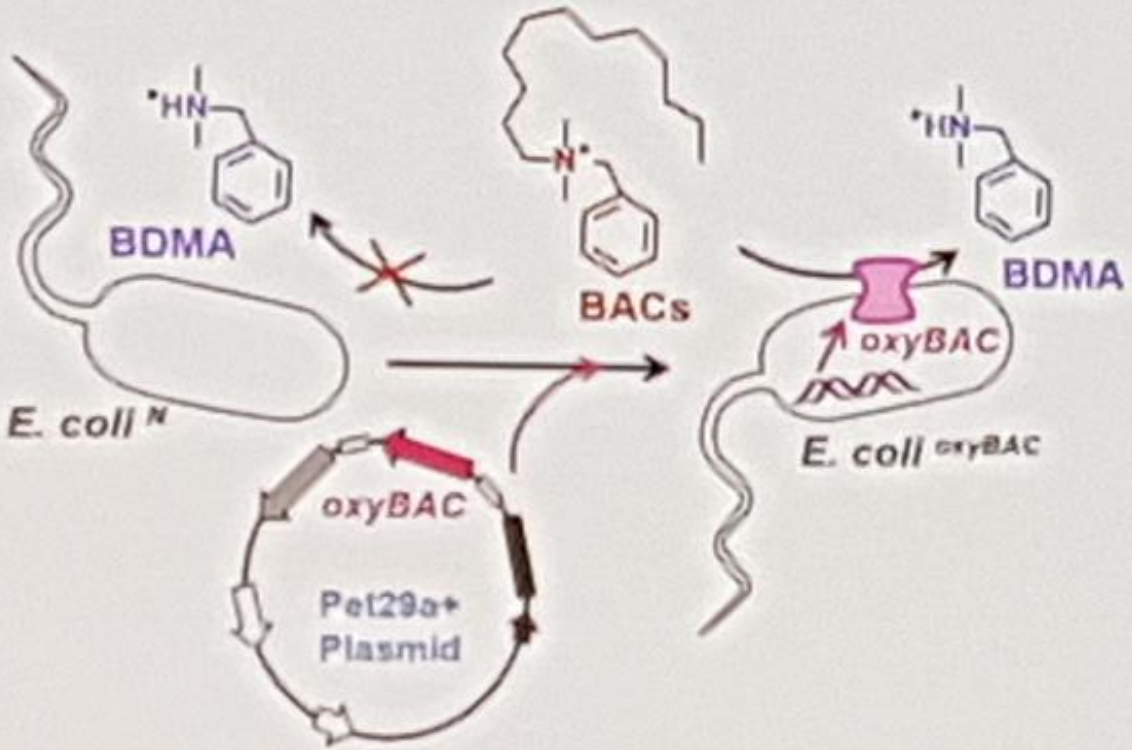
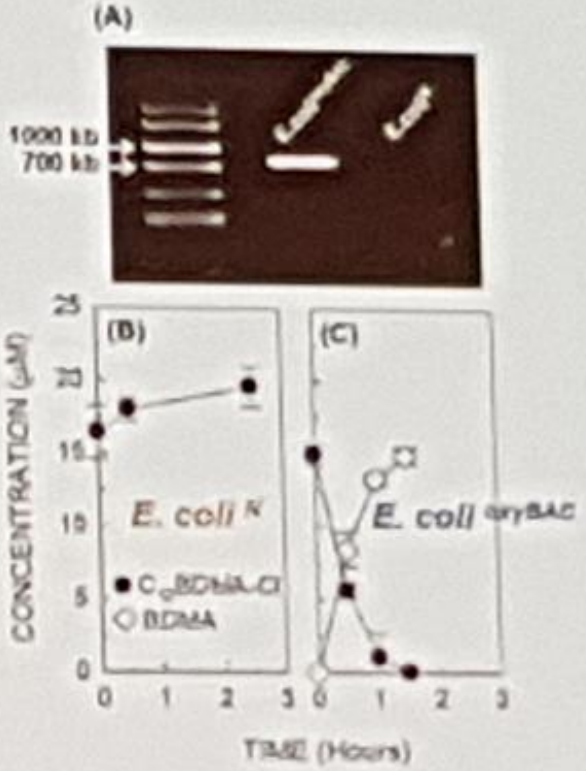


# BIOMIG1 is resistant to many antibiotics

| Antibiotic | MIC (mg/L) | Resistance Breakpoint (mg/L) | Resistance class |
|---|---|---|---|
| **Beta lactams** | | | |
| Penicillin G, P | >256 | 2 | R |
| Amoxicillin, AML | >256 | 8 | R |
| Dicloxacillin, DCX | >256 | - | |
| Carbenicillin, CAR | 256 | - | |
| **Quinolone/Fluroquinolones** | | | |
| Ciprofloxacin, CIP | 0.38 | 0.5 | S |
| Enrofloxacin, ENR | 0.75 | - | |
| Norfloxacin, NOR | 1 | 2 | S |
| Ofloxacin, OFX | 1.5 | 0.5 | R |
| Levofloxacin, LEV | 0.5 | 1 | S |
| **Sulfonamides** | | | |
| Sulfamethoxazole, SMX | 64 | 8 | R |
| Mafenide, MFD | 128 | - | |
| Trimethoprim, TM | >32 | 2 | R |
| **Tetracyclines** | | | |
| Tetracycline, TE | 6 | 16 | S |
| Doxycycline, DXT | 4 | 8 | S |
| **Macrolides** | | | |
| Clarithromycin, CLR | >256 | 32 | R |
| Erythromycin, E | >256 | 16 | R |
| Azithromycin, AZM | >256 | 4 | R |
| **Lincosamides** | | | |
| Clindamycin, CD | >256 | 16 | R |
| **Aminoglycosides** | | | |
| Kanamycin, K | 1 | - | |
| **Glycopeptides** | | | |
| Vancomycin, VA | >256 | 4 | R |
| **Chloramphenicols** | | | |
| Chloramphenicol, C | >256 | 32 | R |

# BIOMIG1 is resistant to many antibiotics

The European Committee on Antimicrobial Susceptibility Testing - **EUCAST**

**Clinical breakpoints database**

# BIOMIG1 multidrug resistance genes

**CARD:** The Comprehensive Antibiotic Resistance Database

**RGI:** Resistance Gene Identifier

| | Resistance Determinant | Target Antibiotic |
|---|---|---|
| Resistance Nodulation Division **(RND)** Multidrug and toxic compound extrusion **(MATE)** | MexCD-OprJ | Fluoroquinolones, chloramphenicol ve macrolides |
| | MexJK-OpmH | Triclosan |
| | TriABC-OpmH | Triclosan |
| | MexEF-OprN | Fluoroquinolones, chloramphenicol ve trimethoprim |
| | MuxABC-OpmB | Tetracycline, erythromycin |
| | PmpM | Fluoroquinolones, benzalkonium chloride, |
| | **oqxAB** | Fluoroquinolones |
| | AdeABC | Tigecycline |
| | **MacAB-TolC** | Macrolides |
| | msbA | Ciprofloxacin |
| | **PDC-5** | Class C beta-lactamase, all beta-lactams |
| | **Cat** | Chloramphenicol |
| | tetX | Tetracycline, doxycycline |
| | **vanW** | Vancomycin |

Most of the **MDR genes** are on mobile genetic elements

# Is there a microbe worse than a SUPER Bug?

**BAD Bug** : microbe that makes you sick



**SUPER Bug** : resistant to many antibiotics



**HYPER Bug** : resistant to antibiotics and degrade disinfectants

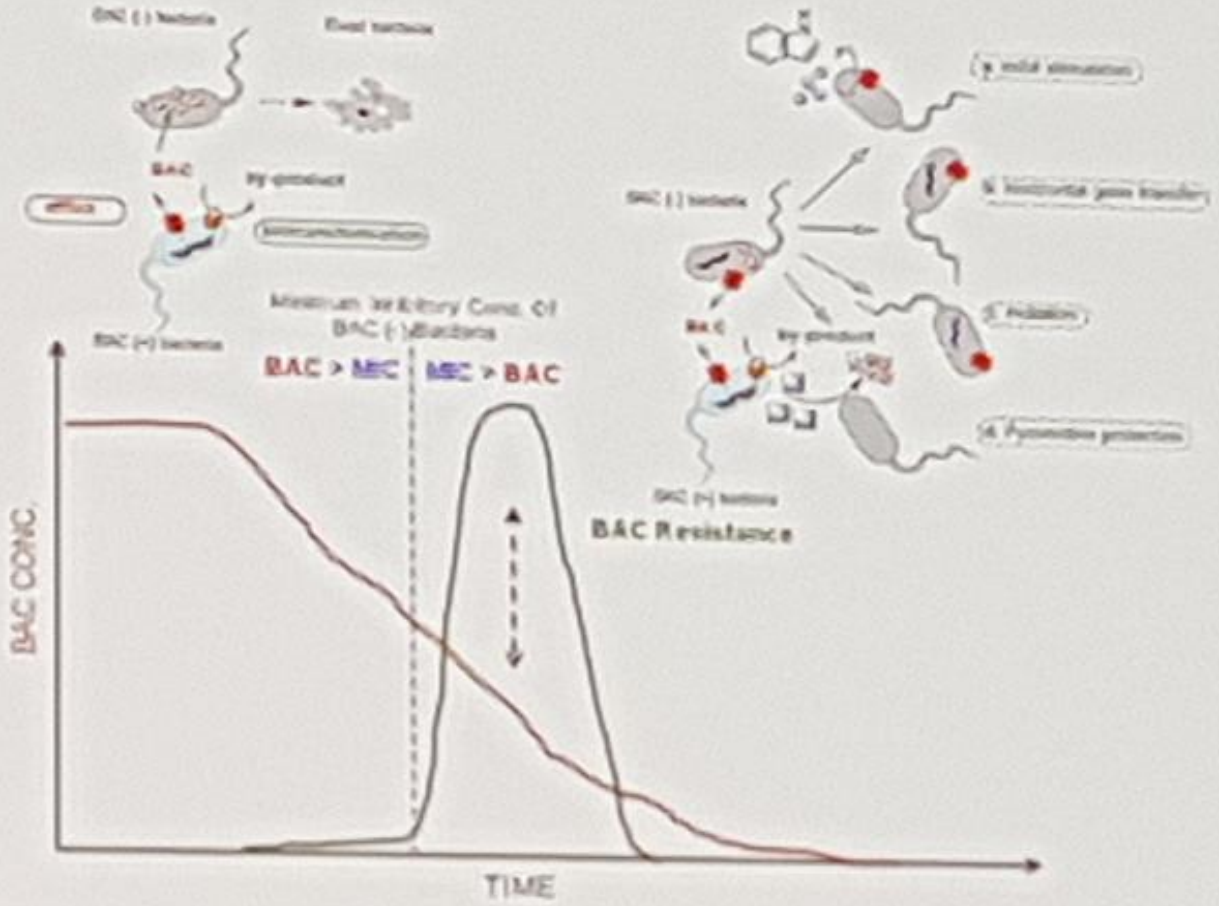
# Assisted Resistance

@ multiple species community

Gamechanger's Game

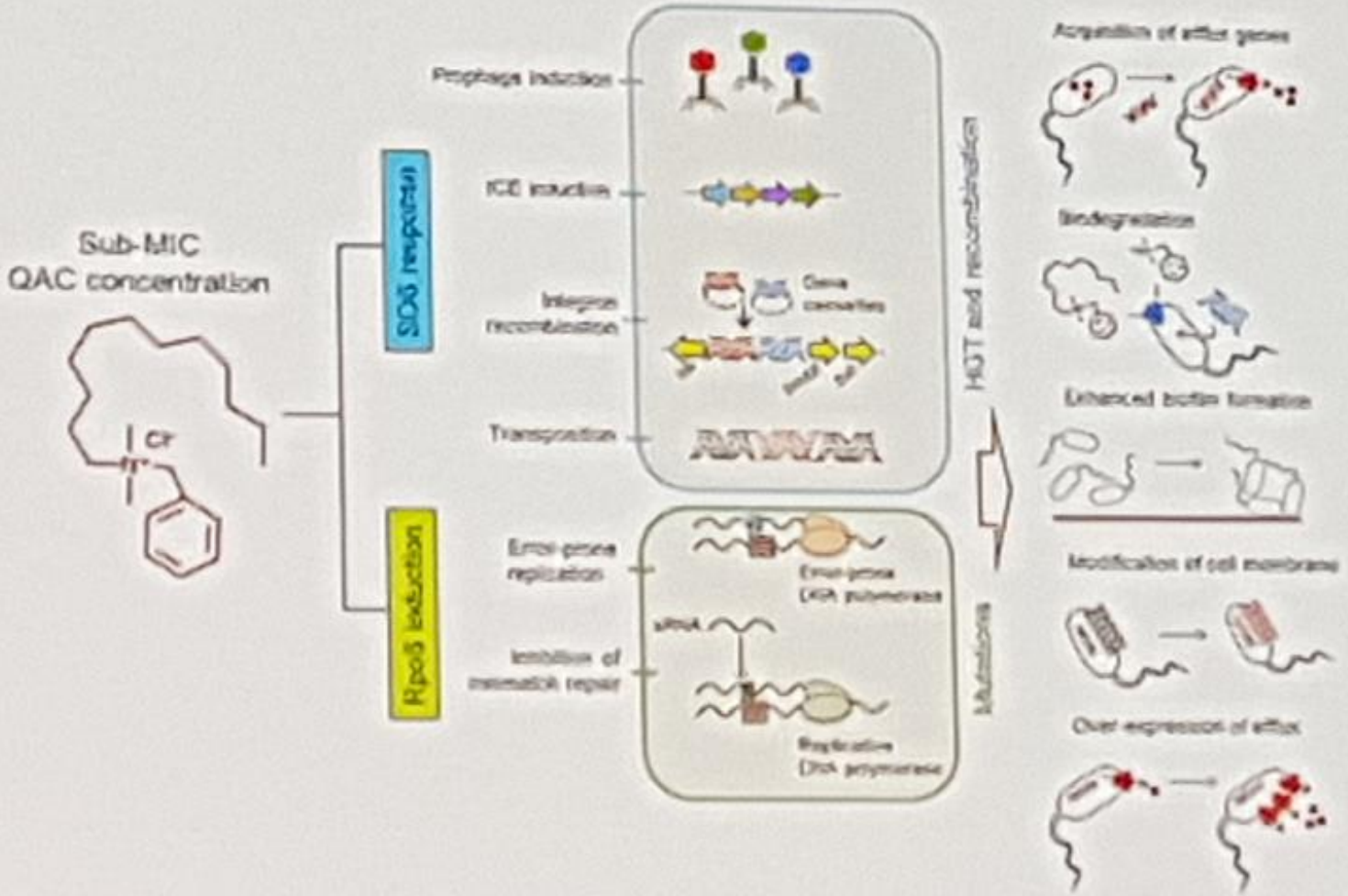
# Assisted Resistance

@ where inhibitor gets consumed



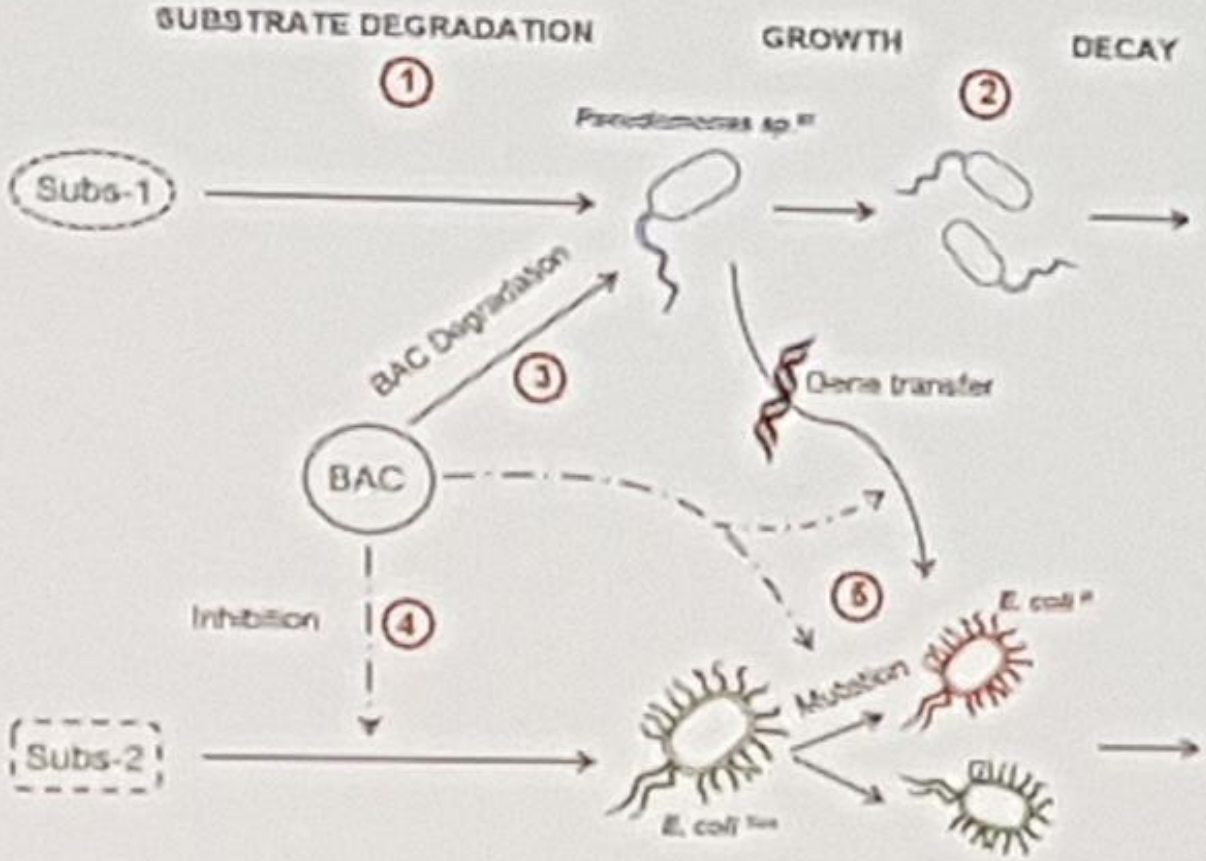
# Assisted Resistance

@ subinhibitory concentration



# Experimental Evidence

*Pseudomonas sp.* BIOMIG1 + *E. coli* in mallose



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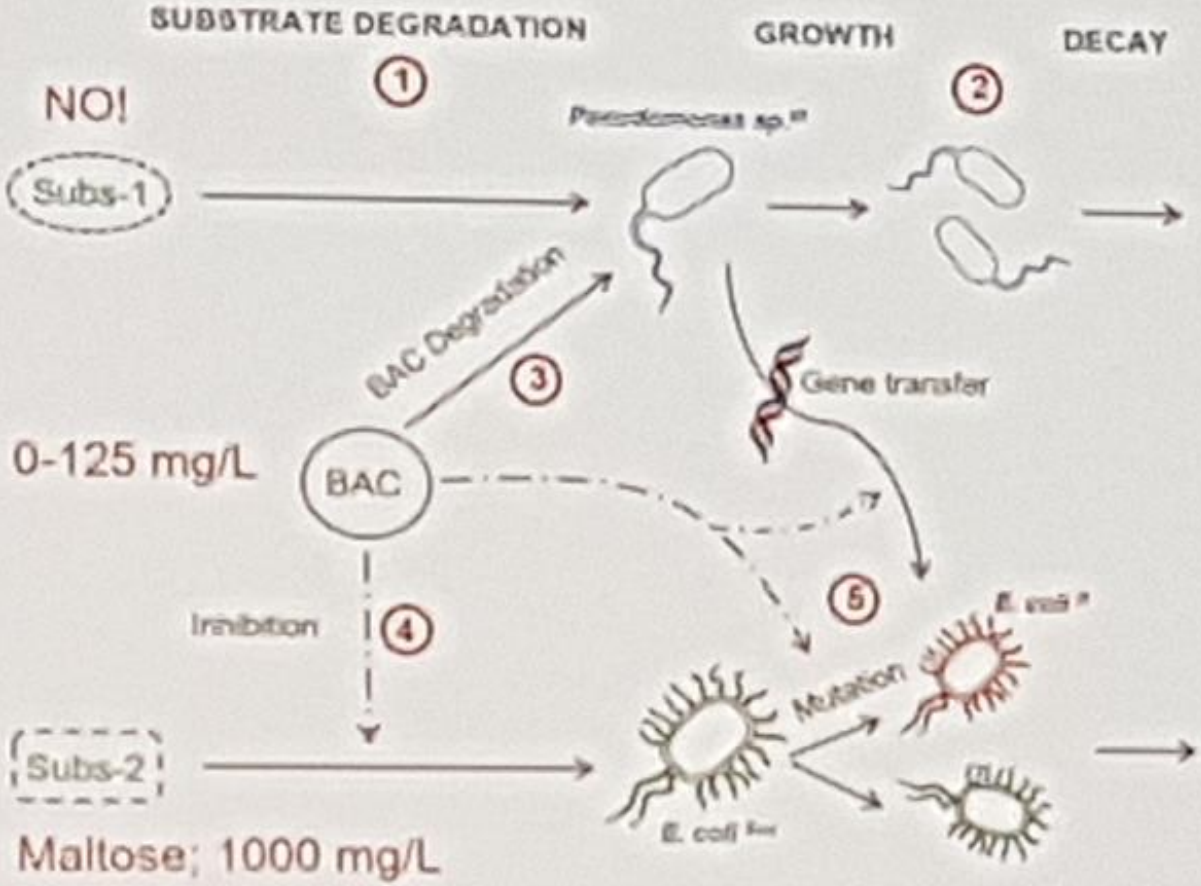

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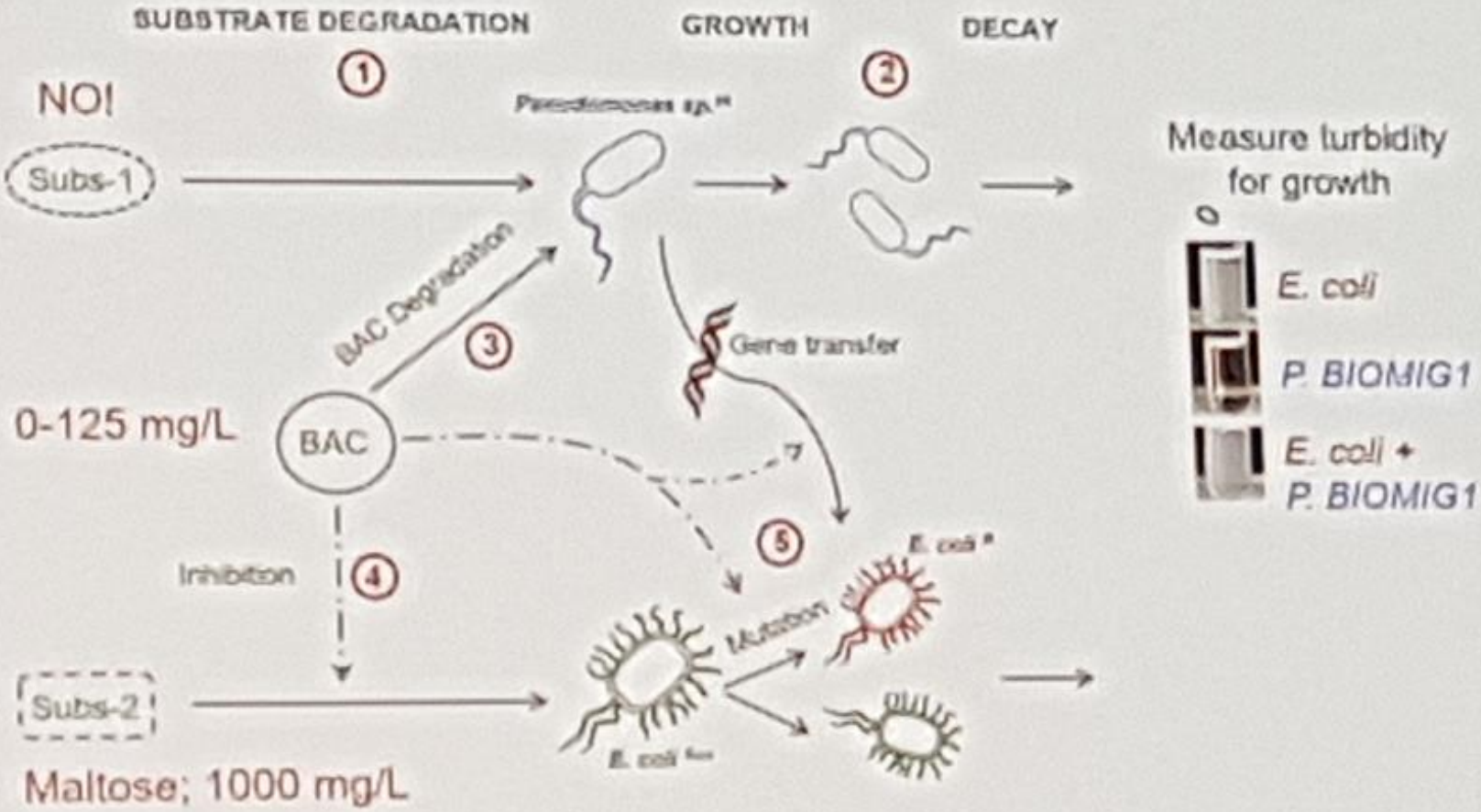
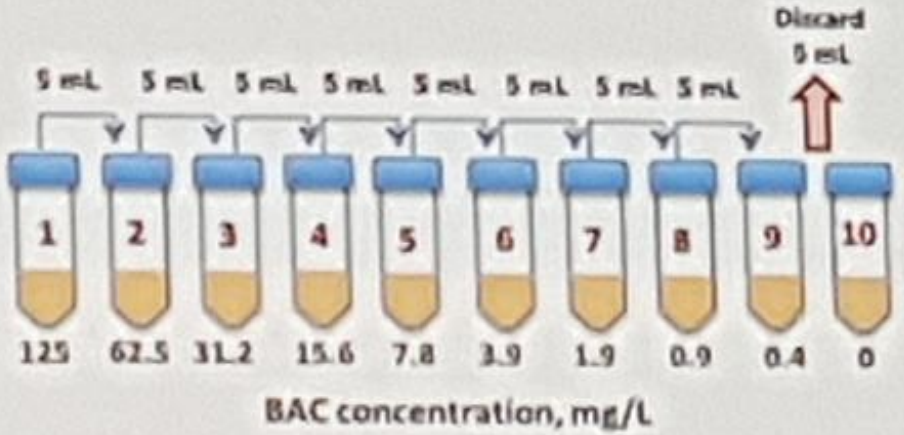


# Experimental Evidence

Maltose can only be used by *E.coli*

Control
*E.coli*
*BIOMIG1*
*Co-culture*

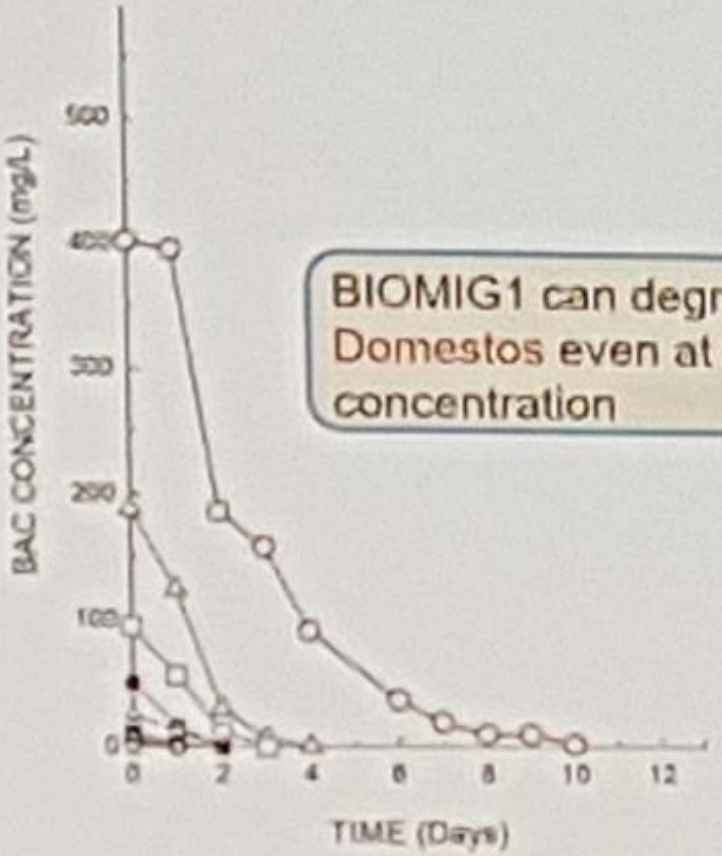



1 000 mg/L Maltose was added to all falcon tubes

- Every day, growth measured with UV/Vis spectrometer at **600nm**
- **BACs concentration** measured with **HPLC method**

Ertekin and Tezel, 2016

# Experimental Evidence



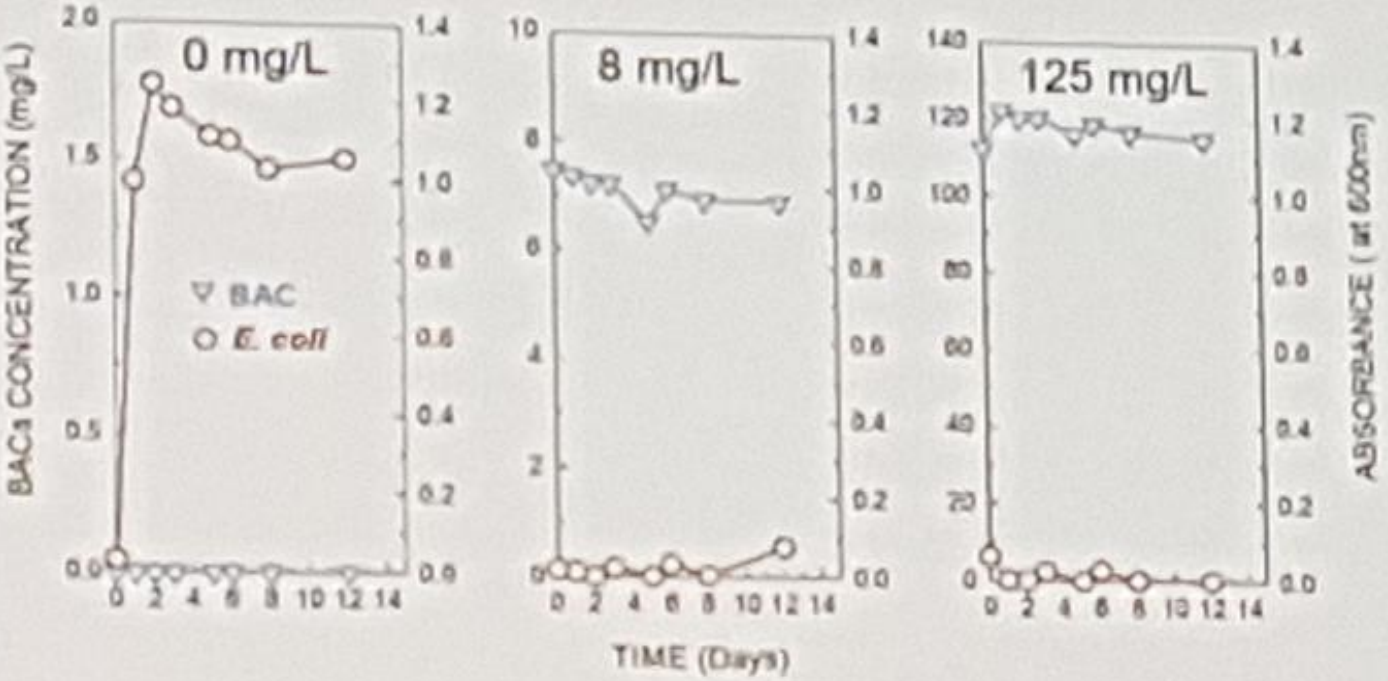

BIOMIG1 can degrade BACs in Domestos even at application concentration

Domestos is commercial disinfectant to clean surfaces

Domestos® contains 16 753 mg/L BACs ($C_{12}$-$C_{16}$)

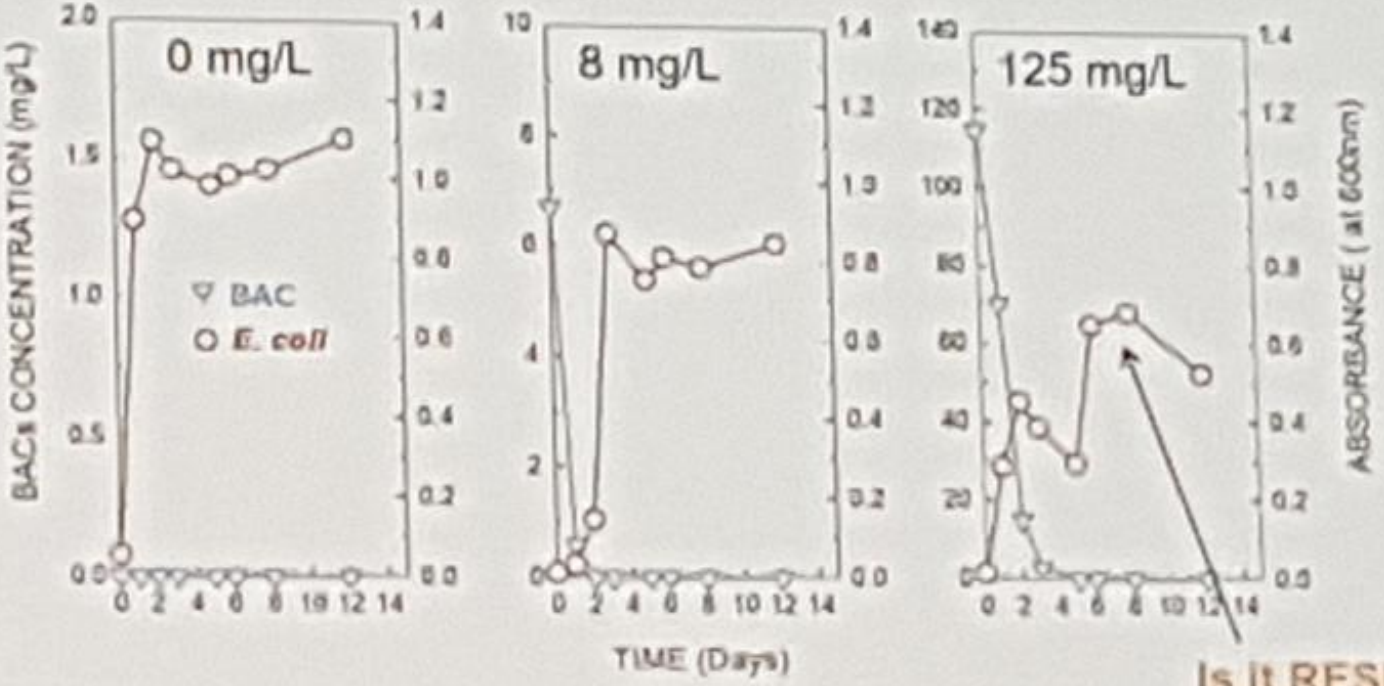
# *E. coli* only

E. coli cannot grow above 8 mg/L BAC when Domestos was applied



# *E. coli* with BIOMIG1

E. coli can grow even at
125 mg/L BAC when
Domestos was applied




**! ALARMING !**

# Recommendations

We have to think about new generation of disinfectants

**HEALTH** vs **REACH**

Or optimize practice: additional ingredients to *inactivate oxyBAC*

**R&D**

BIOMIG

www.biomig.boun.edu.tr

# Thank You

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