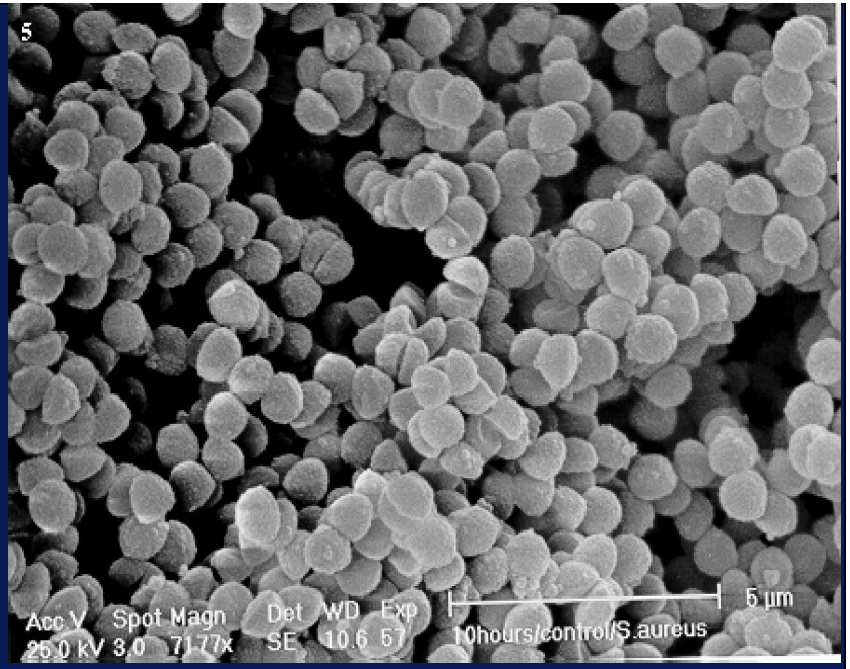
Interactions between biocides & bacteria

Surface disinfection and antimicrobial surfaces



CARDIFF UNIVERSITY
PRIFYSGOL CAERDYDD

Dr Jean-Yves Maillard
Welsh School of Pharmacy
Cardiff University
Wales

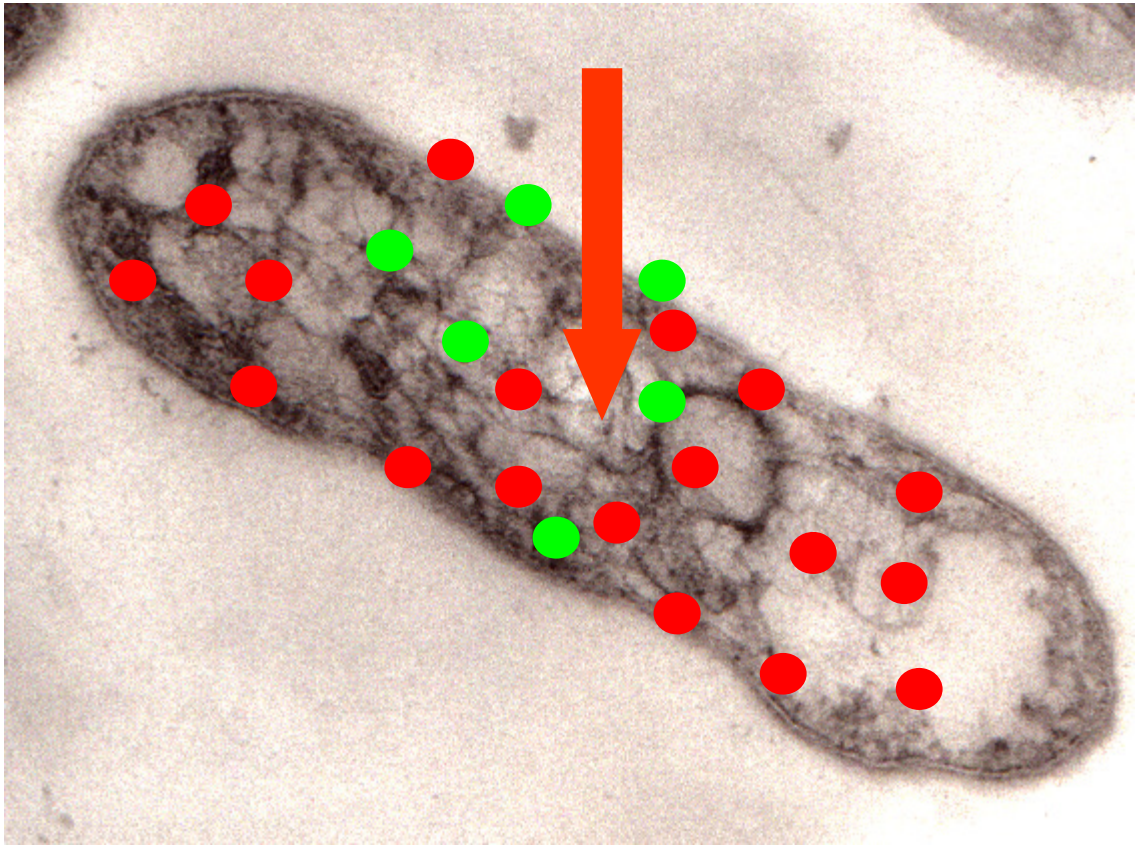
CONTENT

- PRINCIPLE FOR ACTIVITY
- LIMITATIONS IN ANTIMICROBIAL ACTIVITY
- UNDERSTANDING MICROBE-ANTIMICROBIAL INTERACTIONS

BIOCIDES – principles

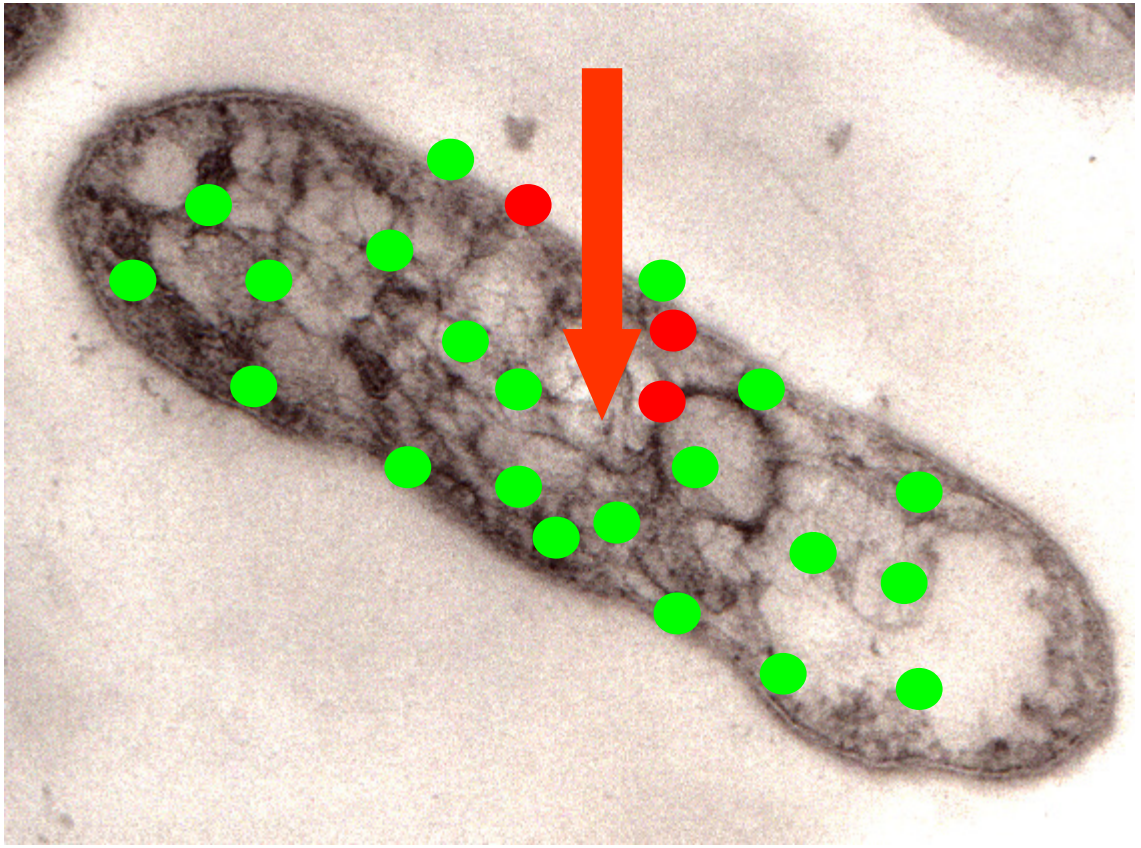
GENERAL CONSIDERATIONS

- Contact
- Penetration
- Accumulation



GENERAL CONSIDERATIONS

- Primary targets
- Secondary targets



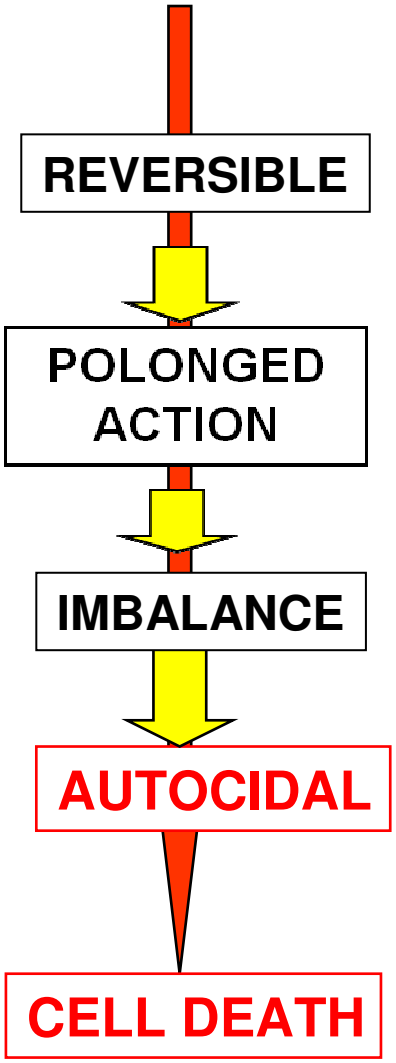
MECHANISMS OF ACTION – principles

EVENTS LEADING TO CELL DEATH - Degree of damage and autocidal activity

- Disruption of the transmembrane PMF leading to an uncoupling of oxidative phosphorylation and inhibition of active transport across the membrane
- Inhibition of respiration or catabolic/anabolic reactions
- Disruption of metabolic processes
- Disruption of replication

- Loss of membrane integrity resulting in leakage of essential intracellular constituents (K^+, inorganic phosphate, pentoses, nucleotides and nucleosides proteins)

- coagulation of intracellular materials
- lysis



LIMITATIONS IN ACTIVITY

FACTORS affecting antimicrobial efficacy

Factors inherent to the product
- concentration
- formulation
- water activity
- pH*

Factors inherent to the application
- surface
- organic load (soiling)
- temperature
- contact time
- humidity

Factors inherent to the micro-organism
- type
- number
- phenotype
- pH*

LIMITATIONS IN ACTIVITY

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Factors inherent to the micro-organism
- type
- number
- phenotype
- pH*

→ concentration exponent

Biocides		Concentration Exponent
Phenolics		4-9.9
Alcohols	Benzyl alcohol	2.6-4.6
	Aliphatic alcohols	6.0-12.7
Cationics	Chlorhexidine	2
	Polymeric biguanides	1.5-1.6
	QACs	0.8-2.5
	Dyes (Crystal violet)	0.9
Aldehydes	Formaldehyde	1
	Glutaraldehyde	1
Peroxygens	Hydrogen peroxide	0.5
Metallic salts	Silver nitrate	0.9-1.0
	Mercurials	0.03-3.0
Organic acids	Parabens	2.5
	Sorbic acid	2.6-3.2

LIMITATIONS IN ACTIVITY

FACTORS affecting antimicrobial efficacy

Factors inherent to the product
- concentration → concentration exponent
- formulation → bioavailability of biocide
- water activity → synergism/antagonism
- pH* → delivery

Factors inherent to the application → stability of biocide
- surface
- organic load (soiling)
- temperature
- contact time
- humidity

Factors inherent to the micro-organism
- type
- number
- phenotype
- pH*

LIMITATIONS IN ACTIVITY

FACTORS affecting antimicrobial efficacy

Factors inherent to the product
- concentration
- formulation
- water activity
- pH*

Factors inherent to the application
- surface
- organic load (soiling)
- temperature
- contact time
- humidity

Factors inherent to the micro-organism
- type
- number
- phenotype
- pH*

Biocides		Soiling
Phenolics		+++
Alcohols	Benzyl alcohol Aliphatic alcohols	+
Cationics	Chlorhexidine Polymeric biguanides QACs Dyes (Crystal violet)	+++
Aldehydes	Formaldehyde Glutaraldehyde	+
Peroxygens	Hydrogen peroxide	-
Metallic salts	Silver nitrate Mercurials	+++
Organic acids	Parabens Sorbic acid	++

LIMITATIONS IN ACTIVITY

FACTORS affecting antimicrobial efficacy

Factors inherent to the product
- concentration
- formulation
- water activity
- pH*

Factors inherent to the application
- surface → Type of surfaces
- organic load (soiling)
- temperature → temperature exponent
- contact time
- humidity

Factors inherent to the micro-organism
- type
- number
- phenotype
- pH*

LIMITATIONS IN ACTIVITY

FACTORS affecting antimicrobial efficacy

Factors inherent to the product
- concentration
- formulation
- water activity
- pH*

Factors inherent to the application
- surface
- organic load (soiling)
- temperature
- contact time
- humidity

Factors inherent to the micro-organism
- type
- number
- phenotype
- pH*

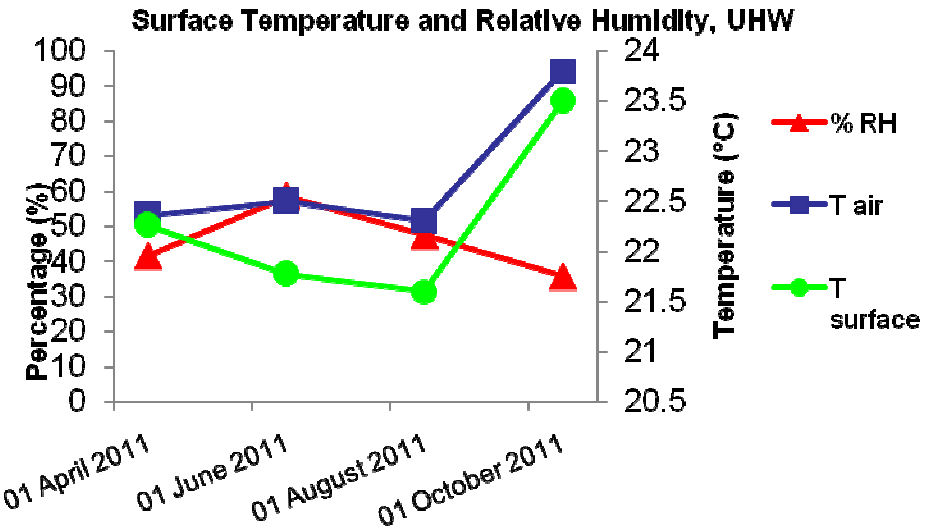
JIS-Z-2801

Temperature: 37°C

Humidity: 100% humidity (covered with film)

Contact time: 24 hours

Inoculum: drop of bacterial inoculum



LIMITATIONS IN ACTIVITY

FACTORS affecting antimicrobial efficacy

Factors inherent to the product
- concentration
- formulation
- water activity
- pH*

Factors inherent to the application
- surface
- organic load (soiling)
- temperature
- contact time
- humidity

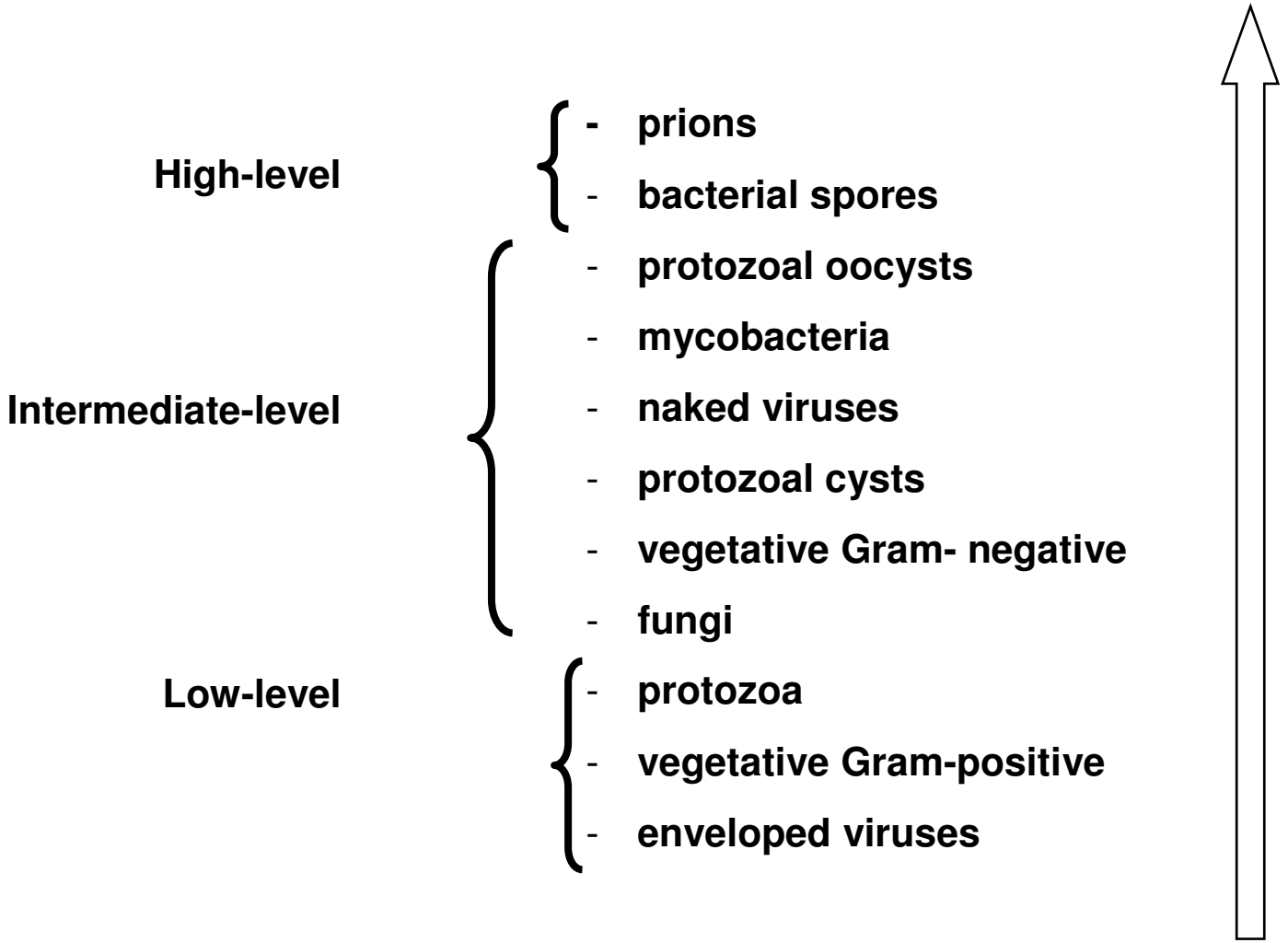
Factors inherent to the micro-organism
- type
- number
- phenotype
- pH*

→ insusceptible/resistant
→ dormant – super-dormant (spores)
→ biofilm

→ growth of microorganisms

LIMITATIONS IN ACTIVITY

FACTORS affecting antimicrobial efficacy



LIMITATIONS IN ACTIVITY - BIOFILMS

Biofilm resistance mechanisms	Observation
Establishing a reduced local biocide concentration	Diffusion gradient Non-specific neutralising interaction with cell constituents Lysed bacterial community offering mechanistic inactivation as a result of increased organic load
Enhanced bacterial insusceptibility	Degradation of antimicrobial Efflux (more effective against lower concentrations) Early stress-response
Slow growth/metabolism	A local chemical gradient (reduced nutrients / O_2) can retard growth rate, mitigating against biocide injury
Selection for increased resistance	Formation of pockets of surviving bacteria Dormant cells (which re-grow rapidly in the presence of exudates released from lysed community)
Acquisition of new resistance determinants	Increased genetic exchange
Intrinsic resistance	Nature of micro-organisms (i.e. some being more resistant than others)

Maillard & Denyer. *Chemica Oggi 2009*; 27:26-8.

MECHANISMS OF ACTION – principles

COMPLEX

- type of biocide
- micro-organisms
- concentration

GENERALISATION IS POSSIBLE (to some extent)

- membrane active agents
- oxidising agents
- alkylating agents

Differences in the fine mechanisms of action

MECHANISMS OF ACTION – penetration

INITIAL INTERACTIONS (initial binding and uptake)

Penetration

- Interactions with outer cell wall structures
- Interactions at the cytoplasmic membrane level
- Interactions with cytoplasmic constituents

} BACTERIA FUNGI PROTOZOA

- Interactions with spore structures

} SPORES

- Interactions with envelope
- Interactions with capsid
- Interactions with nucleic acid

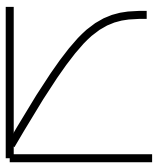
} VIRUSES

MECHANISMS OF ACTION – penetration

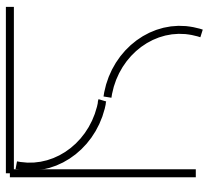
Uptake isotherms

- five main forms of uptake isotherm are recognised

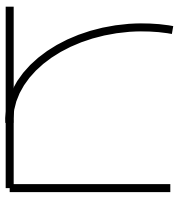
- provide insight to the early stages of biocide-cell interaction



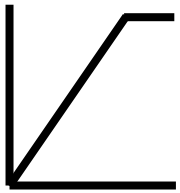
L-shape: normal or “Langmuir” uptake limited by binding sites available; e.g. basic dyes, cetrimide



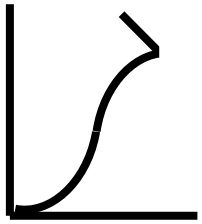
S-shape: initial adsorption is slight; moderate solute-solute interaction facilitates uptake of further molecules (co-operative sorption); e.g. phenols and sodium dodecylsulphate



H-shape: high affinity uptake (adsorbed as ionic miscelles); e.g. chlorhexidine and iodophore



C-shape: constant partition; plateaux reached when site is fully saturated; e.g. some phenols.



Z-shape: pattern shows a sharp break followed by enhanced uptake; possibly caused by concentration of adsorbed species promoting breakdown in cell structure generating new adsorption sites; e.g. 2-phenoxyethanol and triclosan

MECHANISMS OF ACTION – outer cell wall

Outer cell membrane (Gram-negative bacteria)

- Increased permeability

  ethylene diamine tetraacetic acid (EDTA) enhances biocidal activity of biocides (removal of LPS)

  polycations, lactoferrin and transferrin

- Cross-linking agents

Cell wall

- Lysis of bacterial cells
  low concentrations of phenol, formalin and mercuric chloride
  anionic detergents (Sodium Lauryl Sulphate)
  chlorine releasing agents (hypochlorite)

- Formation of long forms of bacteria
  phenol and *m*-cresol
  methyl green, methylene blue, fuchsine, acridines

- Protection from lysis
  alkylating agents

- Disruption of cell wall mycoside C and/or peptidoglycan structure (Mycobacteria)
  ethambutol and *m*-fluoro-DL-phenylalanine, D-cycloserine

MECHANISMS OF ACTION – cytoplasmic membrane

Physical disruption of the membrane

- leakage of intracellular materials

  (i) potassium

  (ii) inorganic phosphates

  (iii) pool of amino acids and materials absorbing at 260nm

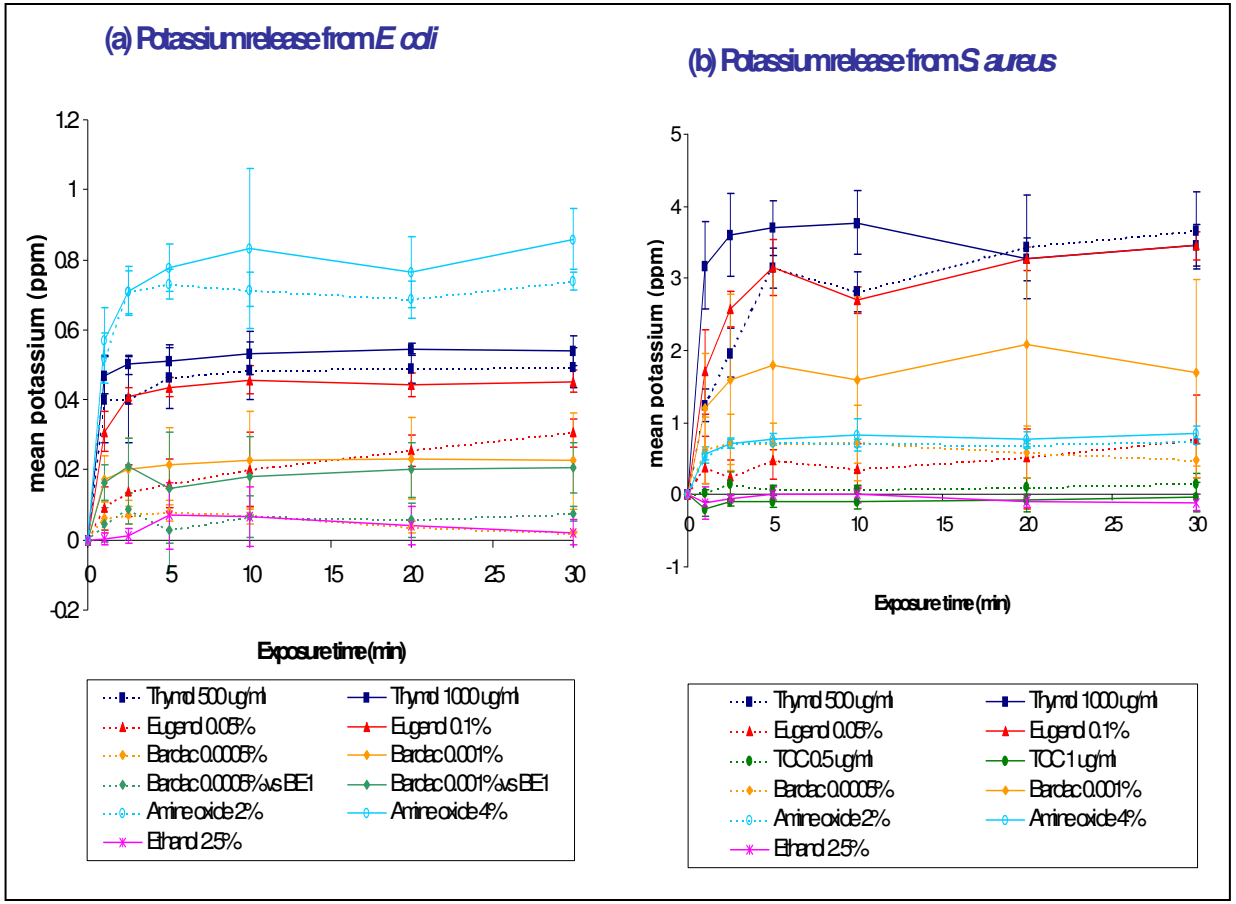
  (iv) nucleic acids and proteins

- gross-disruption ± coagulation

- lysis

MECHANISMS OF ACTION – cytoplasmic membrane

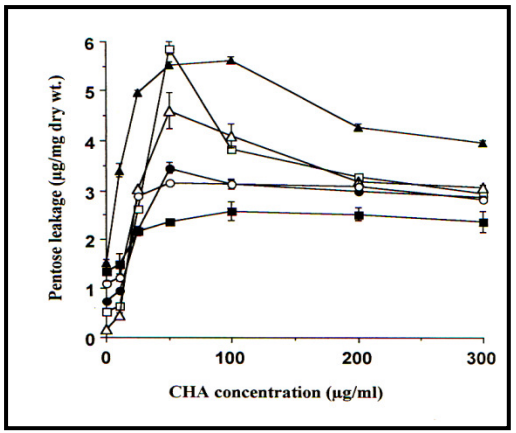
Potassium leakage Walsh et al. JAM 2001 91: 80-92

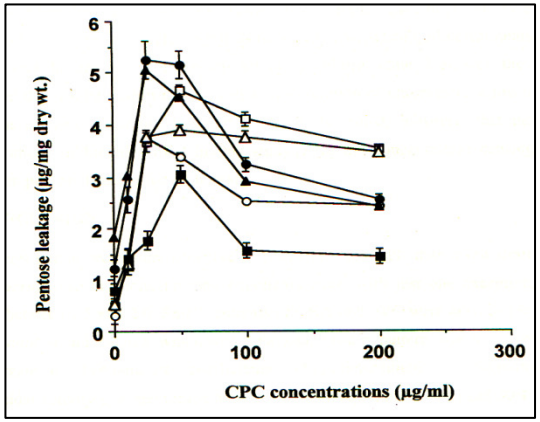


Pentose leakage

Pseudomonas stutzeri strains and *Ps. aeruginosa* 8626

Tattawasart et al. JAM 1999 87:323-331





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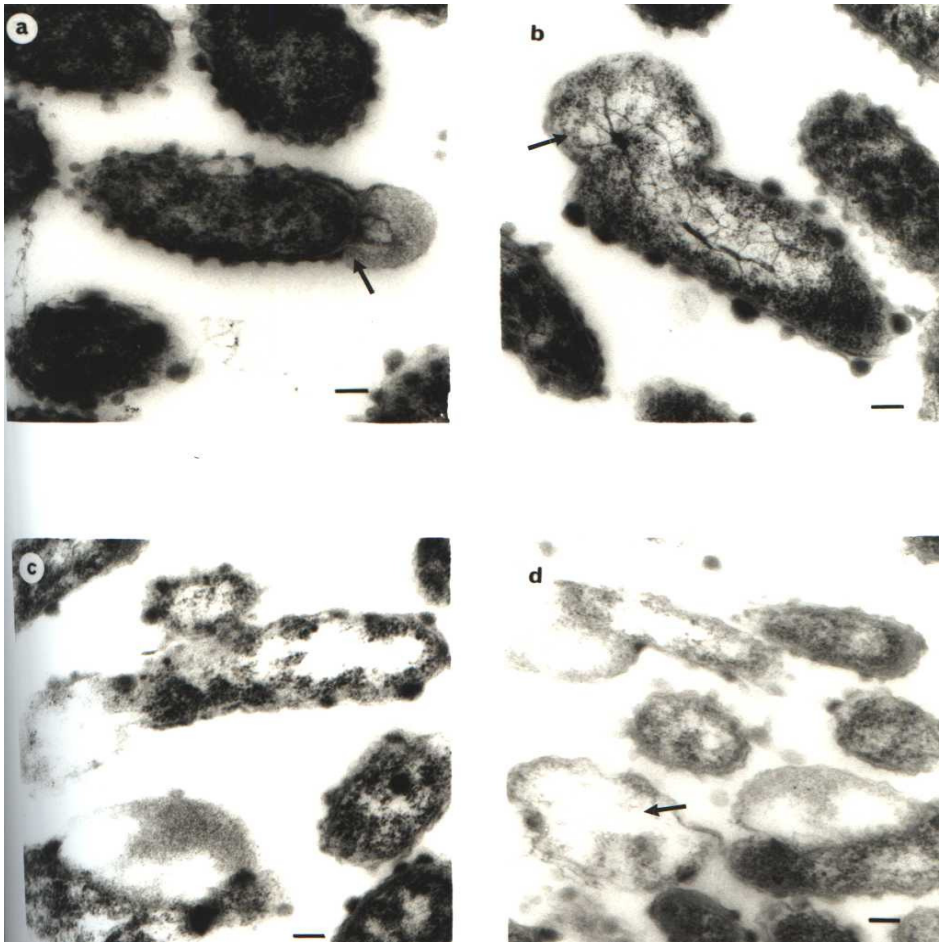
MECHANISMS OF ACTION – cytoplasmic membrane

Physical disruption of the membrane

Effect of chlorhexidine against *Pseudomonas stutzeri* JM302

a) 5 min: blebbing
b) 15 min: cytoplasmic swelling
c) 30 min: cell lysis
d) 60 min: ghost cells



Tattawasart et al. JAM 1999 87:323-331

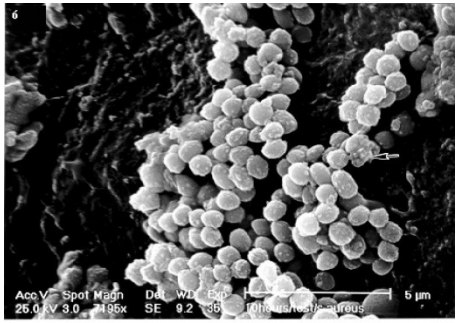
MECHANISMS OF ACTION – cytoplasmic membrane

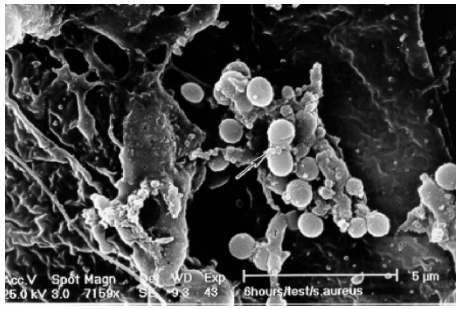
Blebbing and leakage

PCMX
Dettol formulation

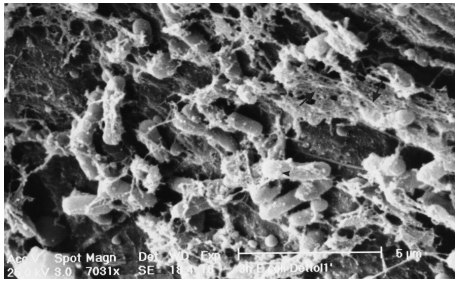
Triclosan in isopropanol

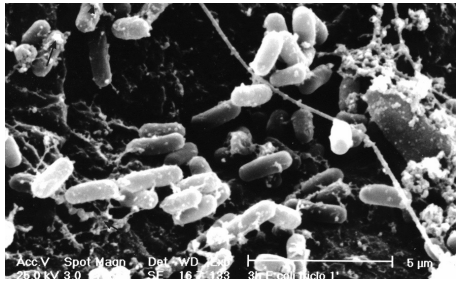
Staphylococcus aureus



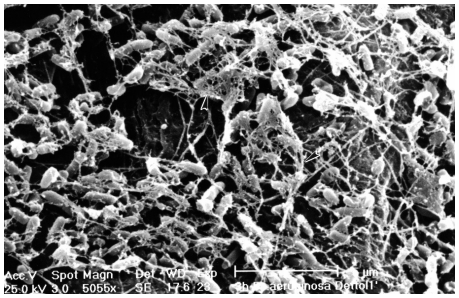


Escherichia coli





Pseudomonas aeruginosa



Messager et al. J Pharm Pharmacol 2000 52S: 124

MECHANISMS OF ACTION – cytoplasmic membrane

Inactivation of membrane-associated enzyme activity

AT A LOW CONCENTRATION

- hexachlorophane: electron transport chain
- ethanol: inhibition of DNA, RNA, protein and peptidoglycan synthesis
- chlorhexidine: inhibition of membrane-bound adenosine triphosphatase
- CTAB / ß-galactosides carrier-mediated process
- triclosan: enoyl-acyl carrier protein reductase

Dissipation of proton motive force

AT A LOW CONCENTRATION

- organic acids and parabens
- acetic acid (other effect, lower pHi; denature proteins)
- phenolics (fentichlor and triclosan): inhibition of ATP synthesis
- cationics

  discharge of membrane potential (CTAB)

  inhibition of ATP synthesis (QAC)

  inhibition of respiratory enzyme (PHMB)

MECHANISMS OF ACTION – cytoplasmic membrane

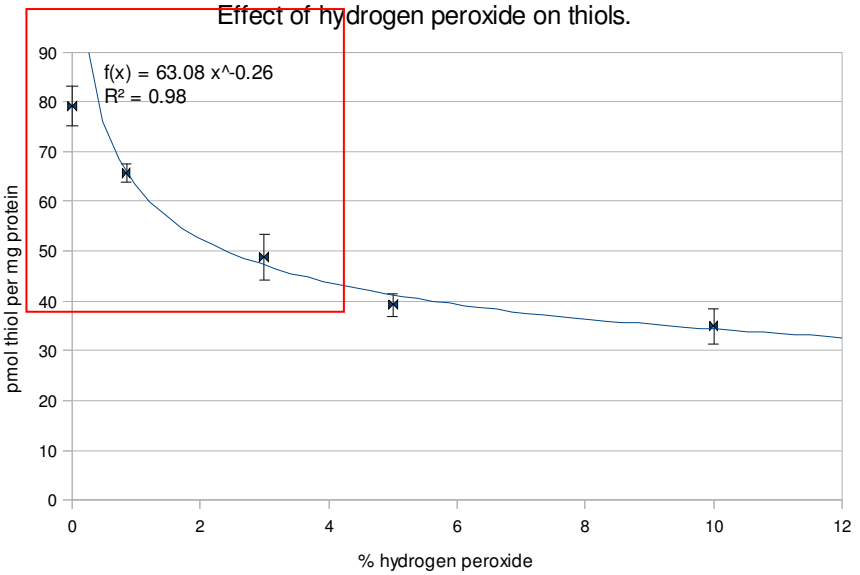
Inactivation of membrane-associated enzyme activity

- interaction with thiol group
  - metallic salts (copper, silver)
  - isothiazolones (BIT, CMIT)
  - oxidizing agents (highly reactive)

THIOL OXIDATION – H_2O_2

Total Thiol oxidation following H_2O_2 intervention

Protein thiols measured by reaction with DTNB (5,5′-dithiobis(2-nitrobenzoic acid) according to the method of Ellman.



Liquid treatment produces decrease in protein thiol

MECHANISMS OF ACTION – cytoplasmic membrane

Inactivation of membrane-associated enzyme activity

- interaction with thiol group
  - metallic salts (copper, silver)
  - isothiazolones (BIT, CMIT)
  - oxidizing agents (highly reactive)

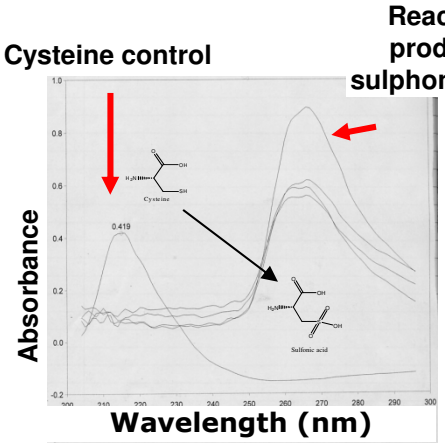
- interaction with amino group
  - alkylating agents (highly reactive)
  - oxidising agents (highly reactive)

- interaction with hydroxy group
  - oxidising agents (highly reactive)

MECHANISMS OF ACTION – cytoplasmic membrane

Amino acid/ Oxidation product	Oxidising agent (ratio amino acid/biocide)						
	H_2O_2	vH_2O_2	ClO_2	PAA	HAMO	SK	S20
Cysteine							
Sulphenic acid	1:2	-[b]	-[a]	2:1	100:1	-[a]	
Sulphinic acid	1:3	-[b]	-[a]	-[a]	1:1	-[a]	1:3
Sulphonic acid	-[a]	-[b]	-[a]	1:2	1:2	-[a]	-[a]
Methionine							
Sulphoxide	1:3	-[b]	-[a]	2:1	10:1	-[a]	
Sulphonic acid	-[a]	-[b]	-[a]	1:2	1:1	-[a]	1:3
Lysine							
N-(Hexanoyl)lysine	-[a]	-[b]	-[a]	2:1	10:1	-[a]	1:3
2' oxo-lysine	1:1	-[b]	-[a]	10:1	-[a]	-[a]	
Aminoadipic semialdehyde	1:3	-[b]	-[a]	-[a]	-[a]	-[a]	
Histidine							
2' oxo-histidine	1:3	-[b]	-[a]	2:1	10:1	-[a]	
Di-histidine	-[a]	-[b]	-[a]	1:2	1:2	-[a]	1:2
Glycine							
Aminomalonate	1:3	-[b]	-[a]	100:1	1:1	-[a]	
Tryptophan							
N-*formyl* kynurenine	-[a]	-[b]	-[a]	1:2	1:5	-[a]	-[a]

Finnegan et al. J Antimicrob Chemother 2010; 65(10): 2108-15



HAMO: PAA formulation
S20: STERIS 20: PAA formulation
SK: SPOR-KLENZ TM; hydrogen peroxide and peracetic acid

[a]: no oxidation observed at a 1:5 ratio

[b]: no oxidation observed at a ratio 100 mM amino acid and 2 g/L vH_2O_2

Hamburg 2011

MECHANISMS OF ACTION – cytoplasmic membrane

Inactivation of membrane-associated enzyme activity

- interaction with thiol group
  - metallic salts (copper, silver)
  - isothiazolones (BIT, CMIT)
  - oxidizing agents (highly reactive)

- interaction with amino group
  - alkylating agents (highly reactive)
  - oxidising agents (highly reactive)

- interaction with hydroxy group
  - oxidising agents (highly reactive)

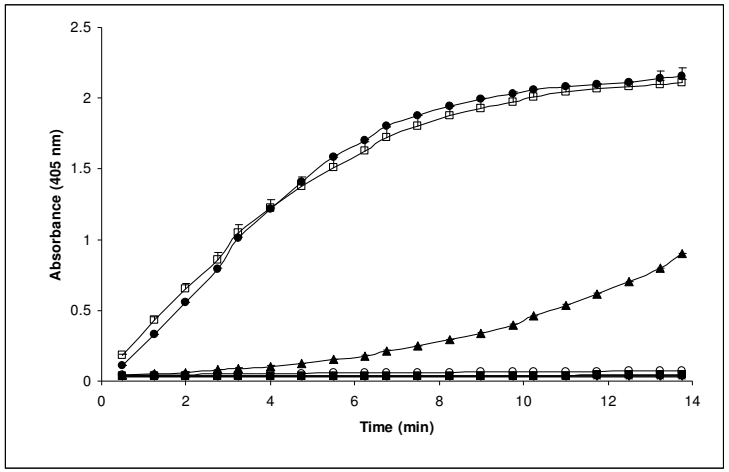
BASED ON THEORY RATHER THAN HARD EVIDENCE OF ENZYME DISRUPTION

MECHANISMS OF ACTION – cytoplasmic membrane

INTERACTIONS WITH ENZYMES - alkaline phosphatase

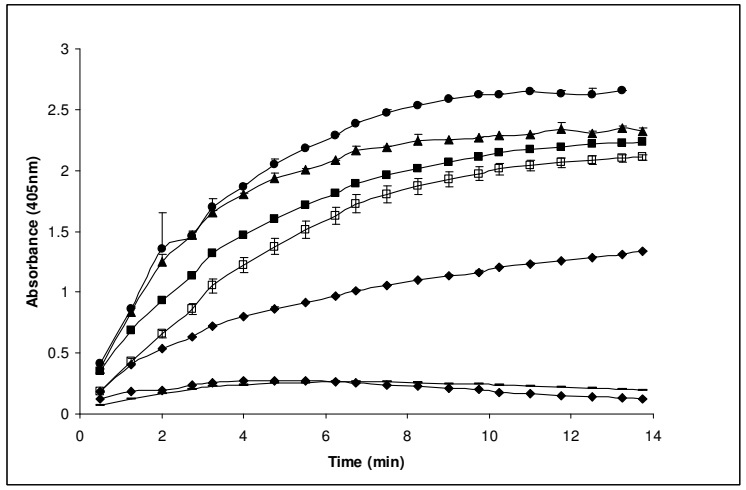
Finnegan et al. J Antimicrob Chemother 2010; 65(10): 2108-15

ClO_2



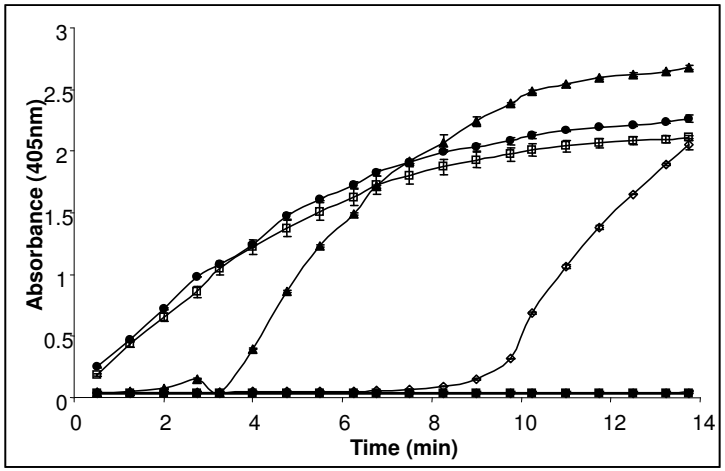
(□) control, (●) 0.33, (▲) 3.3, (○) 10, (.) 20, (◊) 33, (■) 50, and (♦) 330 mM.

H_2O_2



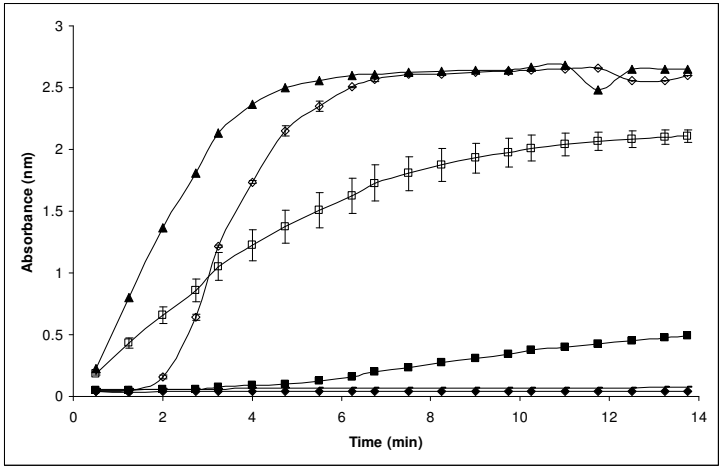
(□) control, (●) 0.33, (▲) 3.3, (○) 10, (■) 50, (-) 100 and (♦) 330 mM.

PAA



(□) control, (●) 0.33, (▲) 3.3, (○) 10, (◊) 33, (■) 50, (-) 100 and (♦) 330 mM

HAMO



(□) control, (▲) 3.3, (◊) 33, (■) 50, (-) 100 and (♦) 330 mM

MECHANISMS OF ACTION – cytoplasm

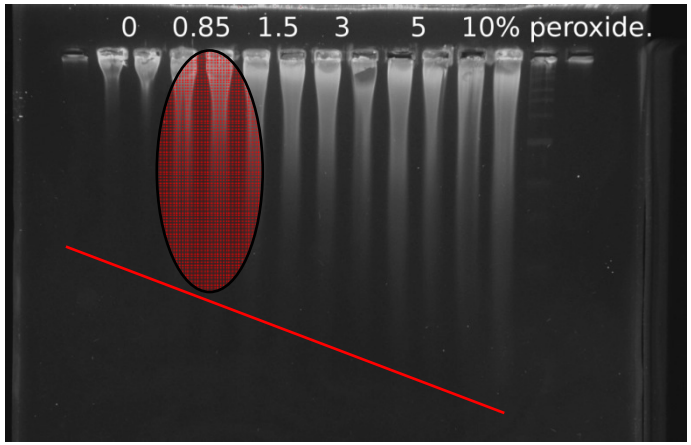
Coagulation
- cationics at high concentrations

Ribosomes
- hydrogen peroxide
- *p*-chloromercuribenzoate

Inhibition of other enzymatic systems: protein synthesis and DNA polymerase
- cationics

Nucleic acid (inhibition of DNA, RNA)
- antibacterial dyes
    - tryphenylmethane group (crystal violet)
    - acridines: intercalation between base pairs
- alkylating agents (?)
- oxidising agents

H_2O_2 intervention



CONCLUSIONS

Biocide interaction with the microbial cell and antimicrobial surfaces

EFFICACY

- Bioavailability
- Sustainable contact with the microbial cell
- Concentration is key for activity
- Number of factors that will decrease efficacy
  - state of the micro-organisms
  - relative humidity
  - soiling (conditioning film)

KNOWLEDGE

- Better understanding of biocide effect at a low concentration
- Emerging microbial resistance?

TESTING FOR ACTIVITY

- Efficacy test protocols
- Matching product and application

THANK YOU

- Unchalee Tattawasart
- Michelle O'Jeil
- Susie Walsh
- Michelle Finnegan
- Ezra Linley
- Syndie Messager