Antimicrobial Mechanisms of Essential Oils

Presented by Jean Bokelmann M.D.

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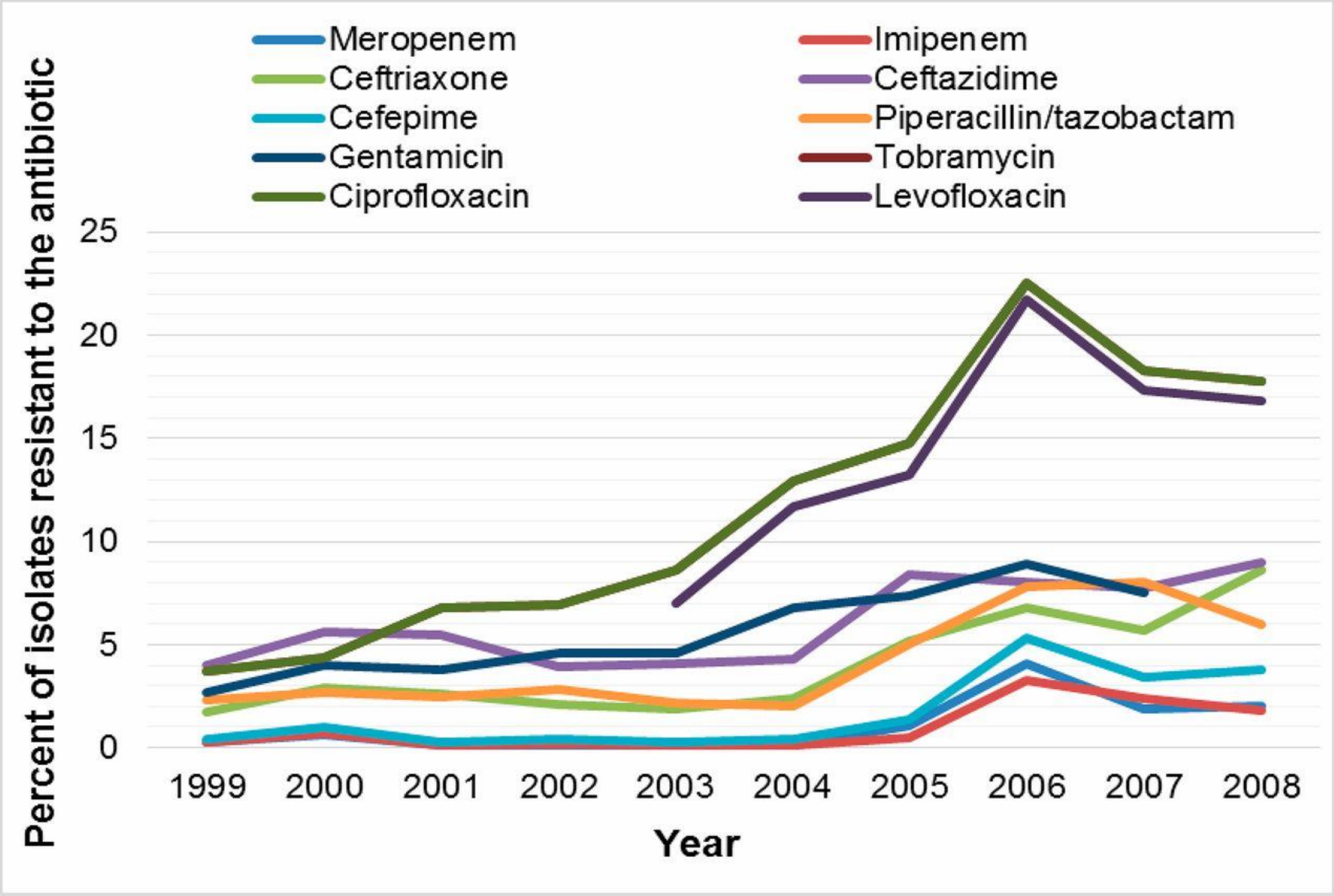
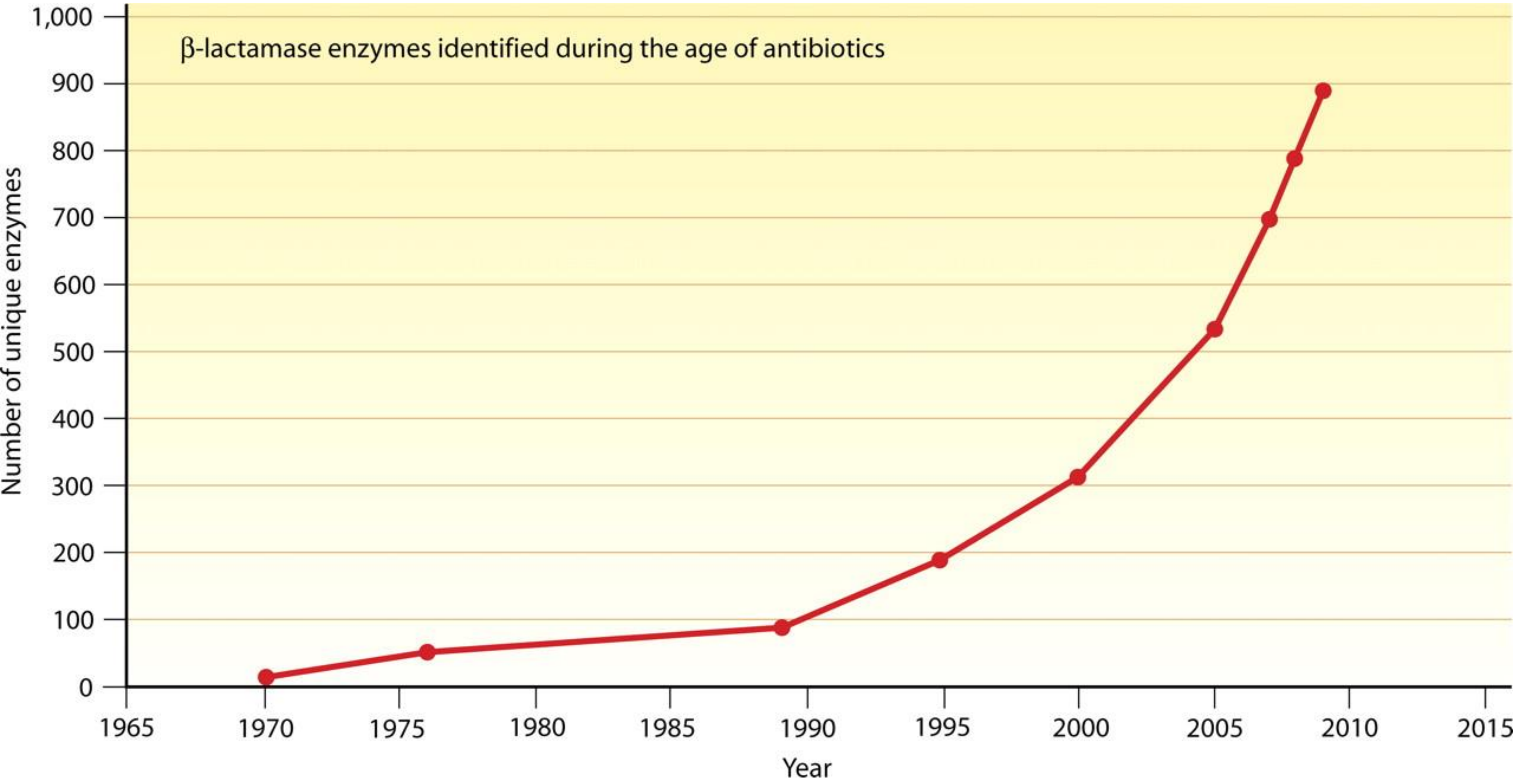
Roadmap

- The valuable role of essential oils as an antimicrobial tool
- Basic features of the microbes and their molecular targets
    - Bacteria
        - Antibacterial essential oils and mechanisms of effect
    - Fungi
        - Antifungal essential oils and mechanisms of effect
    - Viruses
        - Antiviral essential oils and mechanisms of effect

Another type of "pandemic" - Rising bacterial resistance to antibiotics

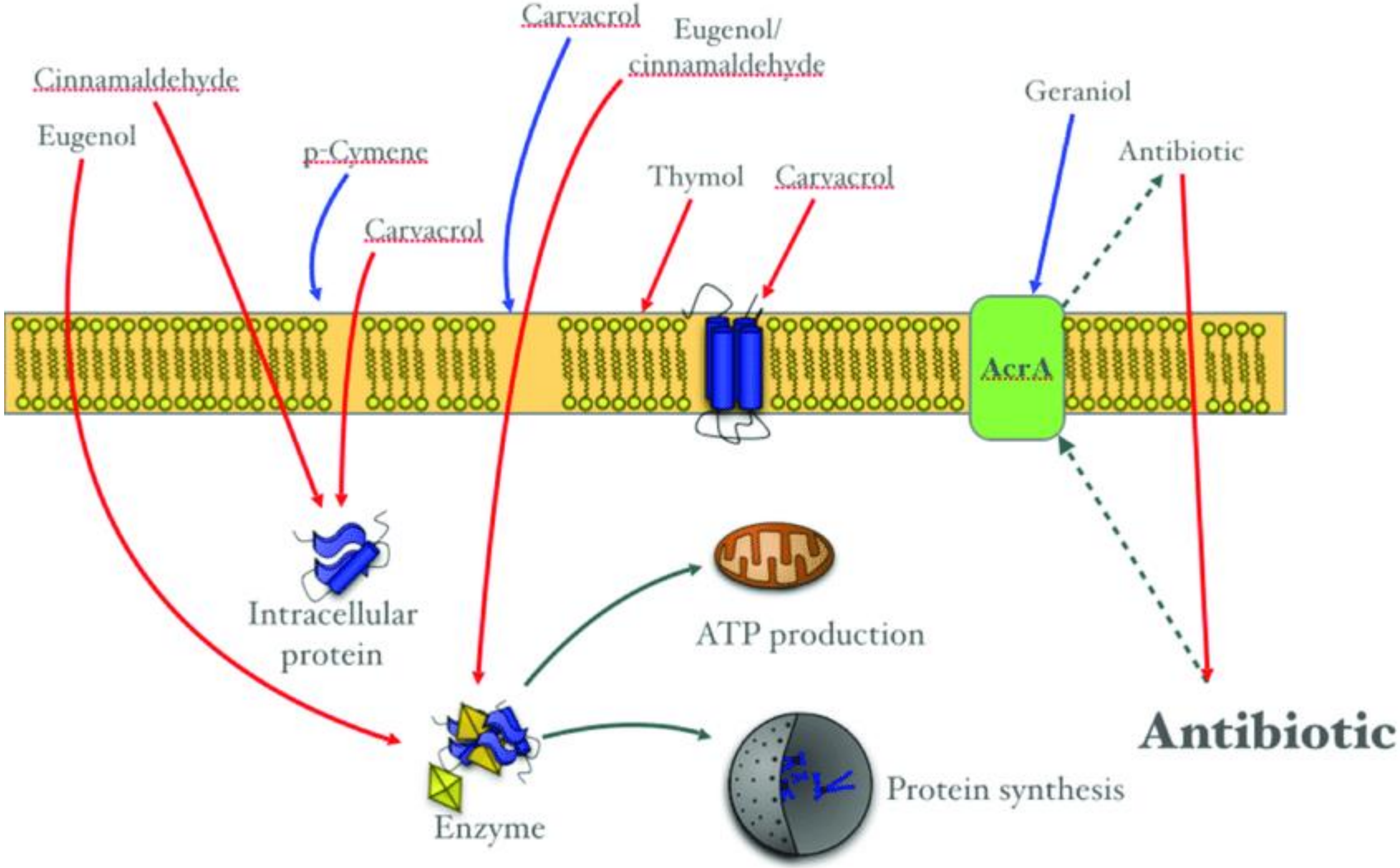
Percentage of Enterobacteriaceae strains from a US surveillance study that show increasing resistance to 10 antibiotics over a 10-year period




β-lactamase enzymes identified during the age of antibiotics
Number of unique enzymes
1,000
900
800
700
600
500
400
300
200
100
0
1965
1970
1975
1980
1985
1990
1995
2000
2005
2010
2015
Year

Essential oils have numerous possible targets for action inside the microbes.

Fewer opportunities for microbes to develop resistance.



Meet the Microbes

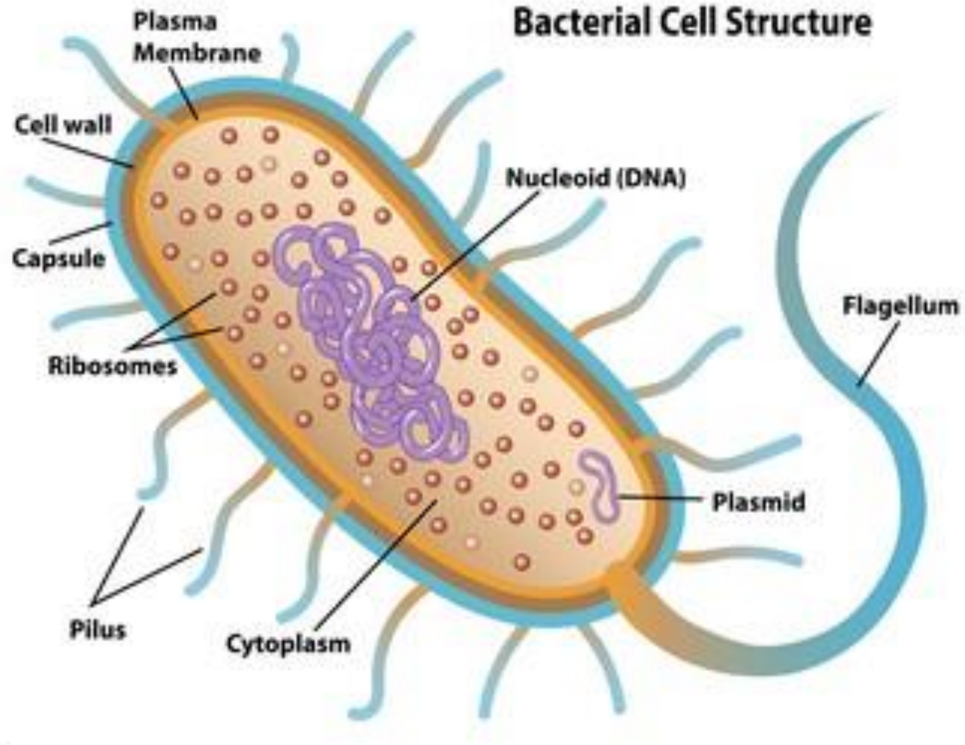
- Bacteria
  - Gram positive and Gram negative
  - Aerobic and Anaerobic
  - Encapsulated and Non-encapsulated

- Fungi
  - Single-celled (yeast)
  - Hyphae (mycelium)

- Viruses
  - Encapsulated and Non-encapsulated
  - RNA Viruses and DNA Viruses

Your Basic Bacteria

- **Prokaryotic**
  - No membrane around the genetic material
  - No interior organelles
  - Plasmids
    - Secondary genetic material
    - Antibiotic resistance



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Essential Oils: Both “Cidal” and “Static”

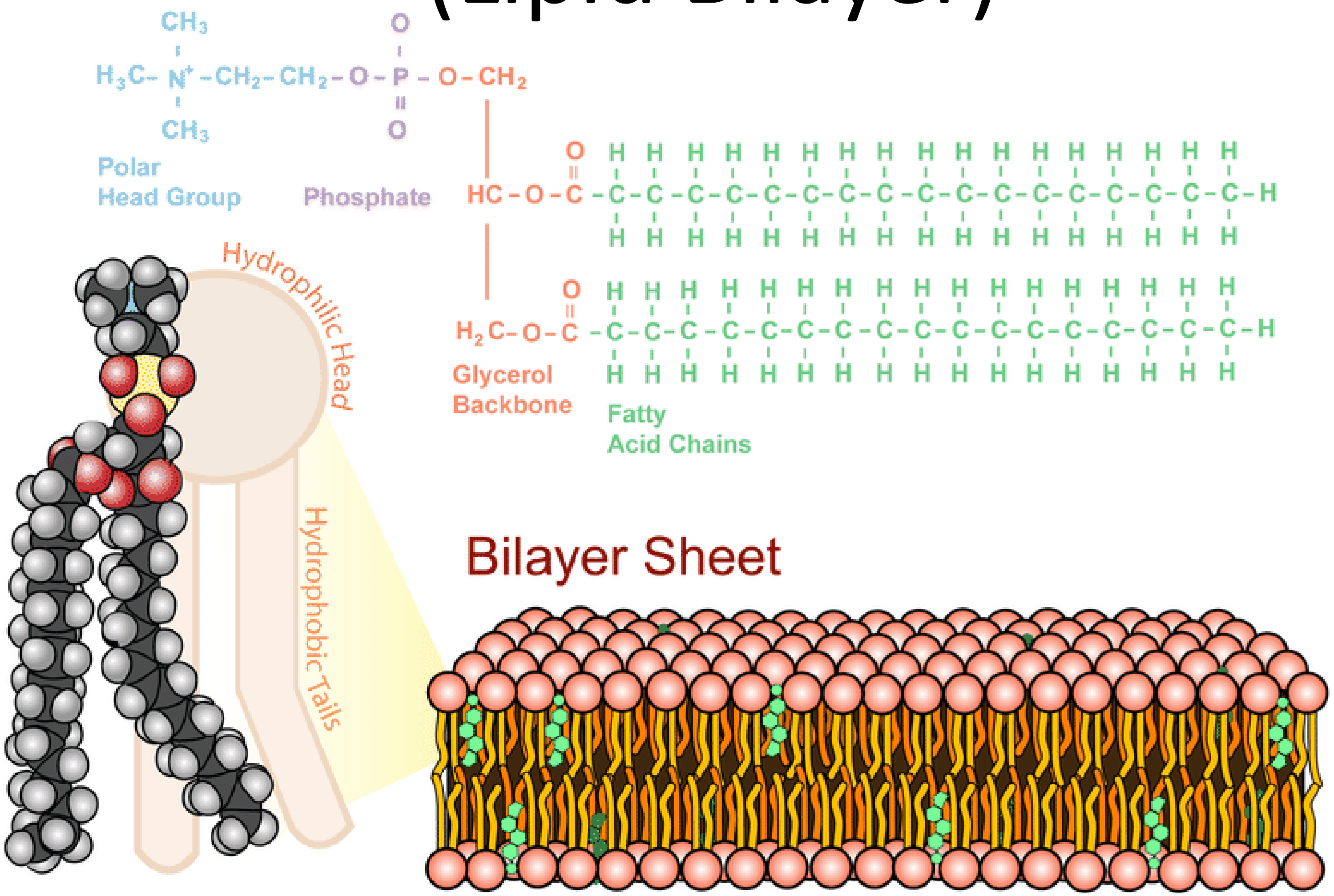
- Bactericidal: destroy bacterial cells
    - Disrupt cell walls or cell membranes
- Bacteriostatic: inhibit the growth of bacteria
    - Stop protein production via genetic downregulation
    - Block protease
    - Stop energy production via ATP cycle

EO Bactericidal Effects

- Cell membrane effects
  - Disruption, expansion, leakage, and lysis
  - Disruption of proton pumps and reduction of membrane potentials
  - Disturbance to insertion and folding of cell membrane proteins
  - Synergism (e.g., P-Cymene increases swelling of the *B. cereus* cell wall, improving transport of carvacrol across the wall.

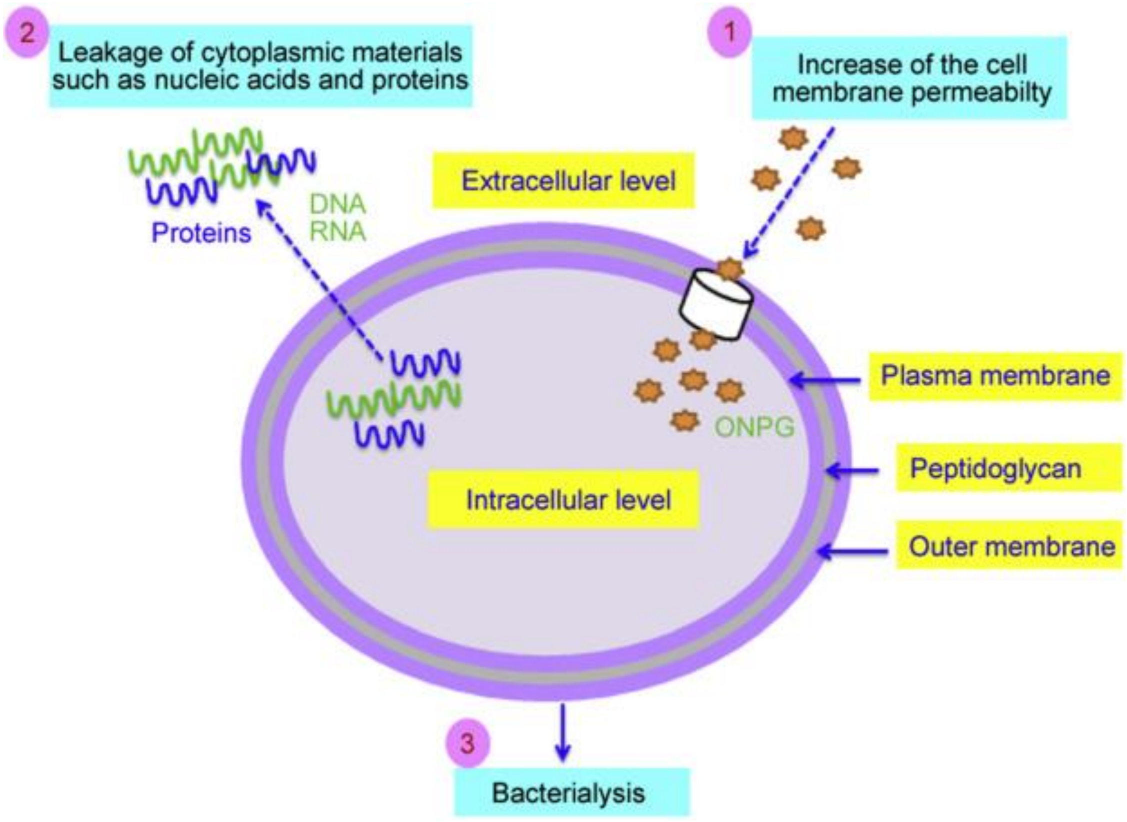
- Periplasm effects
  - Trans-cinnamaldehyde enters the periplasm of the cell and disrupts cellular functions

Main Target: Phospholipid plasma membrane (Lipid Bilayer)



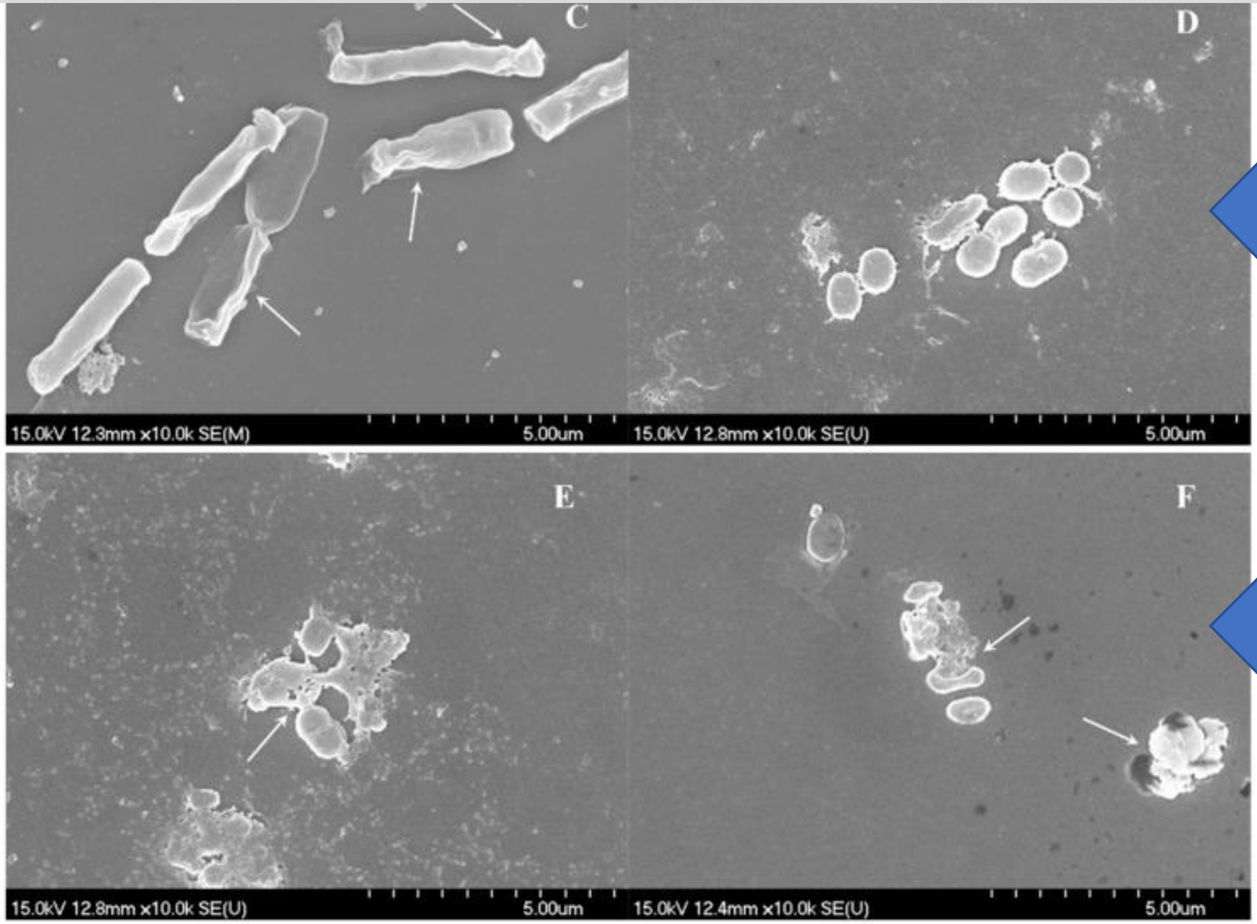
Essential Oils and Plasma Membrane

- Hydrophobicity of EOs enables them to partition in the lipids of the bacterial cell membrane
  - Perturbs the lipid fraction of the bacterial membrane, resulting in the increase of membrane permeability (1)
  - Produces leakage of ions and other cell contents (2)
  - Results in implosion or explosion of the cell



Cell Membrane Disruption

Untreated *S. aureus* cells (upper)
S. aureus cells following 4 hours of exposure to *Citrus medica* EO (lower). Note the cell membranes were pitted and shriveled, with holes on the surface. In addition, bacterial aggregation could be observed.

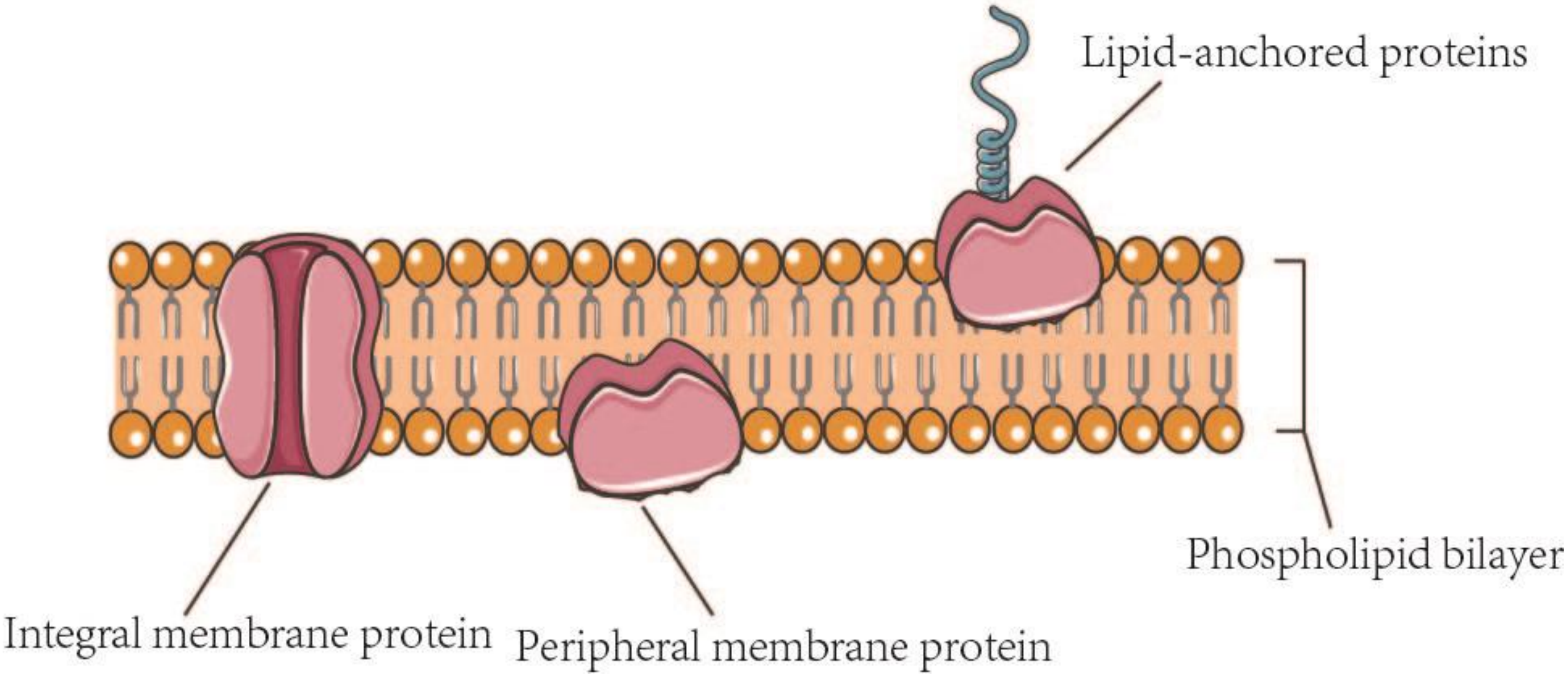


Citrus medica EO is abundant in limonene, γ-terpinene, and dodecanoic acid

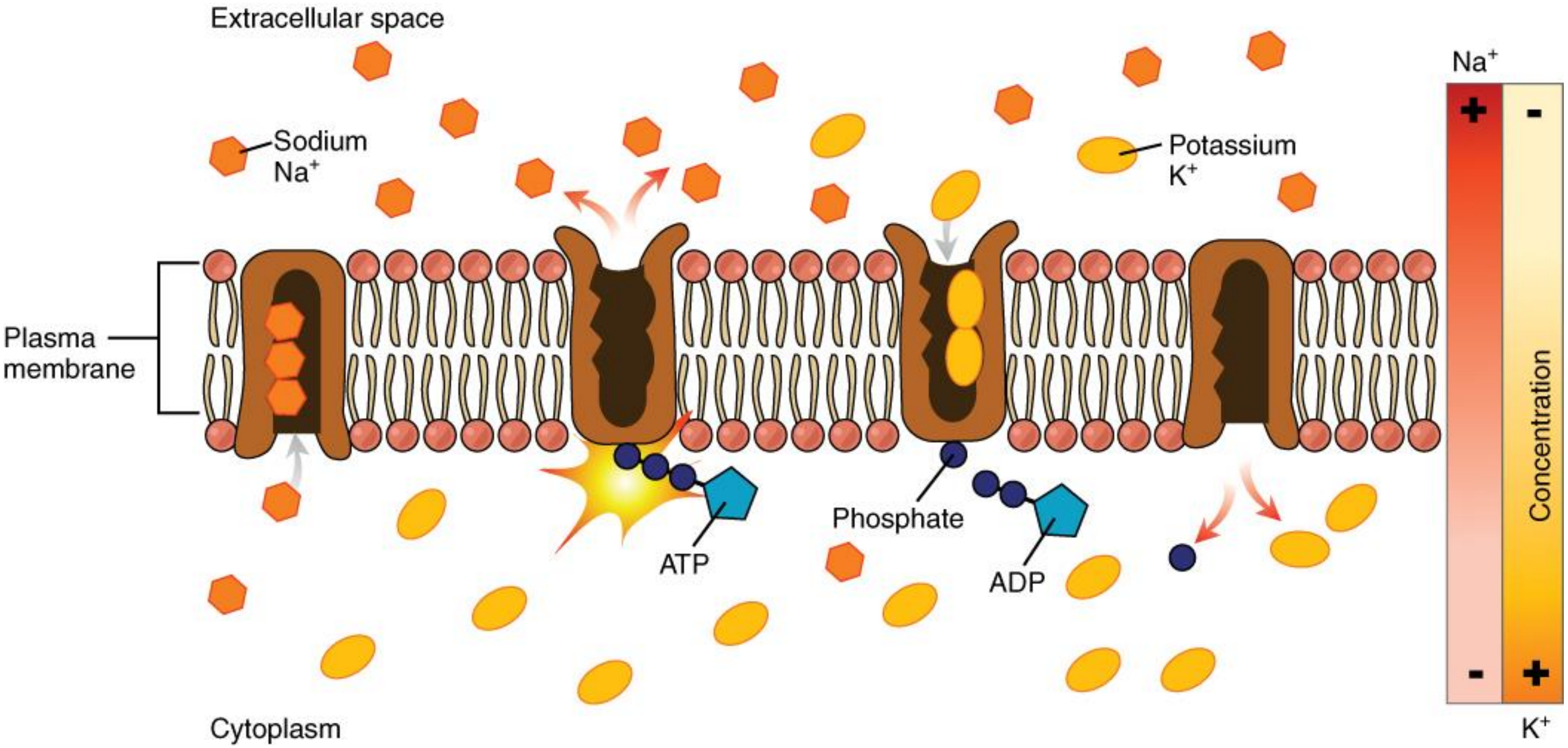
Mechanisms of Antibacterial Activity of Sample EOs

Essential Oil	Bacteria	Mechanism
Allium sativum	*Escherichia coli*	Induced leakage
Litsea cubeba	*Escherichia coli*	Destruction of outer and inner membrane
Foeniculum vulgare	*Shigella dysenteriae*	Loss of membrane integrity
Forsythia koreana	*Foodborne and other pathogenic bacteria*	Loss of membrane integrity and increased permeability
Piper nigrum	*Escherichia coli*	Cell becomes pitted, shriveled and leakage of intercellular material.
Cuminum cyminum	*Bacillus cereus, Bacillus subtilis*	Changes in cytoplasm
Cinnamon	*Escherichia coli, Staphylococcus aureus*	Disruption of cell membrane
Mentha longifolia	*Escherichia coli, Micrococcus luteus, Salmonella typhimurium*	Cell wall damage
Origanum vulgare	*Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa*	Permeabilized membrane

Integral Membrane Protein Disruption



Sodium-Potassium Pump Disruption
Alters membrane potential and other transport proteins



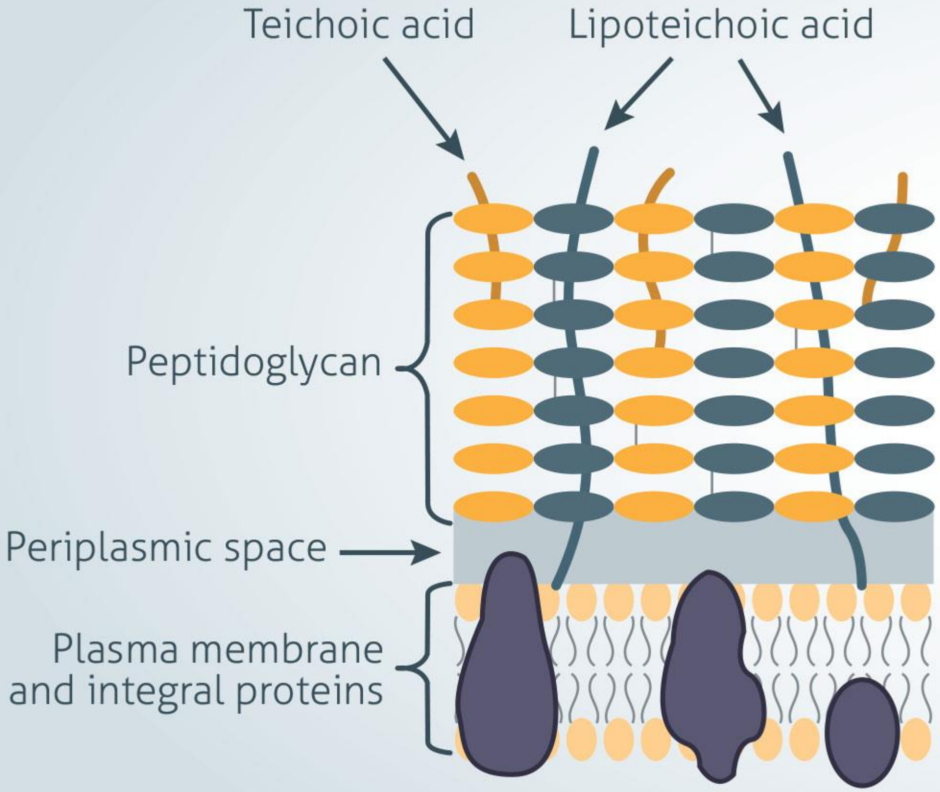
Bacteria Wall Differences: Gram-positive, Gram-negative, and Acid-fast Bacteria

- Gram positive bacteria
  - thick peptidoglycan layer and no outer lipid membrane

- Gram negative bacteria
  - thin peptidoglycan layer and have an outer lipid membrane
  - lipopolysaccharide outer membrane restricts diffusion of hydrophobic compounds → greater resistance than Gram positives

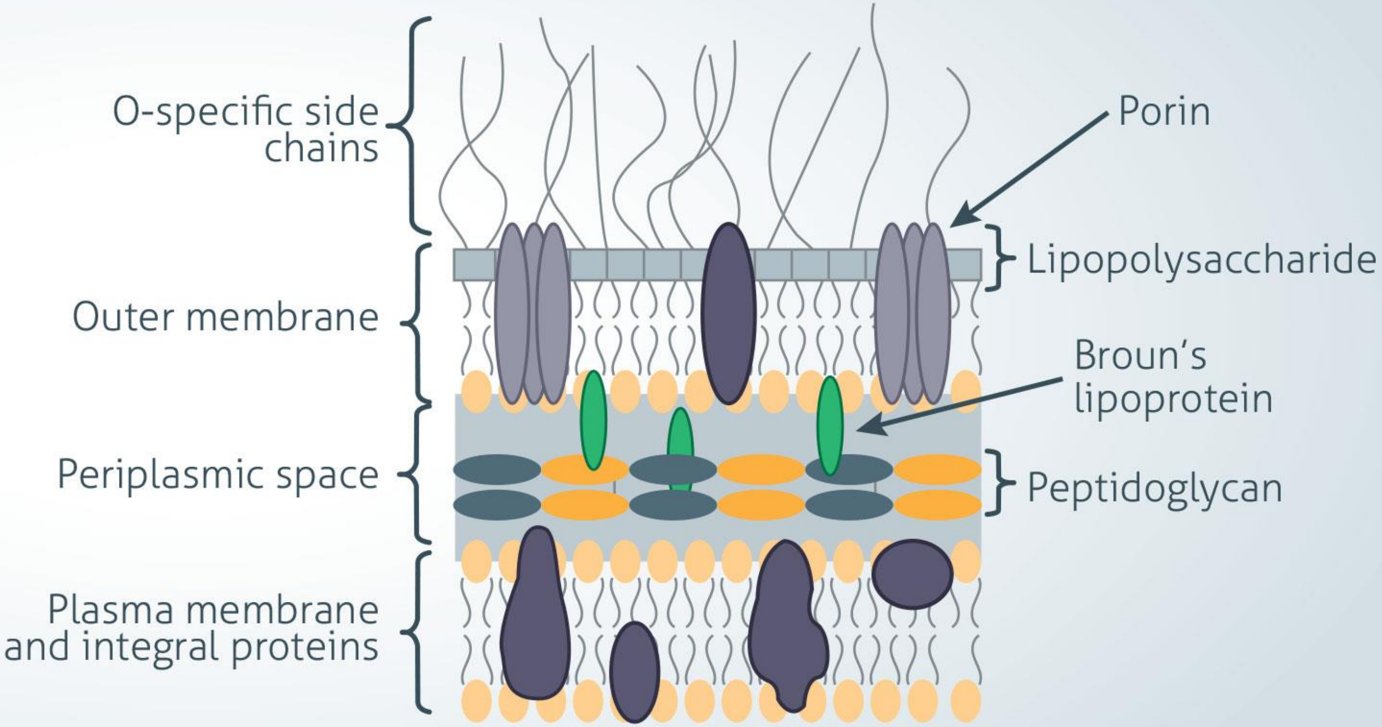
- Acid-fast bacteria
  - Mycolic acid in the cell wall makes it resistant to acid washes

GRAM (+) CELL-WALL



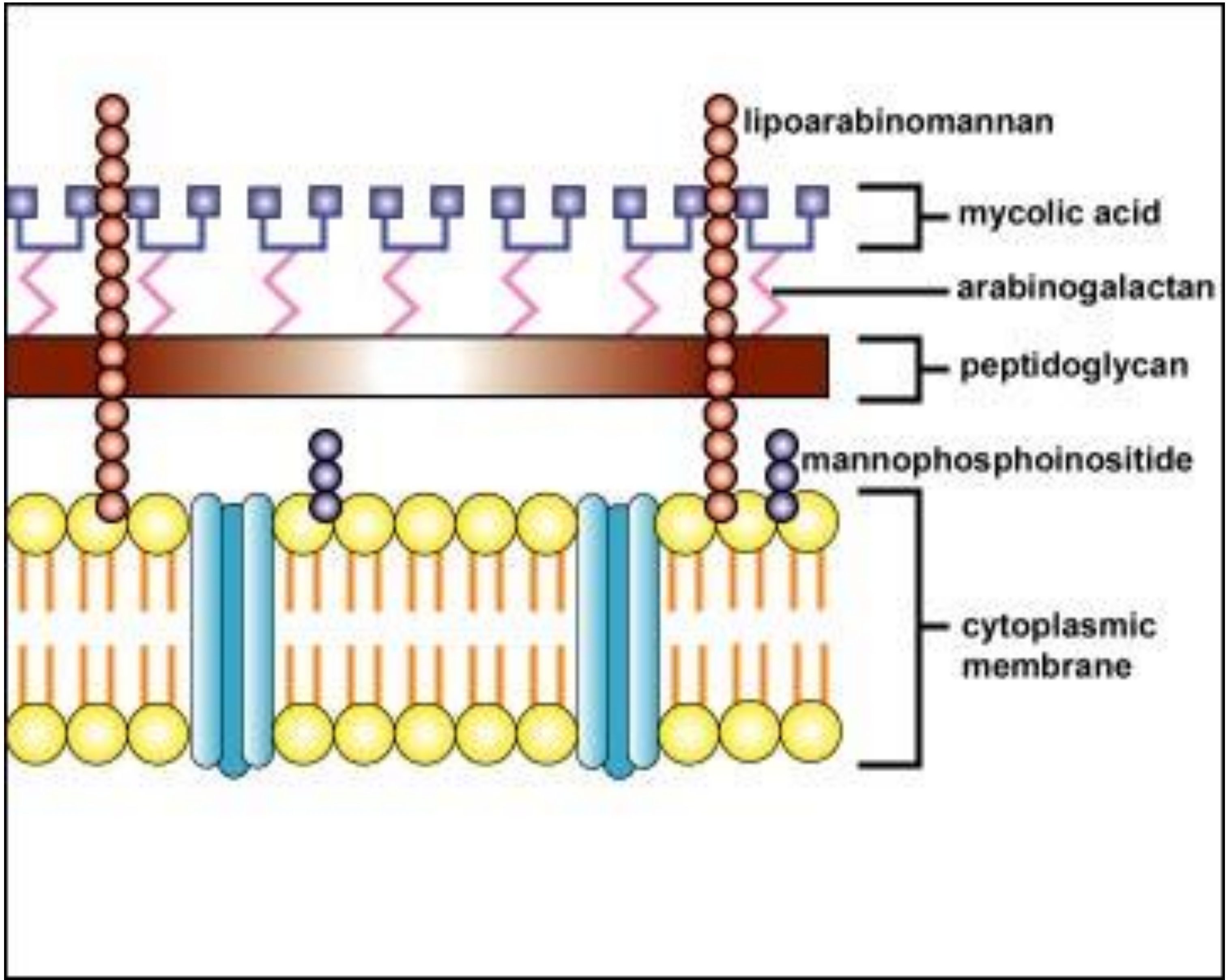
Periplasm contains hydrolytic enzymes, binding proteins, chemoreceptors

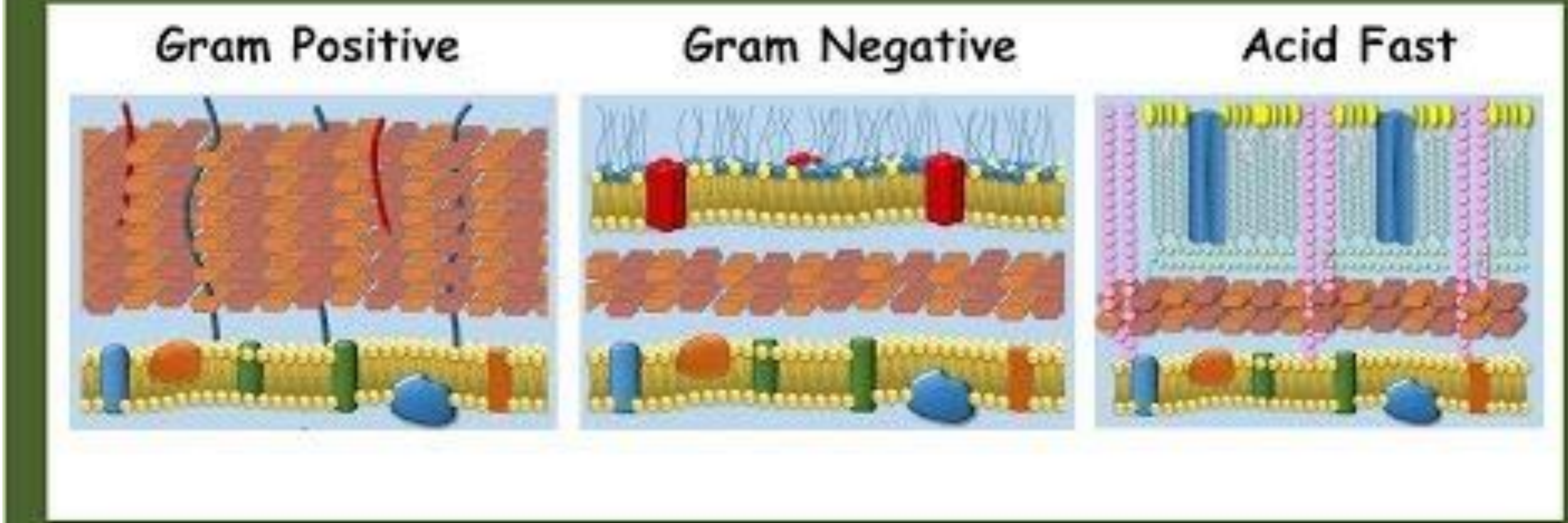
GRAM (-) CELL-WALL



Gram negatives have two lipid bilayers: the outer and plasma membranes. The outer leaflet of the outer membrane contains LPS molecules, which form contacts with outer membrane proteins.

Acid-Fast Bacterial Cell Wall





Cell Walls Compared

Activity of Sample Eos against Gram-positive and Gram-negative Bacteria

- EOs of sandalwood and vetiver inhibit **Gram-positive** bacteria; however, they fail to inhibit Gram-negative bacterial strains.
- EOs of cinnamon, clove, pimento, thyme, oregano, and rosemary show strong antibacterial activity against **Gram-negatives** (*Salmonella typhi and Pseudomonas aeruginosa*) and **Gram-positives** (*Staphylococcus aureus*)
- EO of tea tree and its major constituent, terpinen-4-ol, were shown to possess potential antibacterial properties against **Gram-negatives** (*E. coli, H. influenzae, P. aeruginosa*), **Gram-positives** (*S. aureus, S. epidermidis, E. faecalis, S. pyogenes, and S.* pneumoniae), and **acid-fast bacilli** (*M. avium*).

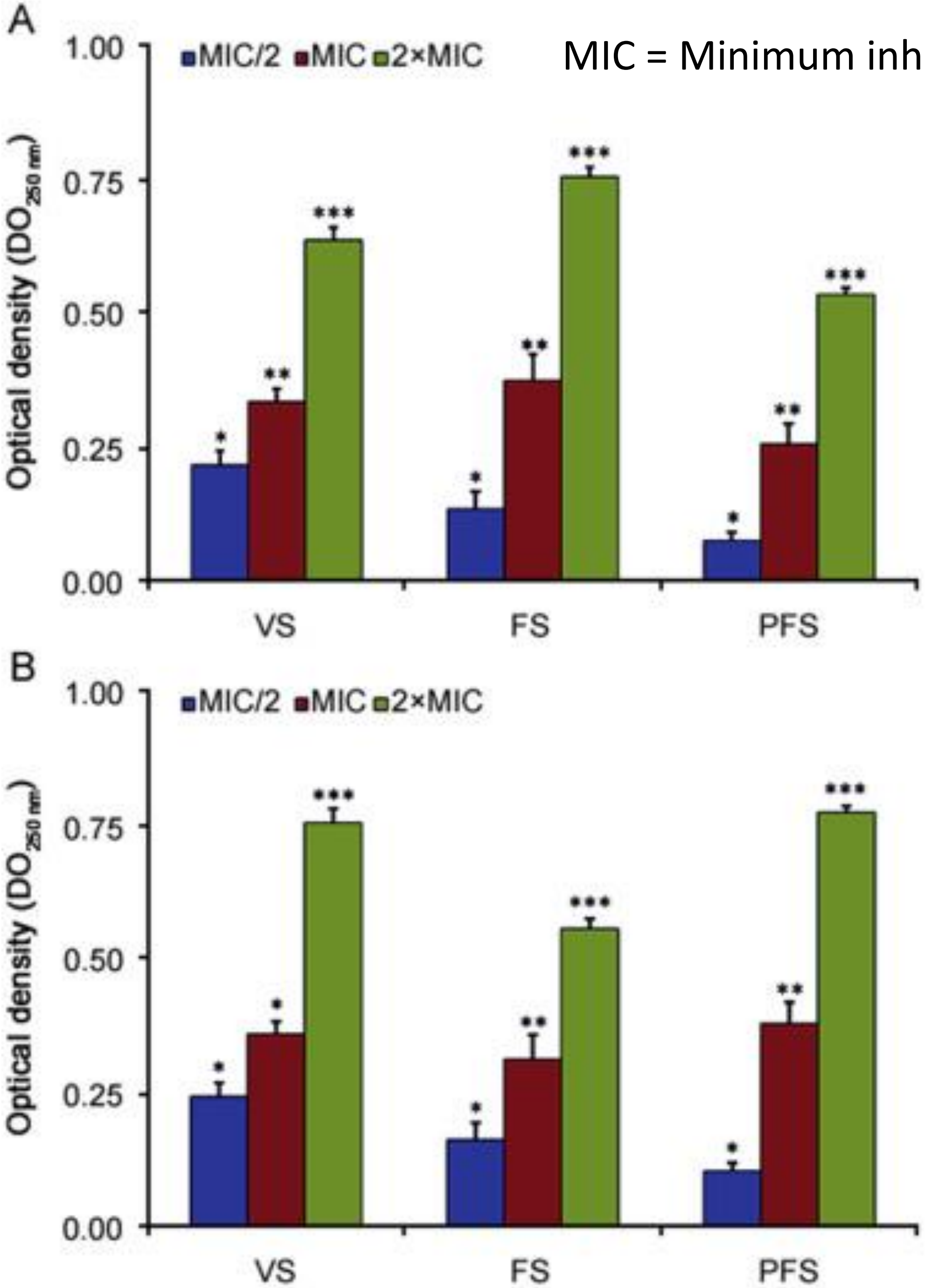
Plant	Chief Chemical Constituents	Susceptible Bacteria
Cinnamomum zeylancium	Cinnamaldehyde	*Enterobacteriaceae, S.aureus, Streptococcus pyogenes, S. pneumoniae, Enterococcus faecalis, E. faecium, Bacillus cereus, Acinetobacter lwoffii, Enterobacter aerogenes, E.coli, Klebsiella pneumoniae, Proteus mirabilis, P.aeruginosa, Salmonella typhimurium, Clostridium perfringens, Mycobacterium smegmatis*
Melaleuca alternifolia	Terpinen-4-ol, 1,8-cineole, γ-terpinene, α-terpinene, terpinolene	*E. coli, S. aureus, S.epidermidis, E. faecalis, P. aeruginosa, M. avium, H.influenzae, S. pyogenes, S. pneumonia*
Origanum vulgare	Carvacrol, thymol, γ-terpinene, trans-sabinene hydrate, cis-piperitol, borneol, terpinen-4-ol, linalool	*Clostridium botulinum, C.perfringens, L.monocytogenes, E. coli, S.choleraesuis, S.typhimurium, S. aureus, B. subtilis, Pseudomonas aeruginosa, Shigella sonnei, Sarcina lutea, M. flavus, K. pneumoniae, K. oxytoca*
Rosmarinus officinalis	Camphor, camphene, limonene, geraniol, myrcene, linalool benzoylacetate, linalool, α-pinene, α-terpinolene, bornyl	*E. coli, S. typhimurium, B.cereus, Bacillus subtilis, S.aureus, S. agalactiae, S.epidermidis, S. aureus, P. vulgaris, P. aeruginosa, K.pneumonia, E. faecalis, B. thermosphacta, L. innocua, L. monocytogenes, P. putida, S. typhimurium, S.putrefaciens, M. smegmatis*
Salvia officinalis	α-Thujone, camphor, 1,8-cineole, α-pinene	*S. aureus, P. stuartii, E. coli, Shigellasonnei, Sarcina lutea, M. flavus, B. thermosphacta, E.coli, L. innocua, L.monocytogenes*
Thymus vulgaris	Thymol, linalool, carvacrol, 1,8-cineole, eugenol, camphor, camphene, α-pinene, borneol, β-pinene	*L. monocytogenes, E. coli, S. typhimurium, S. aureus, C. botulinum, C. perfringens, S. sonnei, S. lutea, M. flavus, B. thermosphacta, L.innocua, L. monocytogenes, P. putida, S. putrefaciens*

Timing of Harvest and Dosage of EO Matters

Measurement of the leakage of DNA (from disruption of cell membrane) of *E. coli* and *B. subtilis* by *Origanum compactum* EO at increasing concentrations (based on mean-inhibitory concentrations).

VS= vegetative stage (carvacrol, thymol)
FS= flowering stage (carvacrol)
PFS = post-flowering stage (thymol)

Bouyaha et al



MIC = Minimum inhibitory concentration

E. Coli
(Gram negative)

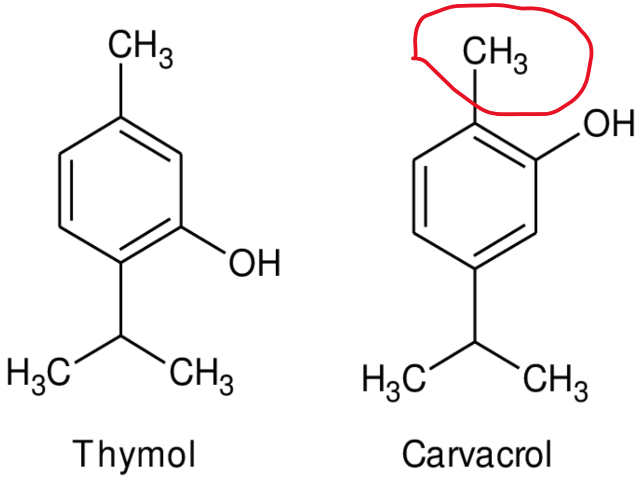
Bacillus subtilis
(Gram positive)

Carvacrol vs Thymol

- *Origanum compactum* at vegetative and flowering stages (carvacrol) caused a greater loss of membrane integrity of *E. coli*
- *Origanum compactum* at post-flowering stage (thymol) was more effective against *B. subtilis*.

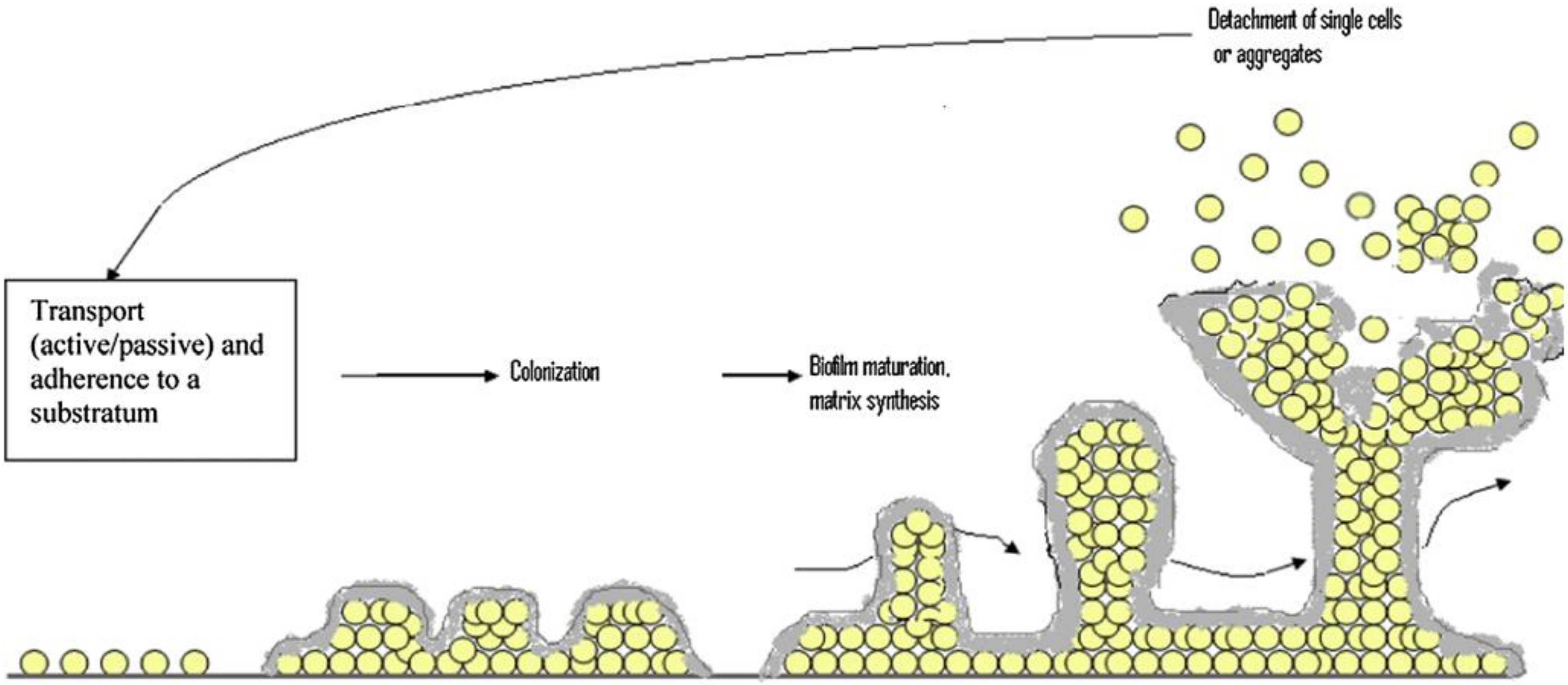
Bacteriostatic Effects of EOs

- DNA downregulation: thymol, carvacrol, eugenol, and benzoic acid have been shown to downregulate the expression of bacterial genes related to
  - Motility (flagellin)
  - Adhesion to enterocytes (various proteins)
  - Toxin secretion
  - Quorum sensing and biofilm formation



- Interference with energy production pathways
  - Interference with TCA cycle→Reduction of ATP production
  - Destruction of Enzymes (e.g., ATPase, amylase, histidine carboxylase, proteases)
- Oxygenation of unsaturated fatty acids
  - Formation of fatty acid hydroperoxide → Free radical damage

Quorum-sensing Stimulates Biofilm Production →Increased Resistance to Antibiotics



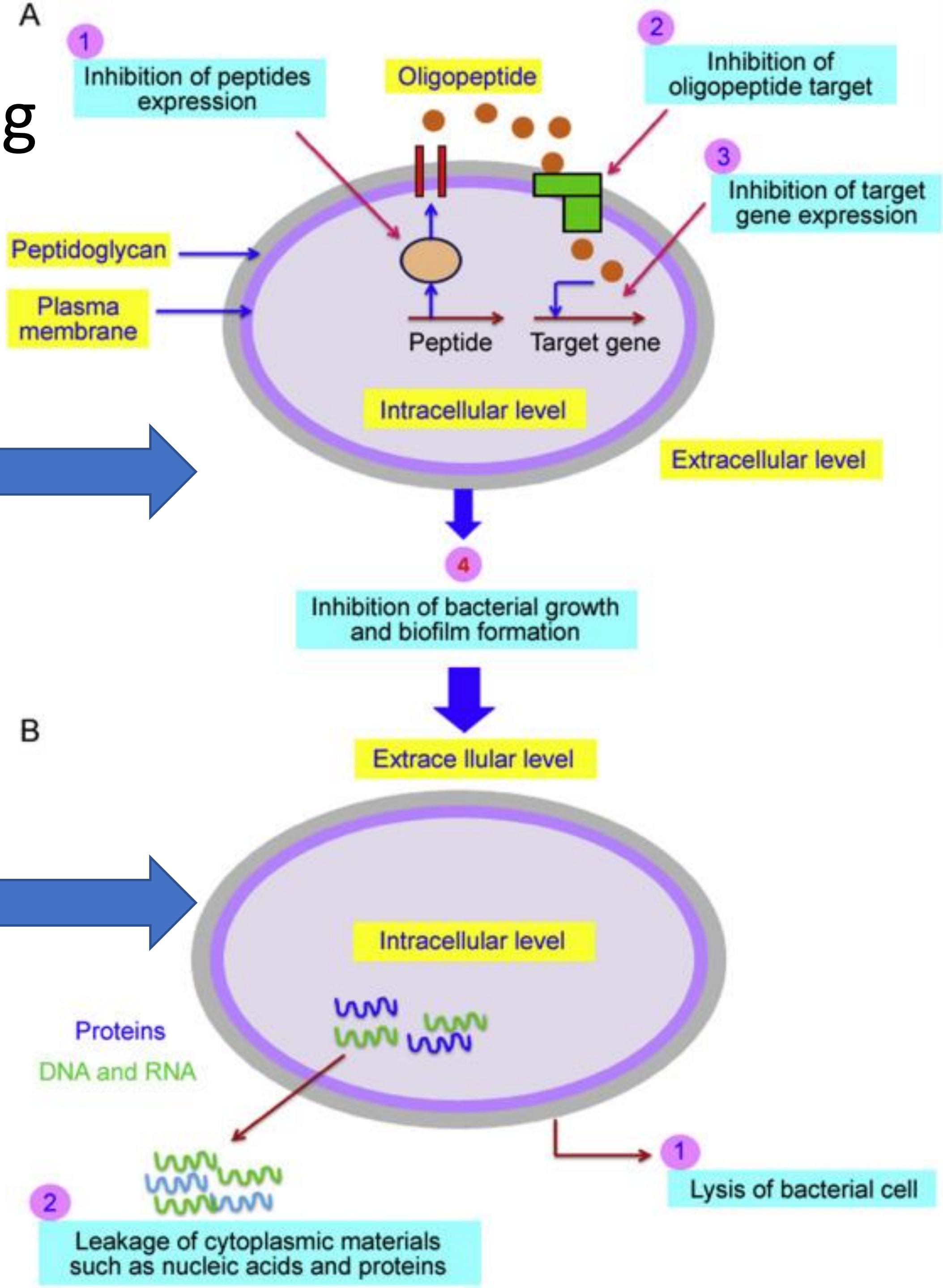
ution of a biofilm at cellular level, from single cells, sedimented and adherent to a conditioning pellicle on a substratum, cellular m

Mechanisms for Quorum-sensing (and Biofilm) Inhibition

(A) Inhibition of peptide expression, oligopeptide formation, oligopeptide receptor, or gene expression which inhibits growth and viability of bacteria

(B) The growth inhibition leads to lysis after some time

Rose, geranium, lavender, clove, caraway, rosemary, oregano, tea tree, and thyme EOs have been shown be effective against biofilms.

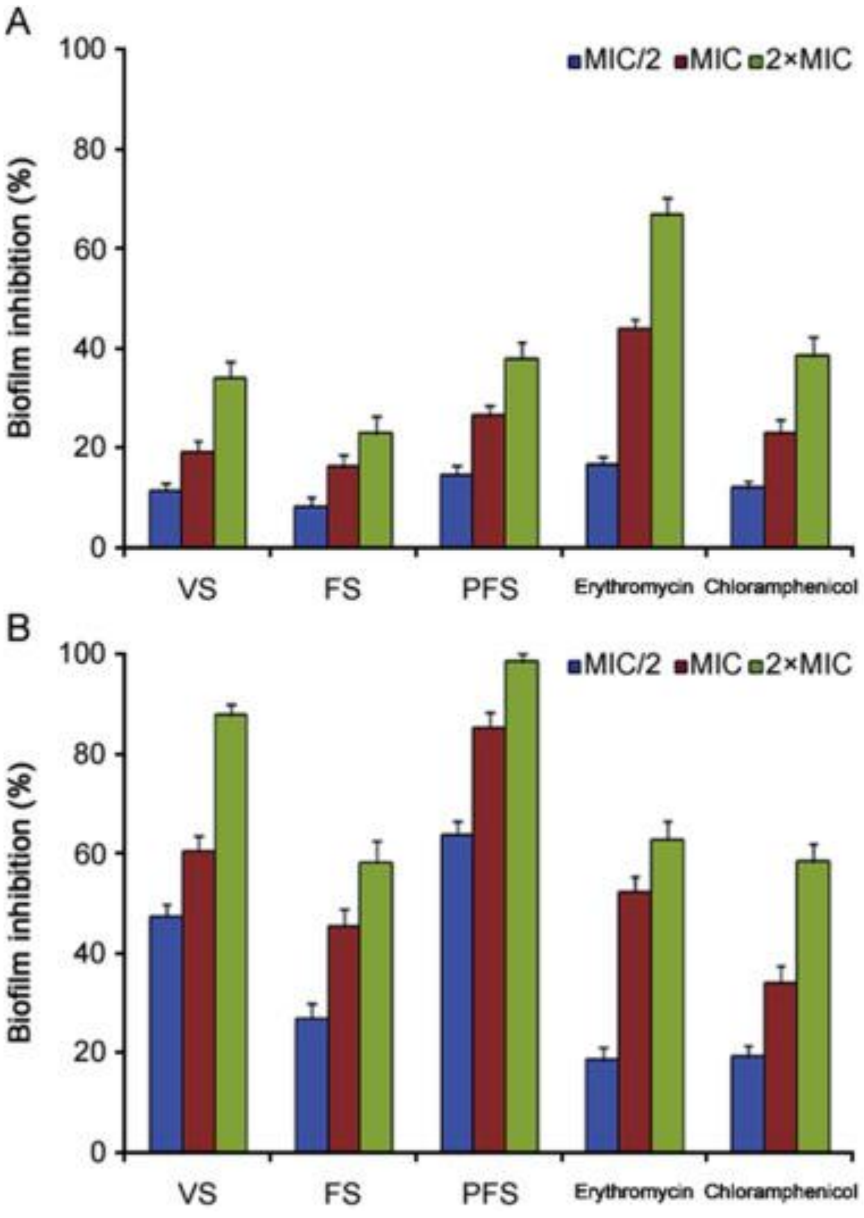


Biofilm Inhibition

Inhibition of biofilm formation of *E. coli* and *B. subtilis* by *Origanum compactum* EO at increasing concentrations * compared with two conventional antibiotics

*(based on miminum-inhibitory concentrations)

Bouyaha et al



E. coli

Bacillus subtilis

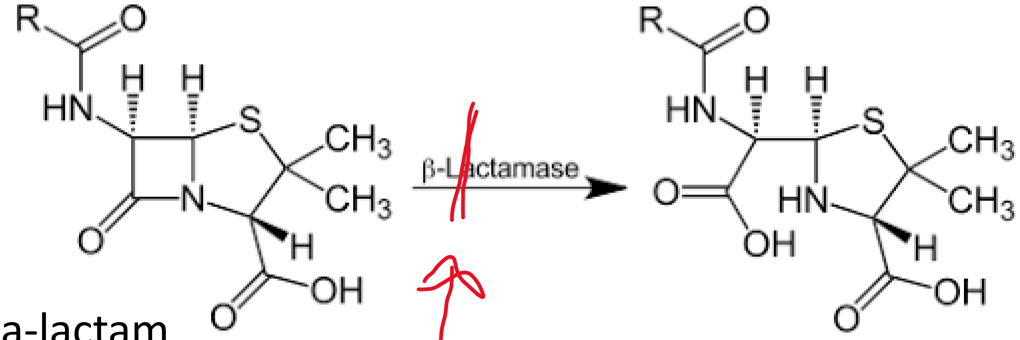
Carvacrol vs Thymol

- *Origanum compactum* at post-flowering stage (rich in thymol) showed a very important anti-biofilm action, especially against *B. subtilis*.

EOs Reduce Antibiotic Resistance

Beta-lactamase Inhibition

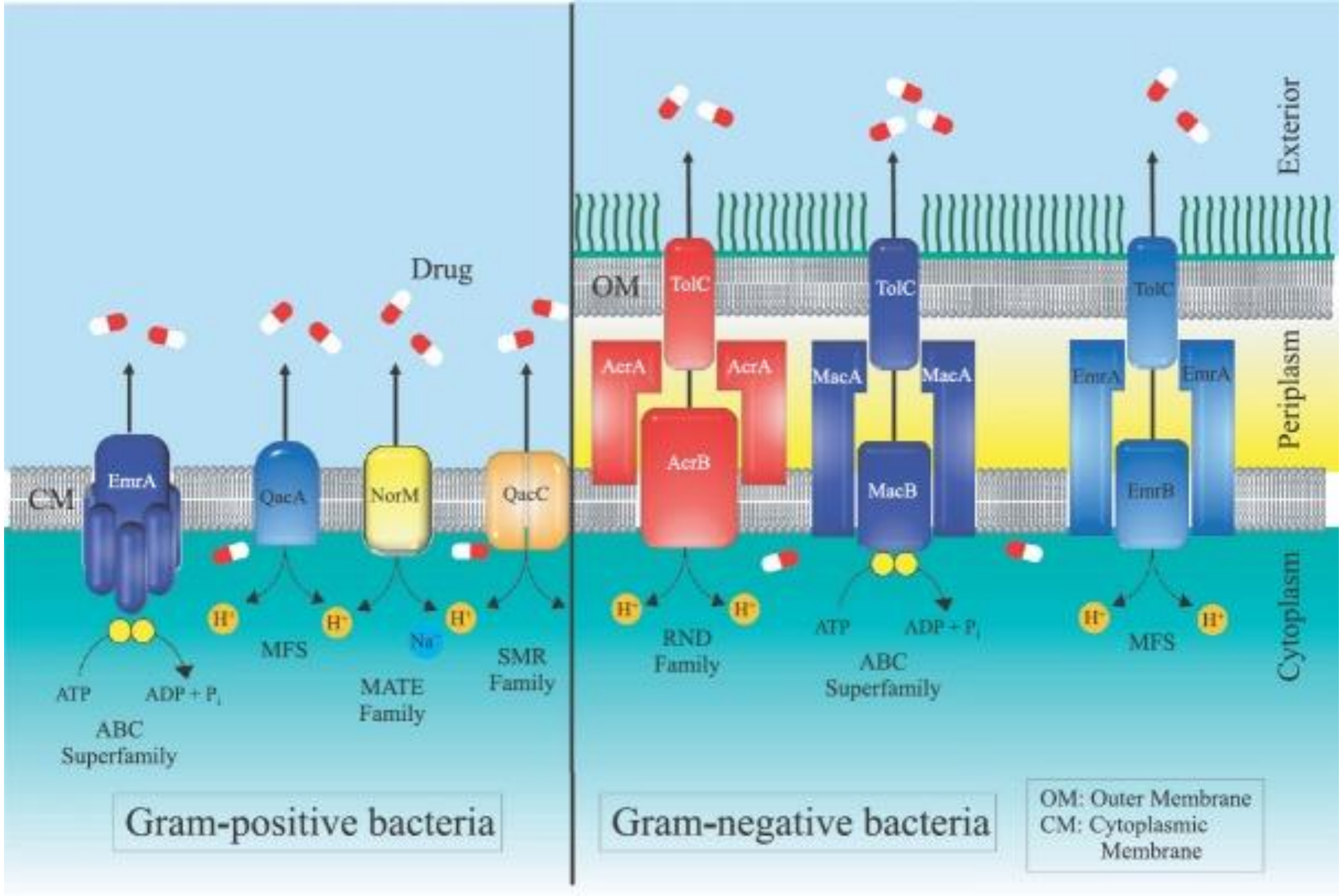
Origanum vulgare
Fissistigma cavaleriei
Foeniculum vulgare
Mentha piperita
Mentha spicata
Ocimum basilicum
Origanum majorana
Origanum onites
Origanum vulgare
Satureja cuneifolia



Beta-lactam
antibiotic

Efflux Pump Inhibition Reduces Antibiotic Resistance

Rosmarinus officinalis
Helichrysum italicum
Eucalyptus grandis
C. ambrosioides
Momordica charantia
Punica granatum
α-Terpinene
15-copaenol
epi-cubenol
geraniol
eucalyptol



Combining EOs with Antibiotics
Synergism vs Antagonism

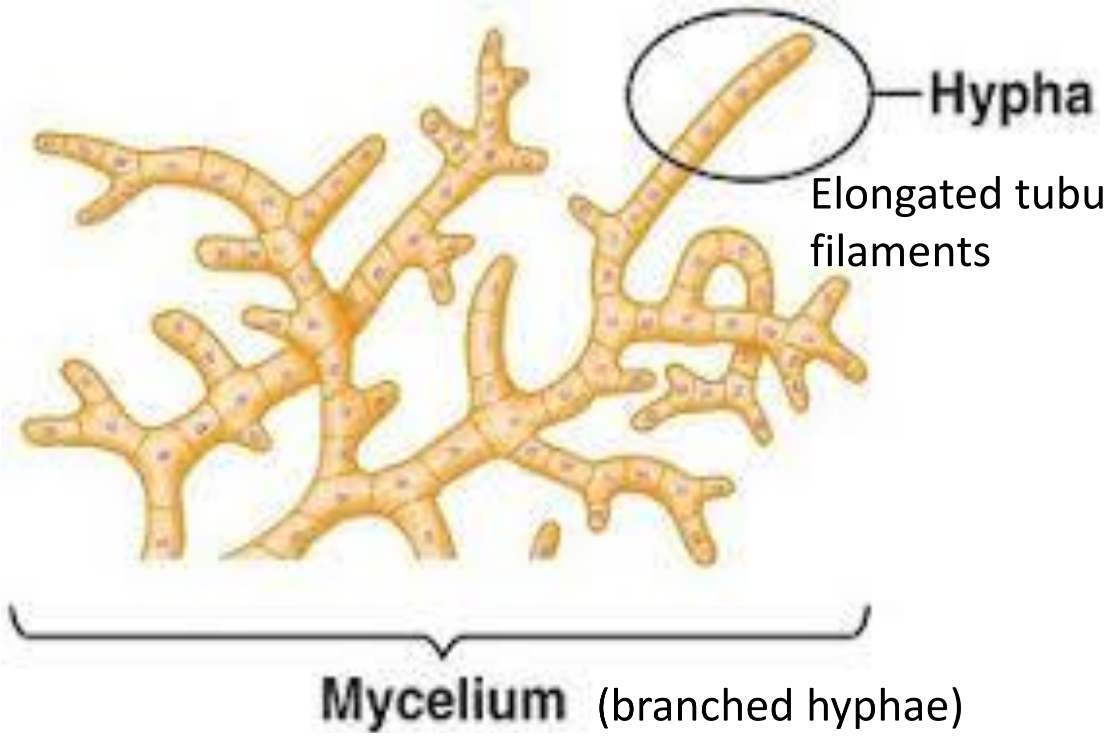
- *Rosmarinus officnalis EO* + ciprofloxacin against gram-negative bacteria displayed a favorable synergistic effect.

- However, *Rosmarinus officnalis EO* + with ciprofloxacin against gram-positive bacteria gave an antagonistic effect.

Vuuren *et al.* (2009)

Next Microbes: Fungi

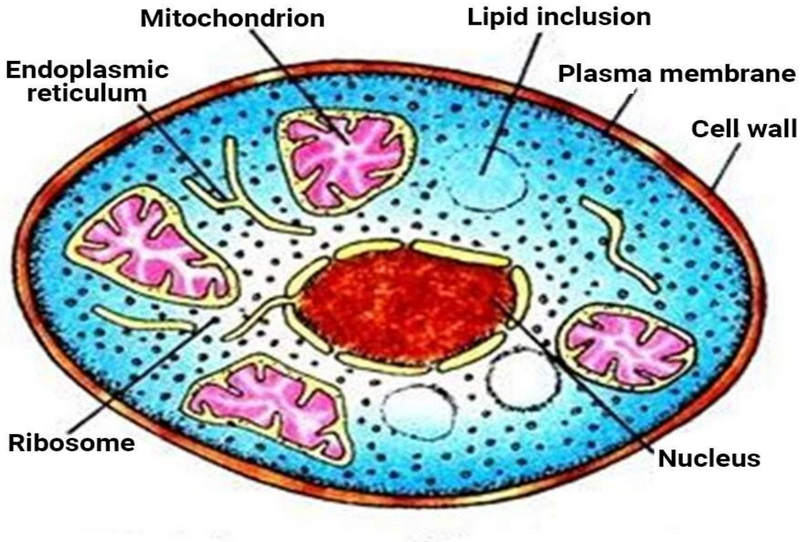
- **Fungus**
  - Eukaryotic (has nuclear membrane)
  - Unlike other eukaryotic organisms has cell wall
  - Chitin in cell wall (N-acetylglucosamine polysaccharide)
  - Unicellular (Yeast)
  - Multicellular (Mycelia, Molds, and Mushrooms)

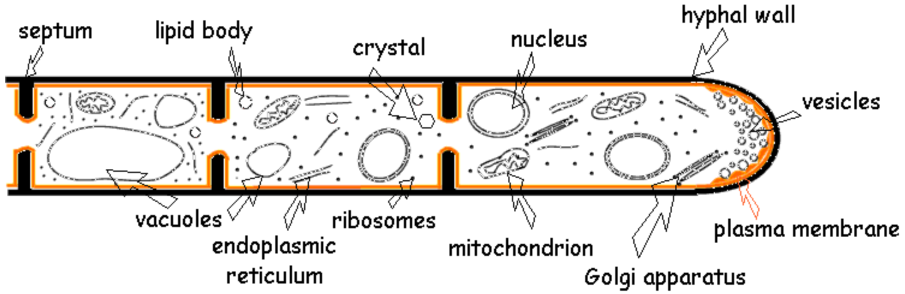


Elongated tubular filaments

(branched hyphae)

Yeast Cell

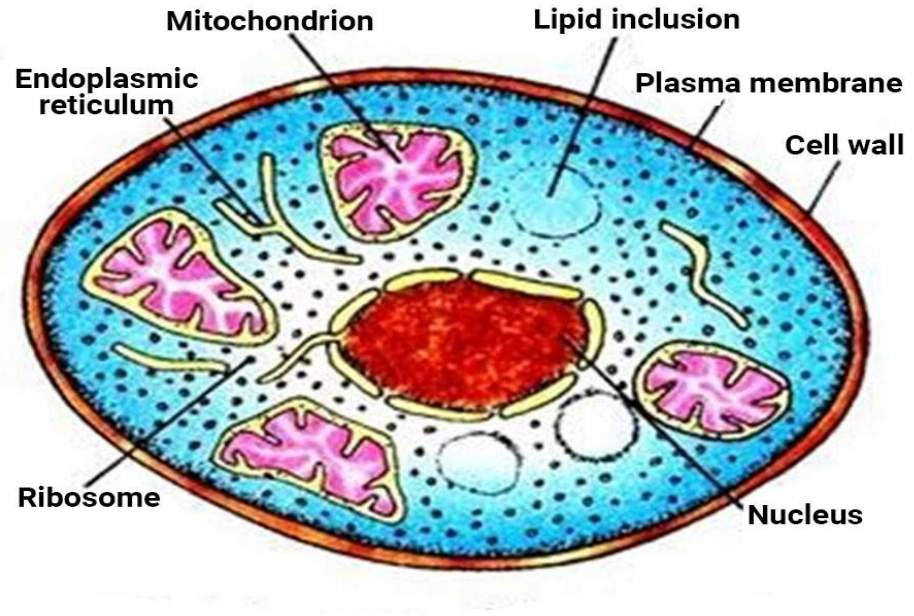




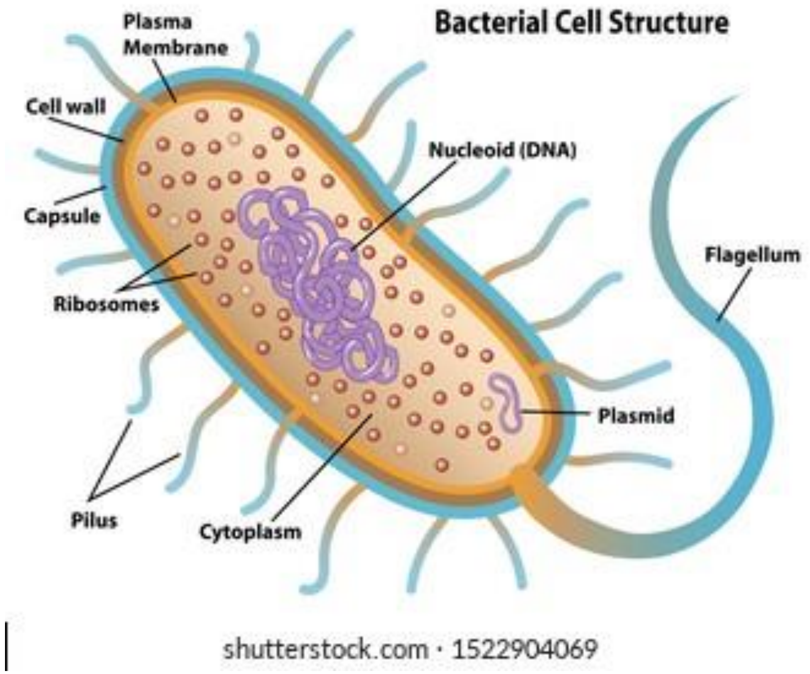
Hypha

Fungus vs Bacteria

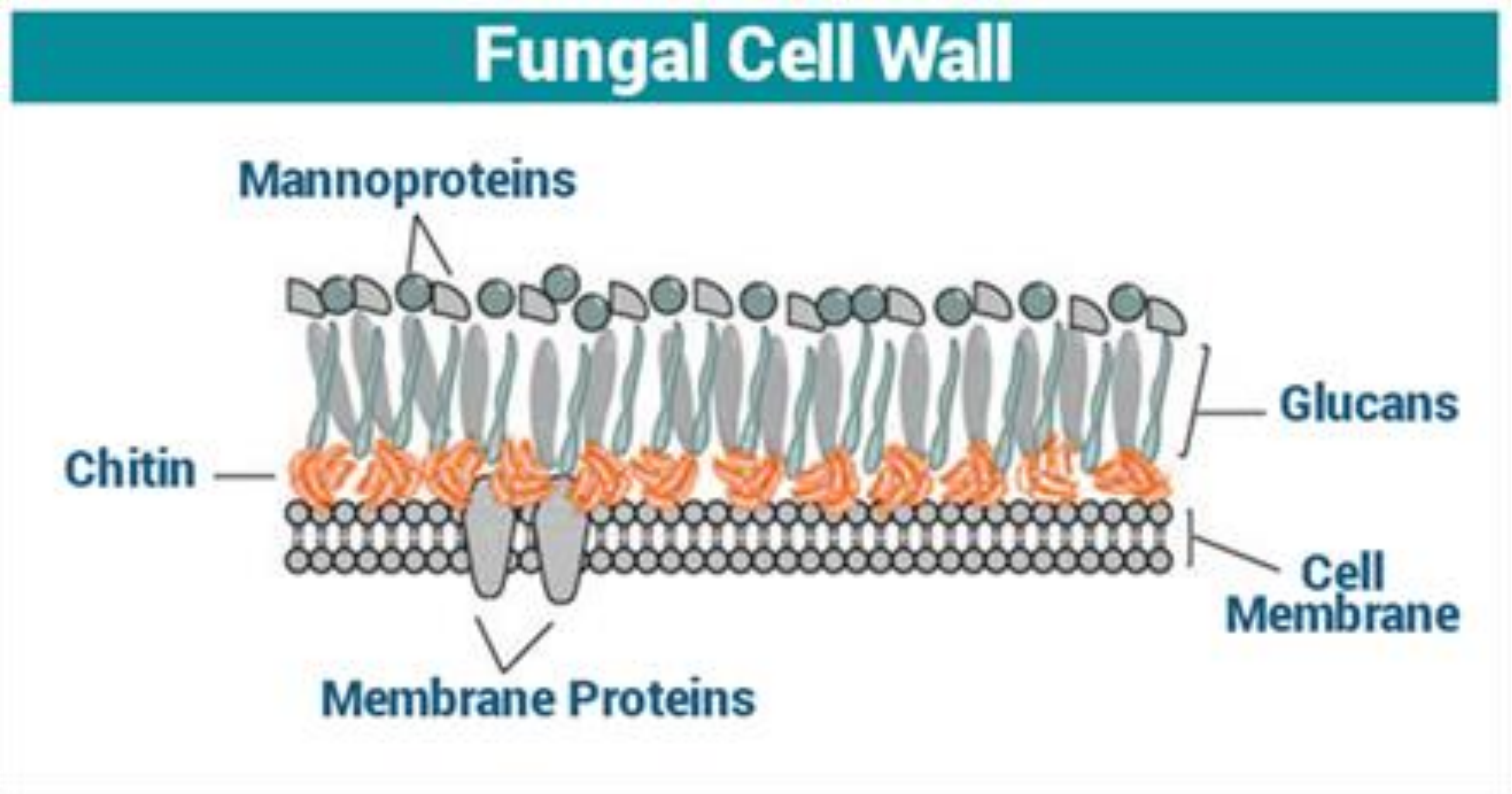
- Fungal Interior membranes
  - Nucleus
  - Mitochondria
  - Endoplasmic reticulum
  - Vacuoles



←Fungus vs Bacteria→



Fungal Cell Membrane Damage



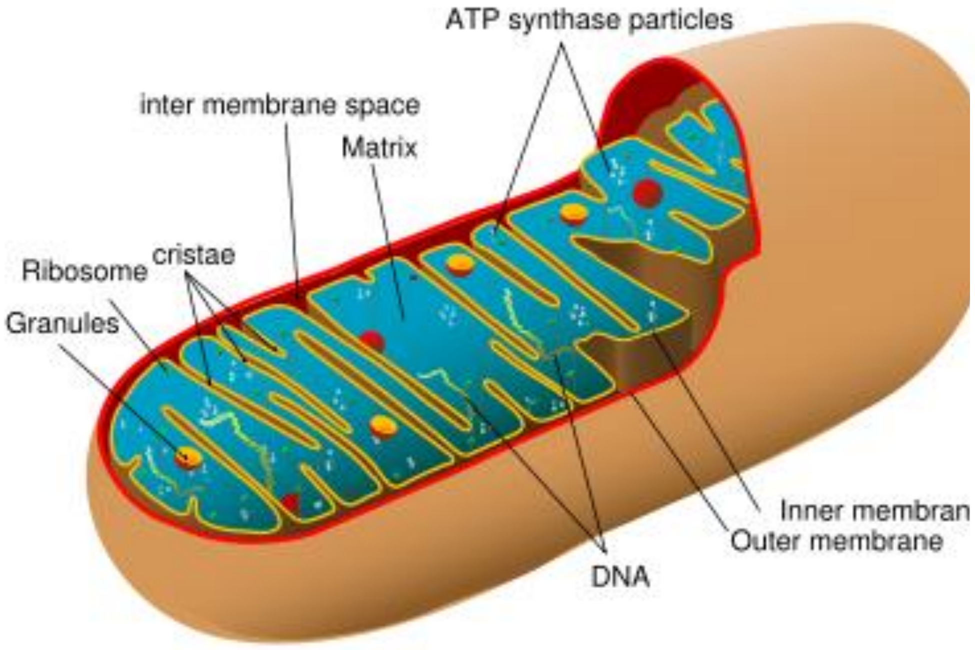
Antifungal Activities of EOs

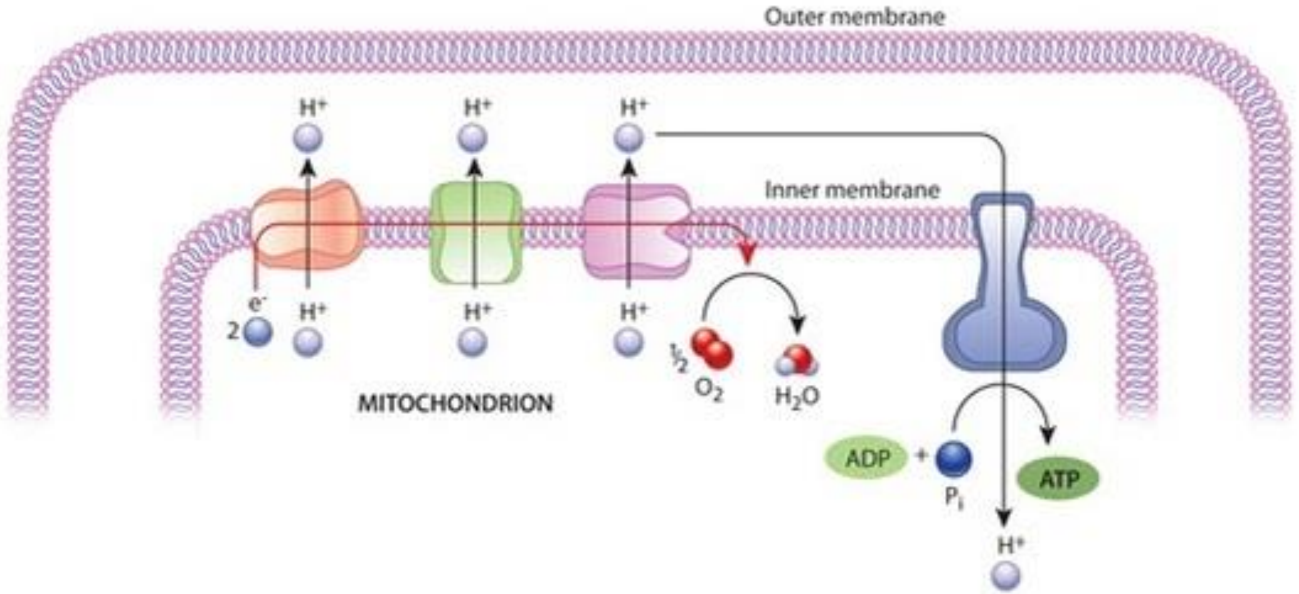
- Cell membrane damage
  - Coagulation of the cellular components
  - Altered membrane potential across the cell membrane

Fungal Interior Membrane Damage

- Inner and outer mitochondrial membrane disruption
  - Disruption of ATP production

Mitochondrial Membrane

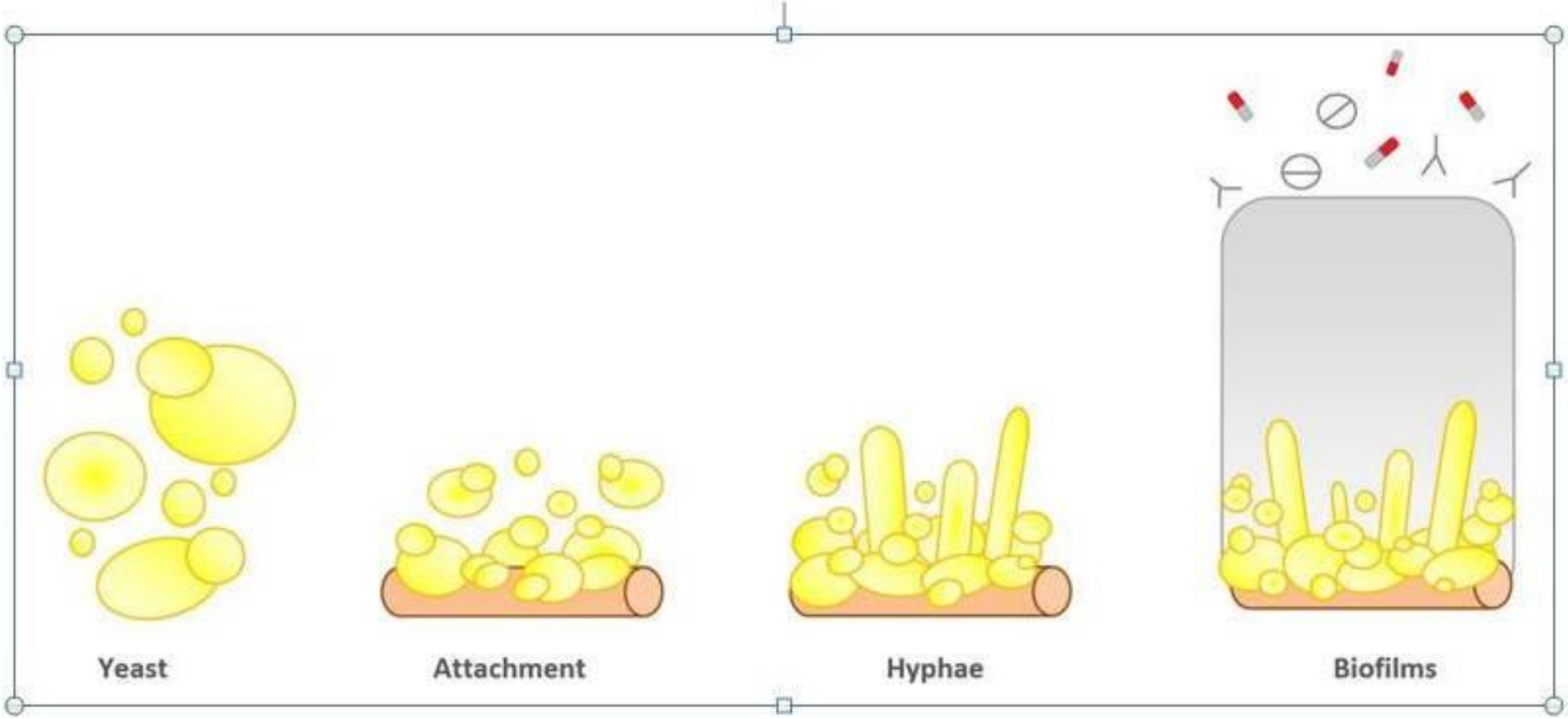




Other Antifungal Activities of Eos

Biofilm

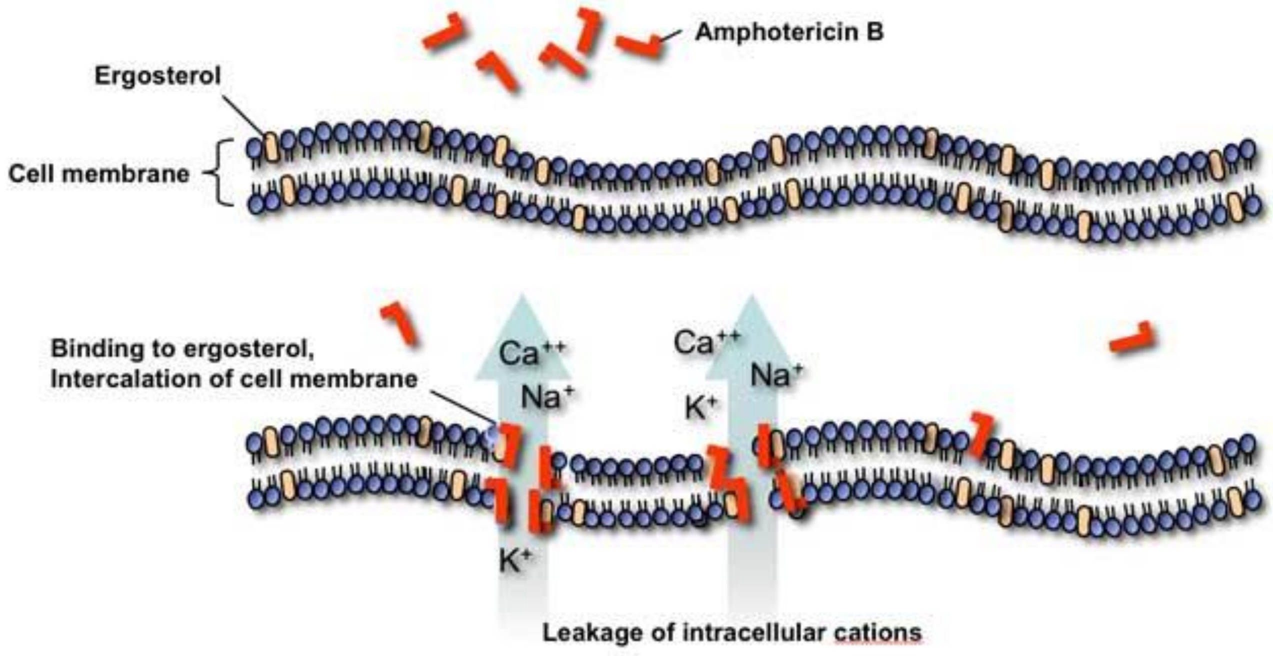
- Disruption of biofilm formation



Other Antifungal Activities of EOs

Ergosterol: The cholesterol of Fungi

- **EOs Possible Effects**
  - Decrease ergosterol biosynthesis
  - Bind to ergosterol in membrane producing leakage



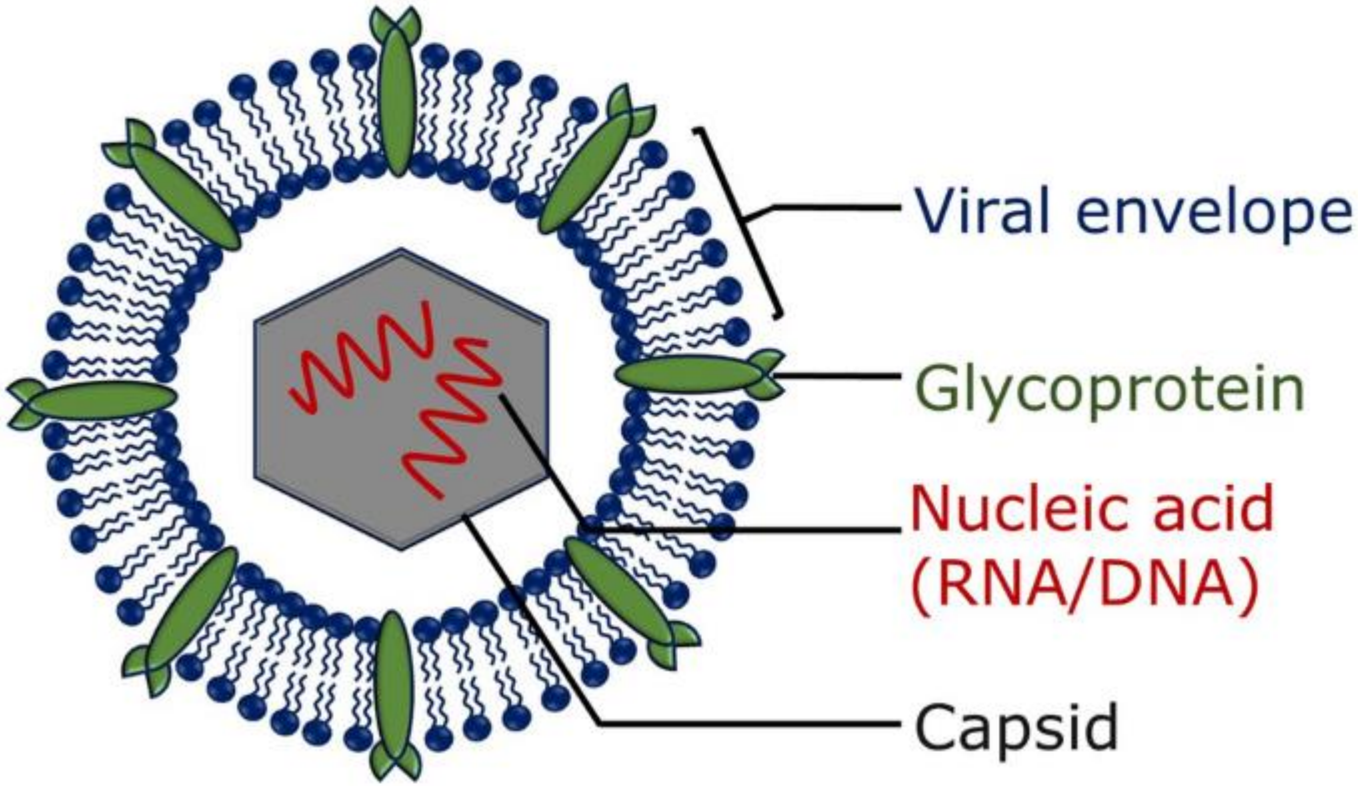
Plant	Chief Chemical Constituents	Susceptible Fungi
Artemisia biennis	(Z)-β-Ocimene,(E)-beta-farnesene, acetylenes, (Z)- and(E)-en-yndicycloethers	*Cryptococcus neoformans, Fonsecaea pedrosoi, A.niger*
Melaleuca alternifolia	Terpinen-4-ol, 1,8-cineole, γ-terpinene, α-terpinene, terpinolene	*Alternaria spp. A. flavus, A. fumigates, A. niger, Blastoschizomyces Capitatus, C. albicans, C. glabrata, C. parapsilosis, C. tropicalis, Cladosporium spp., C. neoformans, Epidermophyton floccosum, Fusarium spp., Malassezia furfur, Microsporum canis, M. sympodialis, M. gypseum, Penicillium spp., Rhodotorula rubra, Saccharomyces cerevisiae, Trichophyton mentagrophytes, T. rubrum, T. tonsurans, Trichosporon spp.*
Ocimum species (Ocimum basilicum and others)	Eugenol, methyl eugenol, cis-ocimene, trans-ocimene, α-pinene camphor	*C. albicans, C. tropicalis, C. glabrata, P. notatum, R. stolonifer, M. mucedo, A. ochraceus, A. versicolor, A. niger, A. fumigates, T. viride, P. funiculosum*
Origanum vulgare	Carvacrol, thymol,γ-terpinene, trans-sabinenehydrate, cis-piperitol, borneol, terpinen-4-ol, linalool	*C. albicans, A. niger, M. gypseum, M. canis, A. cajetani, T. violaceum, T. mentagrophytes, E. floccosum, T. rubrum, T. tonsurans phytopathogens B. cinerea and P. oryzae*
Rosmarinus officinalis	Camphor, camphene, limonene, geraniol, myrcene, linalool benzaylacetate, linalool, α-pinene, α-terpinolene, bornyl acetate, borneol	*C. albicans, M. gypseum, M. canis, A. cajetani, T. violaceum, T. mentagrophytes, E. floccosum, T. rubrum, T. tonsurans, phytopathogens B. cinerea, P. oryzae*
Salvia sclarea	Linalool, linalyl acetate, geranyl acetate, β- ocimene acetate, caryophyllene oxide	*C. albicans, C. tropicalis, C. krusei, C. glabrata, C. parapsilosis*

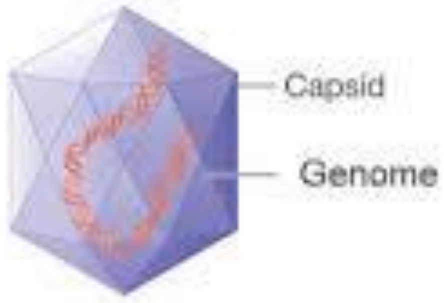
Next Microbes: Viruses

- Enveloped vs Non-enveloped
  - Host cell membrane (phospholipid) plus viral glycoproteins

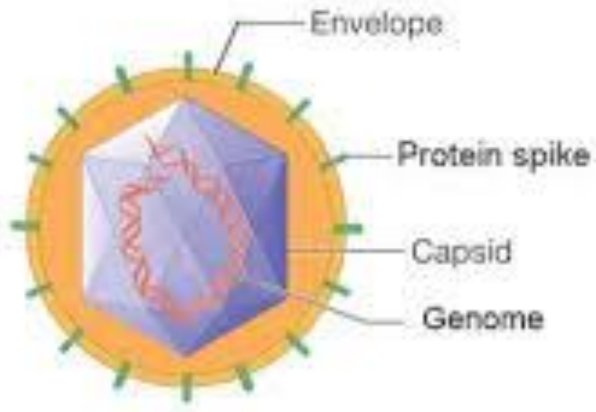
- Capsid (“Nucleocapsid”)
  - Protein subunits

- Genetic material: RNA vs DNA
  - Double stranded
  - Single stranded
  - Positive-sense RNA
  - Negative-sense RNA





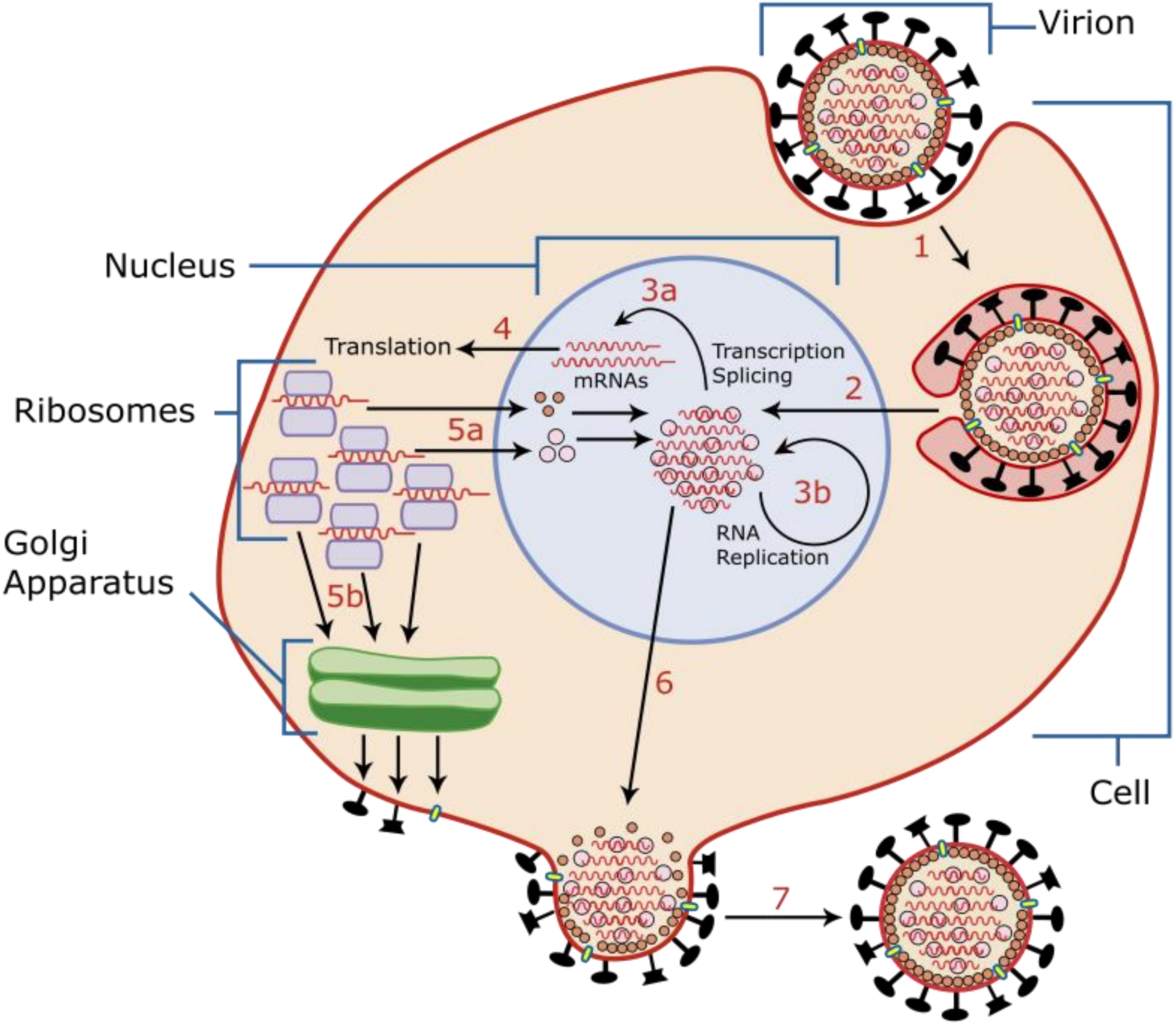
Naked virus



Enveloped virus

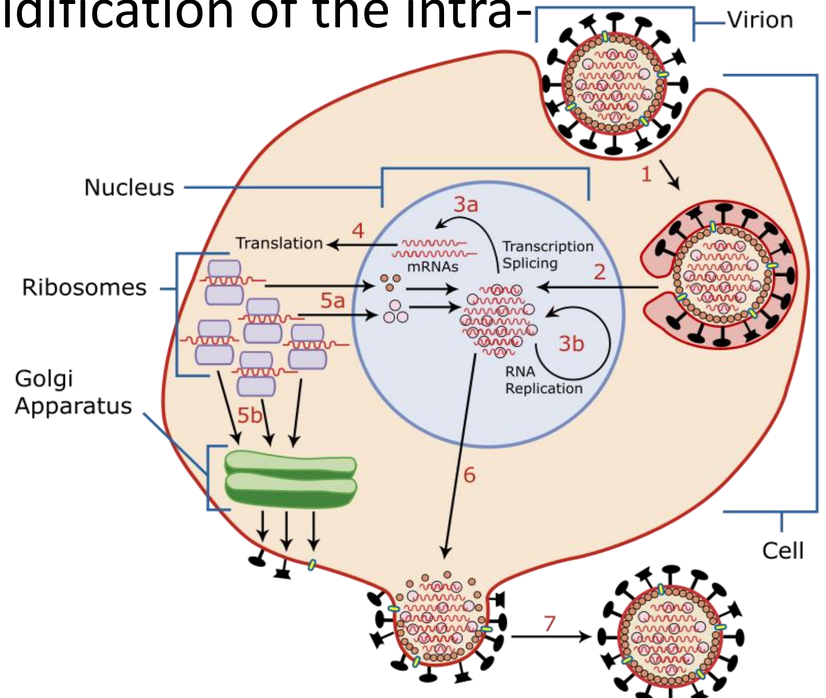
Viral Reproduction Stages

- Attachment
- Entry into host cell (1)
- Uncoating (endosome-lysosome fusion) (2)
- Genome replication
  - Transcription (DNA viruses) (3a)
  - Replication (RNA viruses) (3b &6)
- Synthesis of virus components (4&5a)
- Assembly of parts (5b)
- Release (7)



Antiviral Mechanisms of EOs

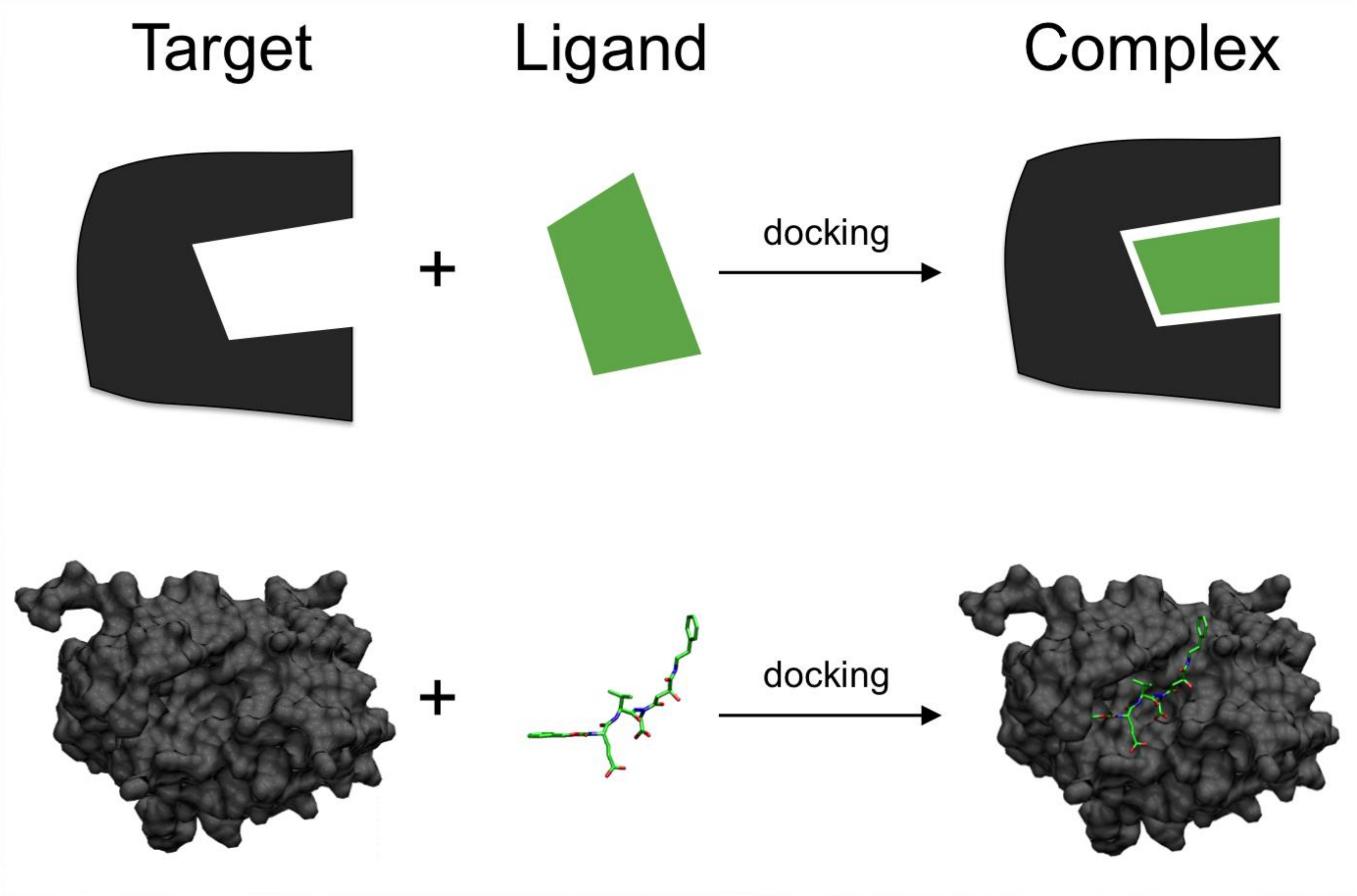
- Direct inactivation outside the cell (disruption of cell membrane integrity)
- Inhibition of attachment to host receptor (attach to binding protein)
  - Blocking host cell receptor
  - Coating capsid
- Inhibition of entry (protease inhibition)
- Uncoating interference (endosome-lysosome fusion or the acidification of the intra-lysosomal compartment)
- Swelling of capsid
- Degradation of RNA or DNA
- Interference with DNA or RNA polymerase
- Inactivation of reverse transcriptase (HIV)
- Suppression of synthesis of viral proteins
- Inhibition of glycosylation of viral proteins



In Silico Research

Molecular docking study

- how two or more molecular structures fit together
- used to predict how a protein (enzyme) interacts with small molecules (ligands)



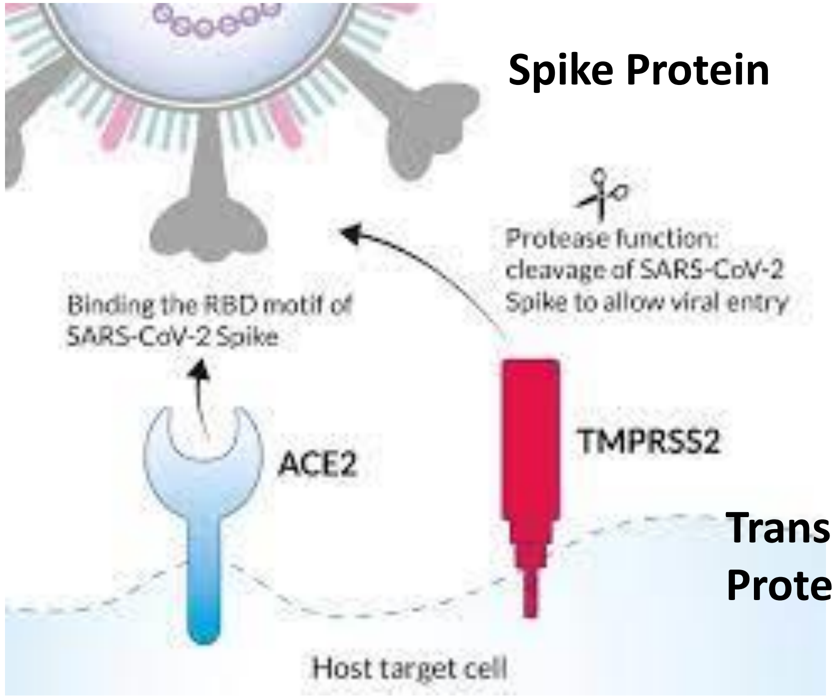
In Silico Molecular Docking Studies for SARS-Cov2

- Eugenol, menthol, carvacrol, garlic oil and other EOs were studied *in silico* against various proteins targets of SARS-CoV-2.
- Docking scores revealed binding affinities towards
    - spike protein
    - main protease
    - RNA dependent RNA polymerase
    - Human ACE-2 receptors

(Silva et al. 2020, Kumar et al. 2020, Thuy et al. 2020)

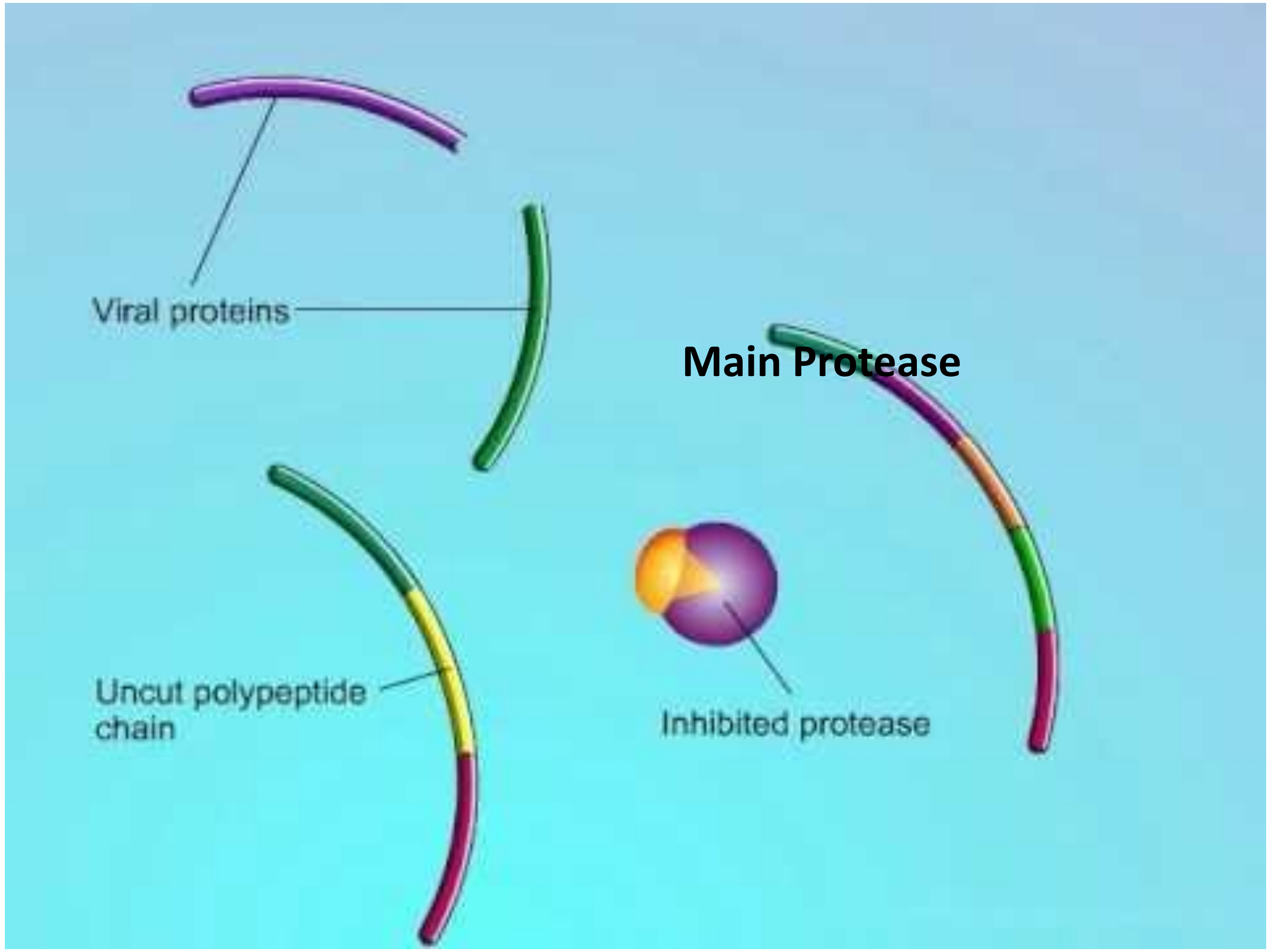
Protease Inhibition: TMPRSS2 and Main Protease

Viral proteases are enzymes that catalyze the cleavage of specific peptide bonds in viral polypeptide precursors or in cellular proteins.



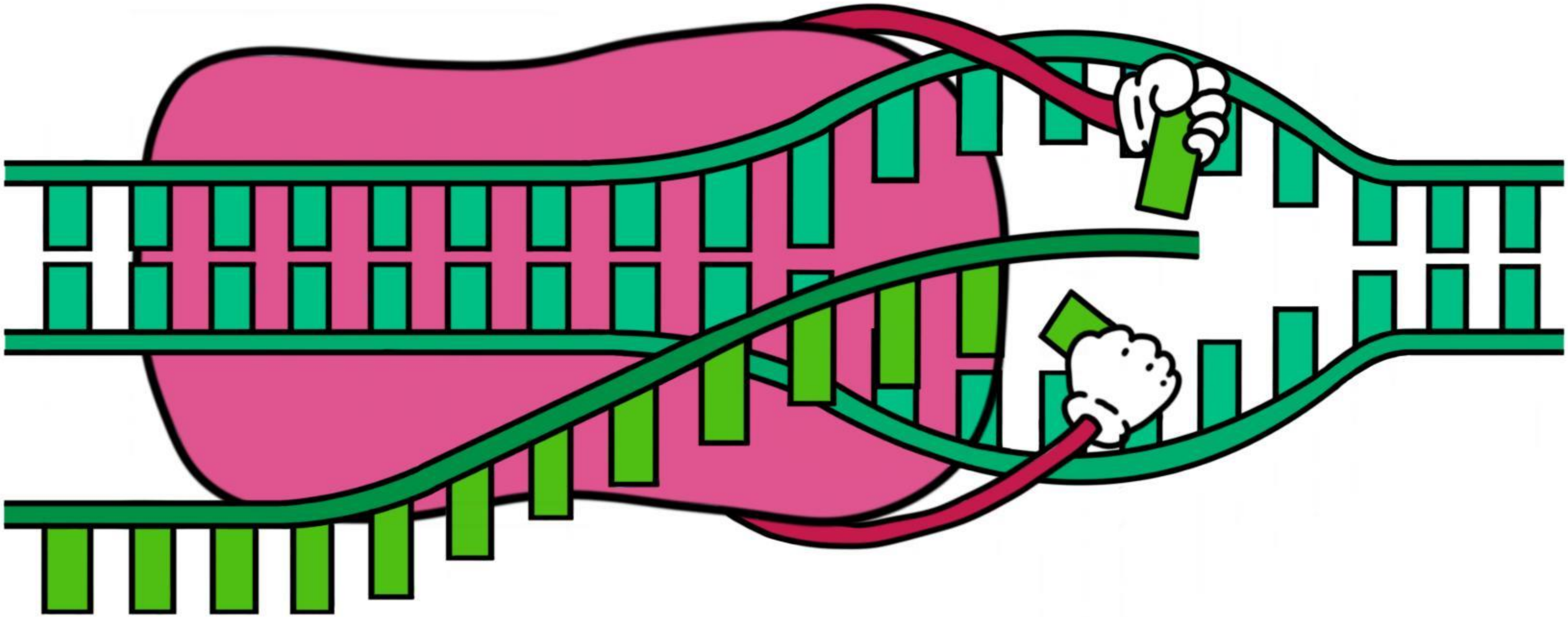
Spike Protein

Transmembrane Protease



Main Protease

Transcription: Role of the RNA Polymerase



RNA polymerase is the main enzyme involved in transcription.
It reads one of the DNA strands and adds complementary nucleotide bases to make an mRNA transcript.
The mRNA will later be translated into protein.

In Silico Molecular Docking Studies for SARS-Cov2

- (E,E)-α-farnesene, (E,E)-farnesol, and (E)-nerolidol showed the best binding with **main protease**.
- (E,E)-αfarnesene, (E)-β-farnesene, (E,E)-farnesol, and (E)-nerolidol showed best binding scores with **non-structural protein 15 (Nsp15), an endoribonuclease**.
- (E,E)-farnesol showed the best affinity for **RNA-dependent RNA polymerase (RdRp)** in RNA viruses.
- α-bulnesene, eremanthin, (E,E)-α-farnesene, (E)-β-farnesene, (E,E)-farnesol, (E)-nerolidol, β-sesquiphellandrene, and (Z)-spiroether showed best binding with human **ACE2 receptor**.
- (E)-cinnamyl acetate, eremanthin, (E,E)-α-farnesene, (E)-β-farnesene, (E,E)-farnesol, and geranyl formate showed best binding of **SARS-CoV-2 spike proteins**.

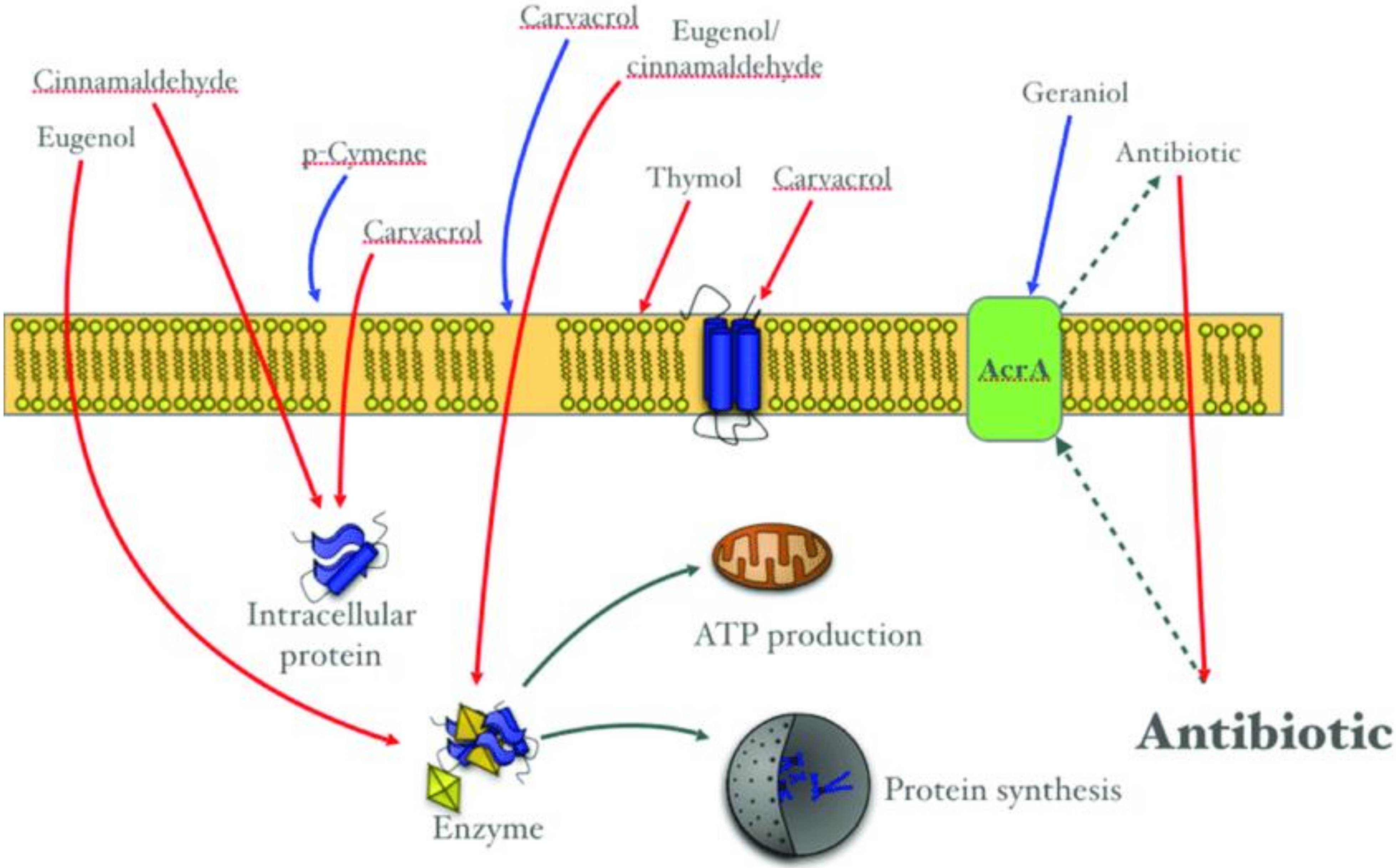
(Silva et al. 2020, Kumar et al. 2020, Thuy et al. 2020)

In Silico Molecular Docking Studies for SARS-Cov2 (continued)

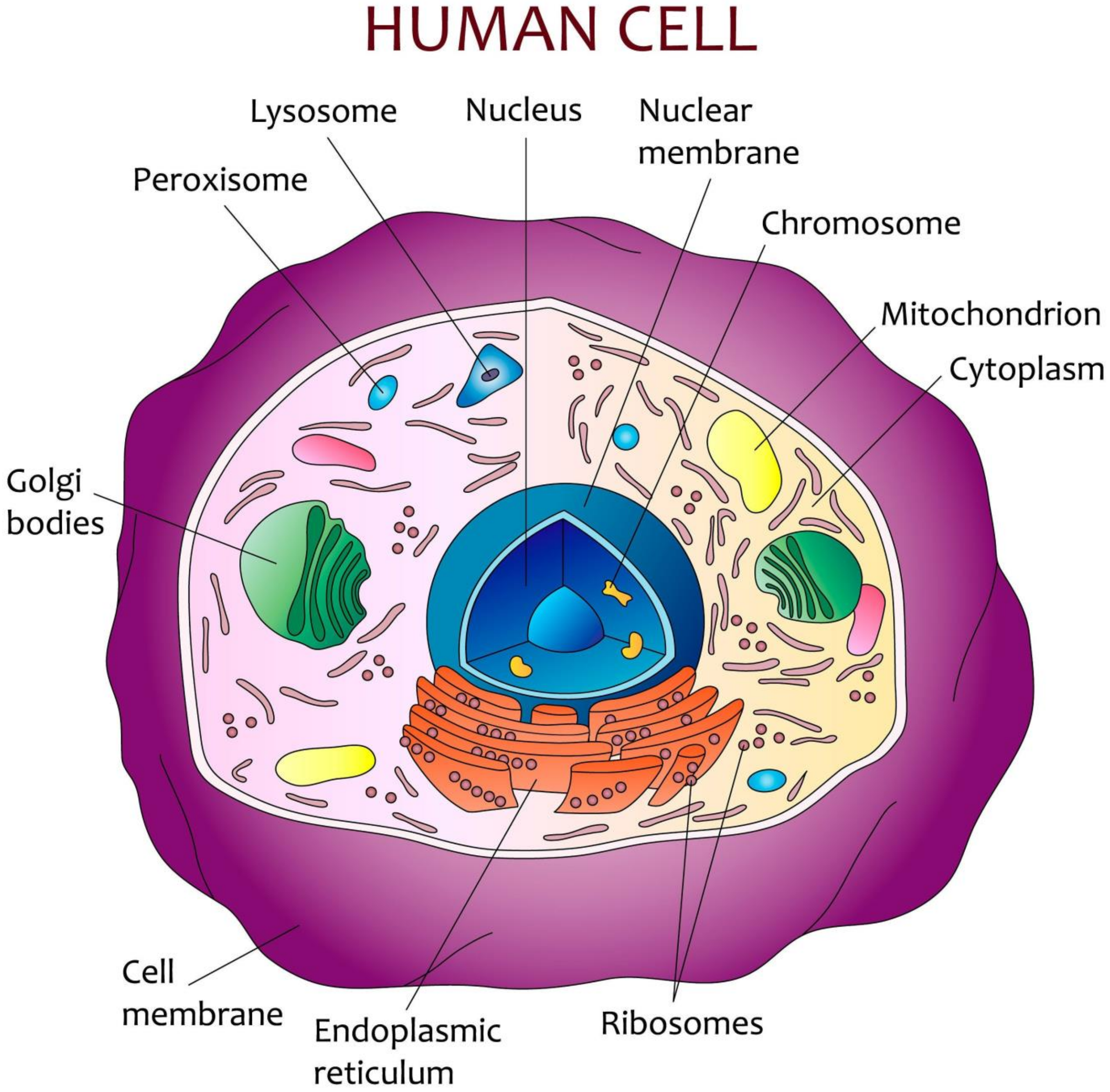
- Anethole, cinnamaldehyde, carvacrol, geraniol, cinnamyl acetate, L-4-terpineol, thymol, and pulegone showed potential to **inhibit S1 subunit of S proteins**. Cinnamaldehyde was the most effective. (Kulkarni 2020)
- Cinnamaldehyde and thymoquinone showed weak binding affinities against SARS CoV **RNA-dependent RNA polymerases**.
  (Elfky 2020)

Plant	Chief Chemical Constituents	Susceptible viruses
Fortunella margarita	Gurjunene, eudesmol, muurolene	*Avian influenza A virus (H5N1)*
Syzygium aromaticum	Eugenol, eugenyl acetate, caryophyllene	*adenovirus type 3, poliovirus, and coxsackievirus B1*
Melissa officinalis	Myrcene, linalool, camphor, citronellal, β-caryophyllene, caryophyllene oxide, citral	*HSV-2, avian influenza virus (AIV) subtype H9N2*
Mentha piperita	Menthol	*HSV-1 and HSV-2*
Ocimum campechianum	Linalool, eugenol	*HSV-1*
Origanum vulgare	Carvacrol, β-fenchyl alcohol, thymol, and γ-terpinene	*adenovirus type 3, poliovirus, and coxsackievirus B1*
Pogostemon cablin	Patchoulol, δ-guaieno; gurjunene-α, α-guaiene, aromadendrene, β-patchoulene	*Influenza A (H2N2) virus*
Trachyspermum ammi	Thymol, α-pinene, p-cymene, limonene	*Japanese encephalitis virus(JEV)*
Thymus vulgaris	Thymol, linalool, carvacrol, 1,8-cineole, eugenol, camphor, camphene, α-pinene, borneol, β-pinene	*SARS-CoV-2, HSV-1, Influenza A*

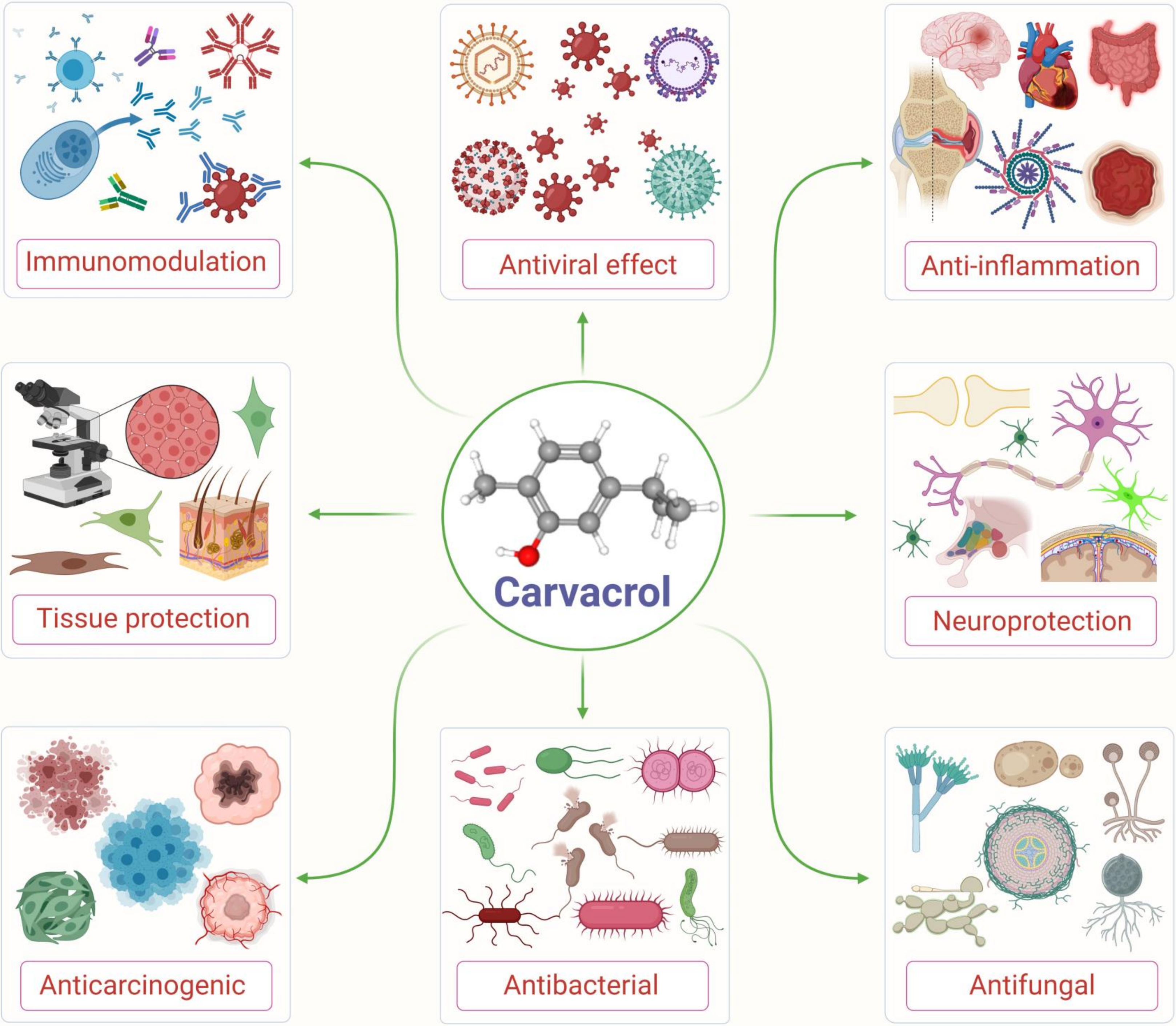
RNA virus in red; DNA virus in black; enveloped virus underlined


Carvacrol
Eugenol/
cinnamaldehyde
Cinnamaldehyde
Eugenol
p-Cymene
Carvacrol
Geraniol
Antibiotic
Thymol
Carvacrol
AcrA
Intracellular
protein
ATP production
Enzyme
Protein synthesis
Antibiotic

Final Cautionary Note: Human Cells Have Membranes Too!



Antimicrobial effects are only a part of the EO story!



Stay Tuned!

Thank You ☺

Different Units for Mean Inhibitory Concentrations (MICs) of Sample EOs

Plant	Bacteria	MIC
Cymbopogan citratus	Escherichia coli	0.6 µL/mL
	Salmonella typhimurium	2.5 µL/mL
	Staphylococcus aureus	0.6 µL/mL
Satureja montana	Pseudomonas aeruginosa	23.33 µg/mL
	Streptococcus pyogenes	116.67 µg/mL
	Streptococcus mutans	60.00 µg/mL
	Streptococcus sanguis	23.33 µg/mL
	Streptococcus salivarius	23.33 µg/mL
	Enterecoccus feacalis	53.33 µg/mL
	Lactobacillus acidophilus	125.00 µg/mL
Origanum vulgarae	Escherichia coli	1600–1800 ppm
	Staphylococcus aureus	800–900 ppm
Lavandula officinalis	Escherichia coli	2000 ppm
	Staphylococcus aureus	1000–1200 ppm
Cinnamomum zeylanicum	Acinetobacter	8 mg/mL
	Klebsiella pneumoniae	2 mg/mL
	Protreus vulgaris	8 mg/mL
	Enterococcus fecalis	4 mg/mL
	Staphylococcus aureus	0.5 mg/mL
	Staphylococcus epidermidis	1 mg/mL