

FAPC and IFT-OK 2014 Research Symposium· Feb 18th, 2014

A RESEARCH STORY

Delivering Lipophilic Food Antimicrobials in Emulsions



Kanika Bhargava, PhD, CHA

Assistant Professor, Food Science

Nutrition and Food Management Program

University of Central Oklahoma

Are you feeling like this?

- Food Science
- Animal Science
- Nutrition
- Dietetics



- Students
- Faculty and Staff
- Food Industry

Transform!

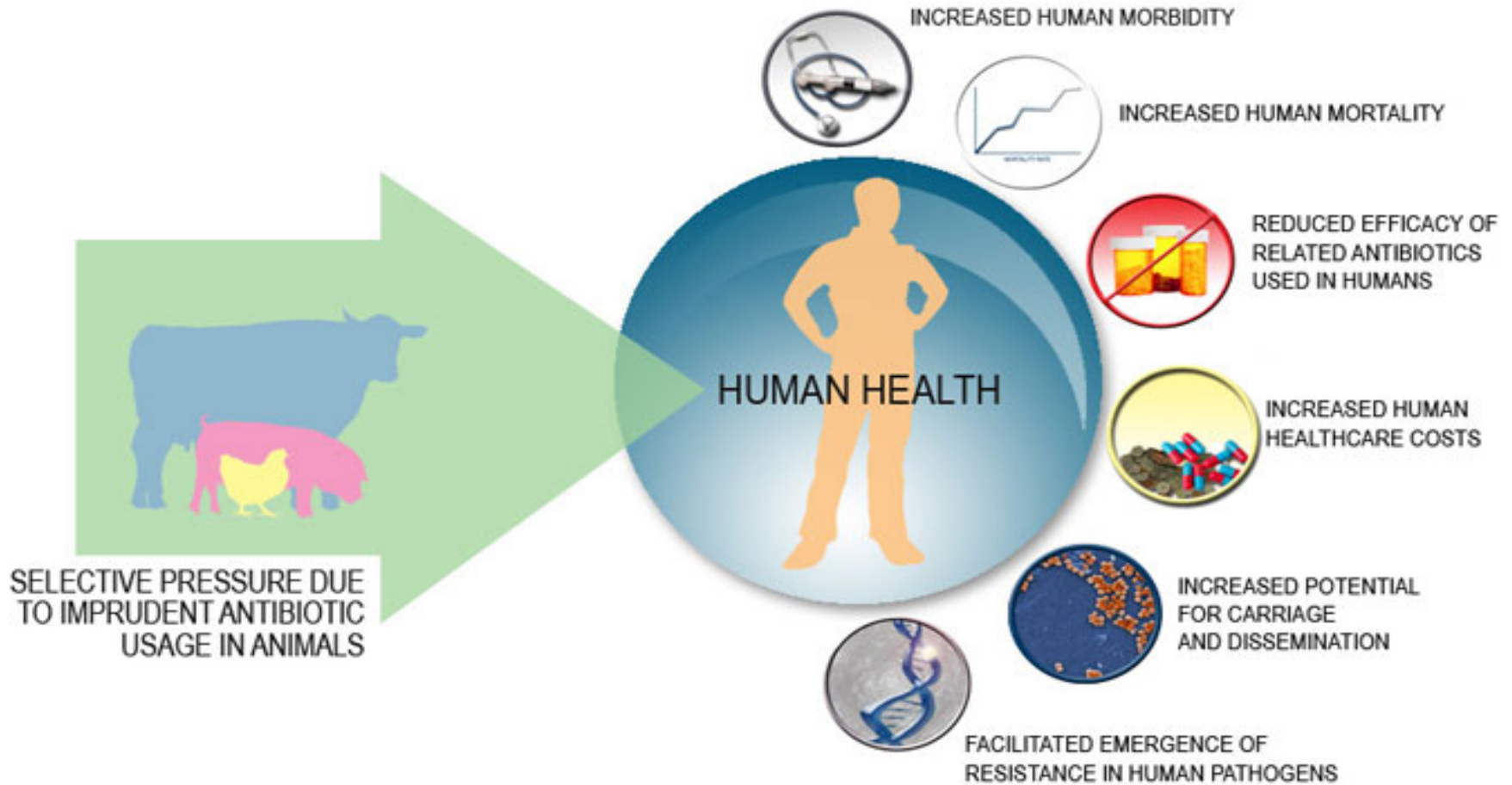




How I got interested in this
research?

Research Journey Begins...

Antimicrobial Resistance (AMR) Global Concern



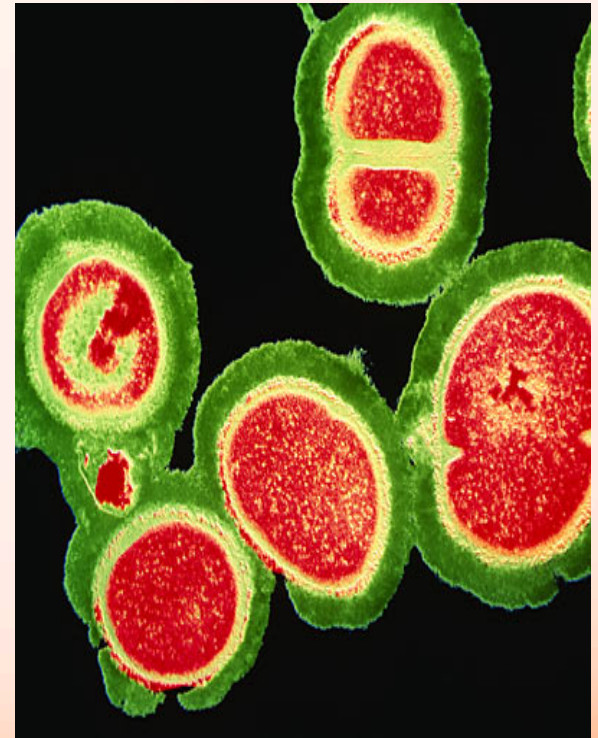
Bill introduced: **Preservation of Antibiotics for Medical Treatment Act**

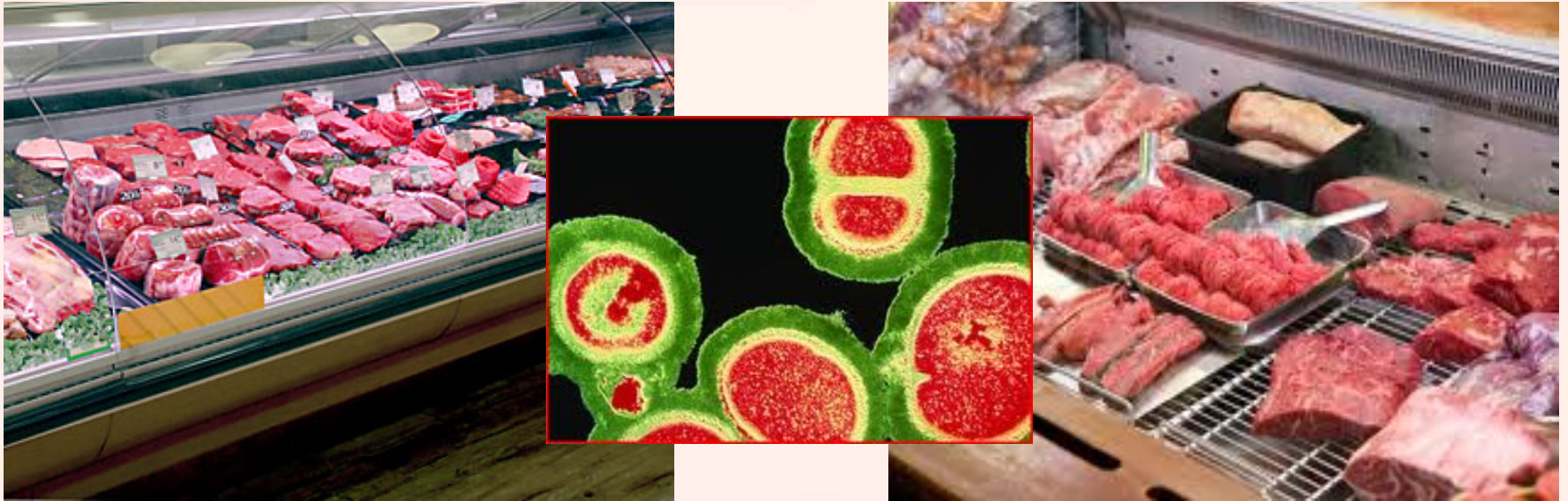
Signature of AMR MRSA

- **Methicillin Resistant**
Staphylococcus aureus (MRSA)

TYPES

- **Hospital Acquired (HA)**
- **Community Acquired (CA)**
- **Livestock Acquired (LA)**
- **Leading cause of HA infections
but not only hospital problem**





How prevalent is MRSA in retail meat?

What is the molecular composition of these isolates?

Sampling



Beef

• 156

Chicken

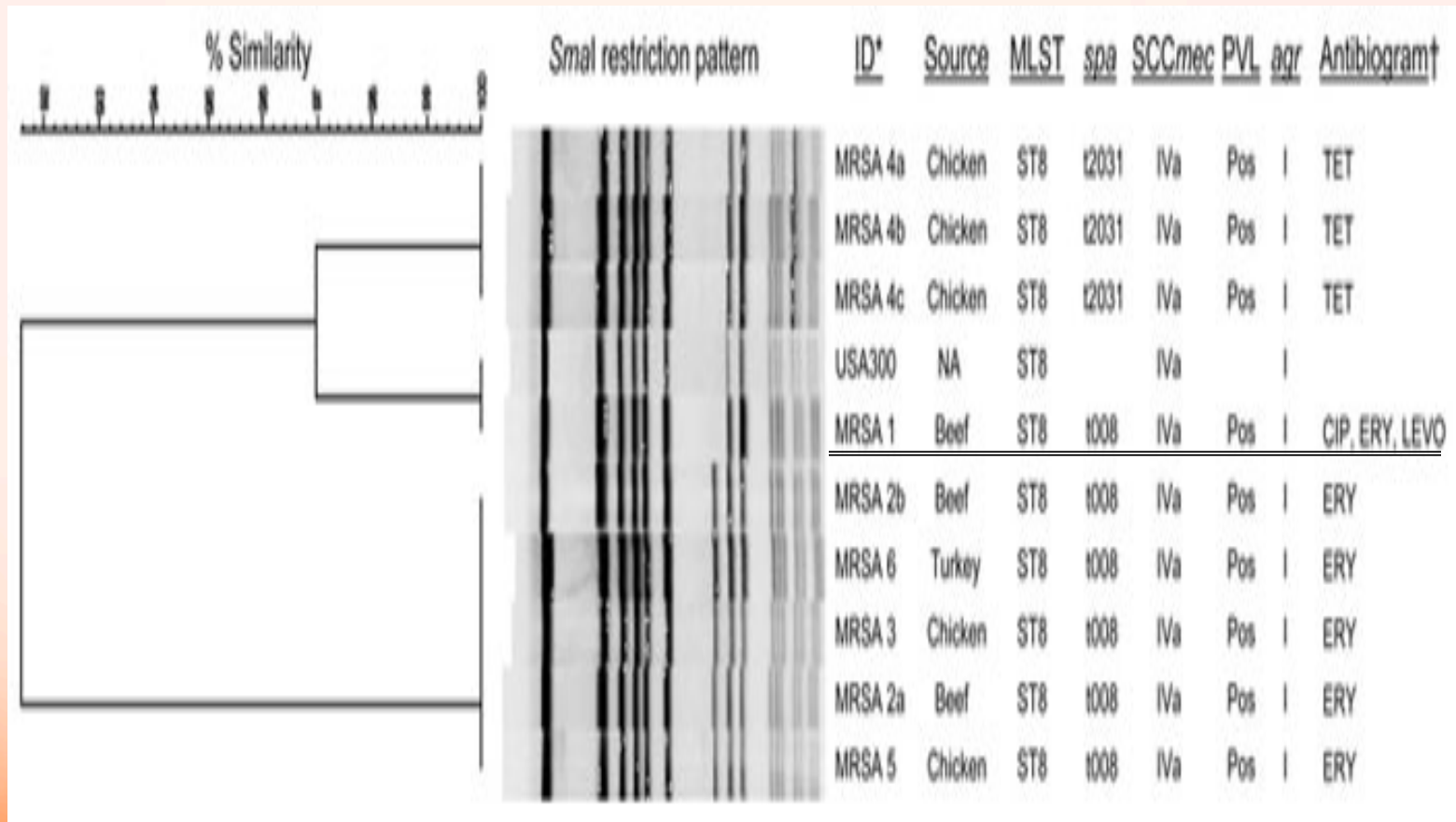
• 76

Turkey

• 57

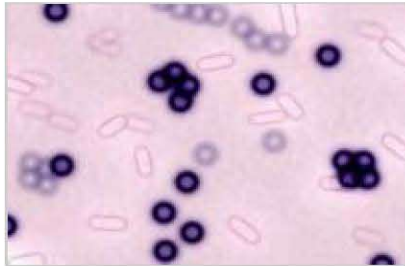
Raw meat samples were collected from 30 randomly-selected retail meat stores in Detroit, Michigan during August 2009 to January 2010.

MRSA USA 300



MRSA 'Superbug' Bacteria Found in Detroit Meat

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Chad Baker/Photodisc/Thinkstock

(DETROIT) -- First they were riding on bedbugs. Now, drug-resistant superbugs are showing up in supermarket meat. Raw beef, chicken and turkey from Detroit grocery stores contained methicillin-resistant *Staphylococcus aureus* (MRSA), a sinister strain of bacteria that doesn't respond to typical antibiotics, researchers reported Wednesday.

It may sound scary, but it's no reason to go vegetarian, experts say.

"We've known for a long time that raw meat and poultry purchased in supermarkets can be contaminated with bugs that can make us sick, like salmonella and *E. coli*. As long as we clean our hands and our utensils and we cook the food, we kill the bacteria," said Dr. William Schaffner, chair of preventive medicine at the Vanderbilt University Medical Center in Nashville. "Even though this is a new bug, that shouldn't change anything. It should just reinforce all those messages."

The study, reported online Wednesday in *Emerging Infectious Diseases* -- the Center for Disease Control and Prevention's journal, is not the first to find MRSA in meat. But very few have ever come out of the United States, so it's making headlines nationwide.

Transformation

Epidemiological
Preventive



Natural Plant Antimicrobials

- Essential Oil (EO) and Spices are being used from centuries in our cuisine
- Clove, Cinnamon, Mustard, Garlic and Mint are still applied as Alternative Remedies
- Medical Application of EO became secondary to their use of flavor and



Essential Oils

- Essential oils are plant essences distilled or pressed from stems, leaves, fruits and flowers and they are rich in chemicals like phenols, monoterpenes, and ketones
- Almost every essential oil contains natural chemicals that act as antimicrobial agents, protecting their native plants and acting as the key ingredients in human medicine for millennia.

COMBINATION OF ANTIMICROBIAL AGENTS!!



Importantly!

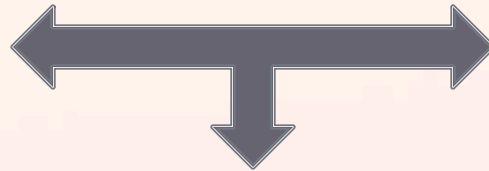
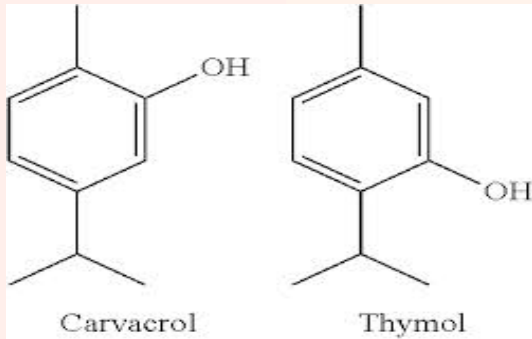
- Potential for development of bacterial resistance to essential oil is very less...

WHY?

*Unlike drugs, whose chemical formulas are precisely modulated and measured, the chemical constituents of an essential oil will vary

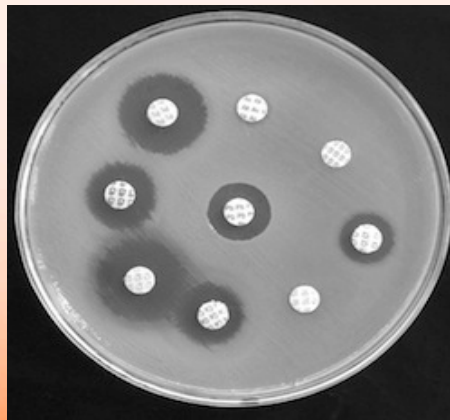
Why doesn't modern medicine consider natural options like essential oils a viable alternative for fighting drug-resistant strains of bacteria?

Issues!

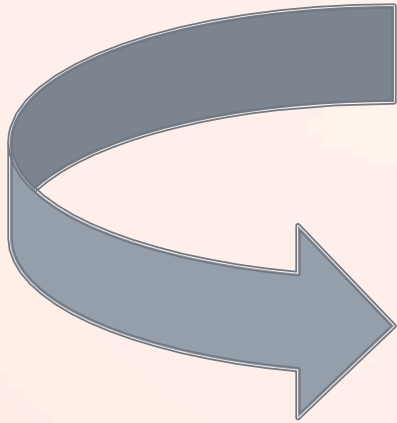
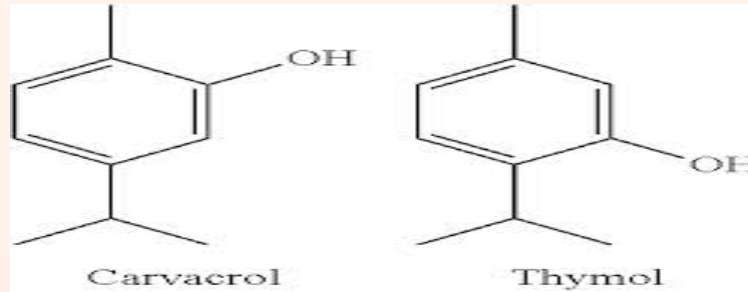


In-vitro
systems

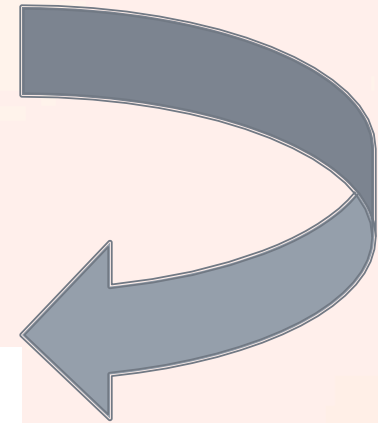
Solubilization



Issues!



Broth-model System



Food



Need of Encapsulation System..

- Modify molecular interactions that are of key importance to functionality
- Maintain integrity and bioactivity of food antimicrobials upon addition to foods

Emulsions???

Food Emulsions

- Milk and Beverages
- Mayonnaise and Salad Dressing
- Butter and Spreads
- Ice Cream and Deserts

A diverse group of products with various appearances, textures, stabilities and flavors, but

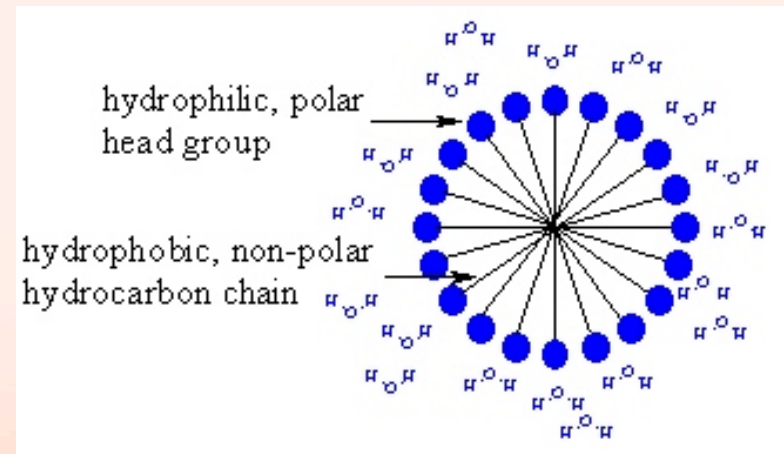


Emulsions

- An emulsion consist of two immiscible liquids (usually oil and water), with one liquid dispersed as small spherical droplets in the other liquid.
- Emulsifier is required
- Particle Diameter (0.1 to 100 μm)
- Optically opaque

Emulsifier

- Amphiphilic Molecules
- Not highly soluble in either water or oil
- Form micelles
 - Polymeric
 - Proteins, Polysaccharides
 - Small molecules
 - soap; phospholipids;
 - glycerol monostearate





Can phytochemicals (essential oils) emulsions serve as antimicrobial alternatives to inhibit MRSA strains?

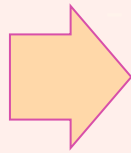
Will they work synergistically with commercial antimicrobials for which MRSA has developed resistance?

Formulation of oil in water emulsions

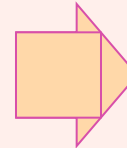
**Essential
Oil/
Derivatives**



**Tween 80
(0.5%)
Non-ionic
emulsifier**



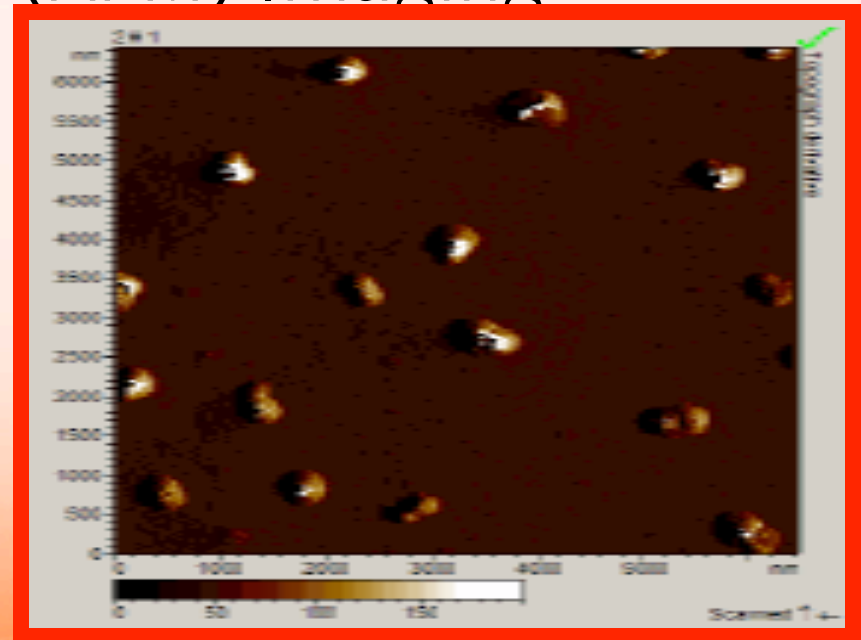
Ultrasonication



Characterization of oil in water emulsions

- Dynamic Light Scattering (DLS) measurements
- Atomic Force Microscopy (AFM) Imaging

**Average Diameter:
130nm**



Methods

Phytochemicals:

Oregano oil
Cinnamon oil
Thyme oil
Clove oil
Lemongrass oil
Rosemary oil
Basil oil
Sage oil
Carvacrol
Eugenol
t-cinnamaldehyde

MRSA Strains:

ATCC 43300
6 HA and 6 CA

Antimicrobials:

Cefoxitin
Tetracycline
Erythromycin
Ciprofloxacin
Vancomycin

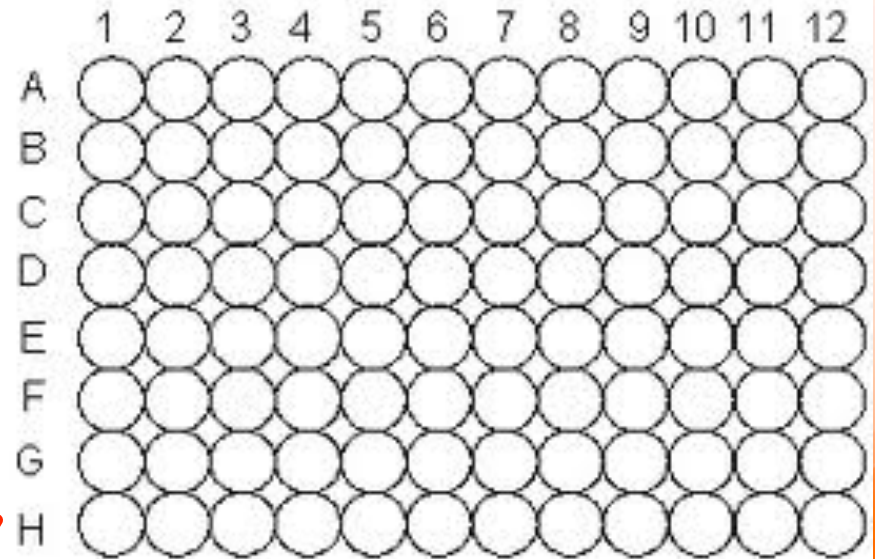
Minimum Inhibitory Concentration
Broth Microdilution test

Selected Phytochemicals and antimicrobial resistant MRSA

Synergism Test
Checkerboard Assay

Methods

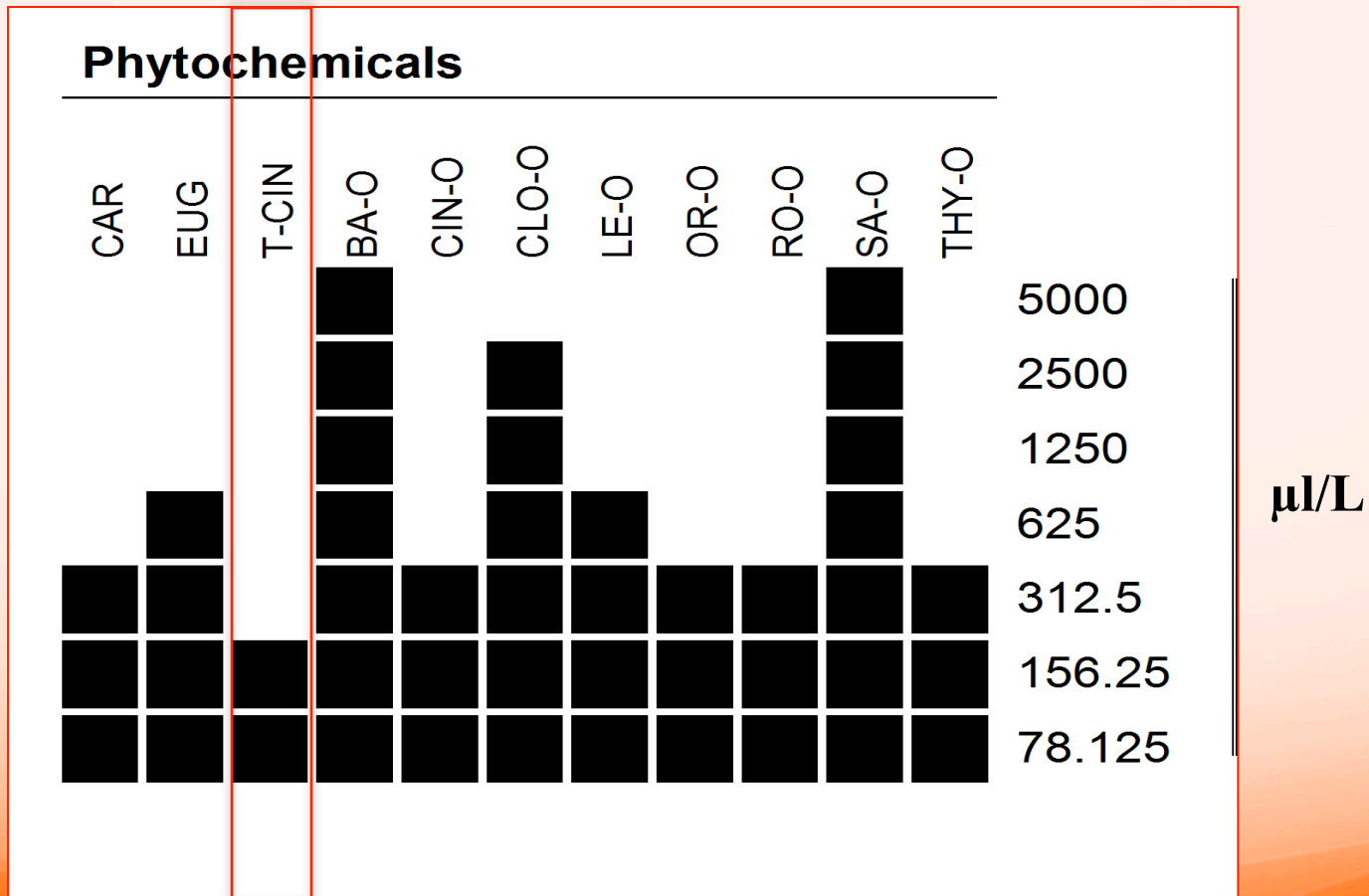
- Determination of MIC and MBC by Broth microdilution test
- Guidelines: National Committee for Clinical Laboratory Standards (NCCLS)



5000ppm till 78.125 ppm in CAMHB

*Final Concentration of bacteria in each well: 5×10^5 CFU/ml

MIC of phytochemical emulsions

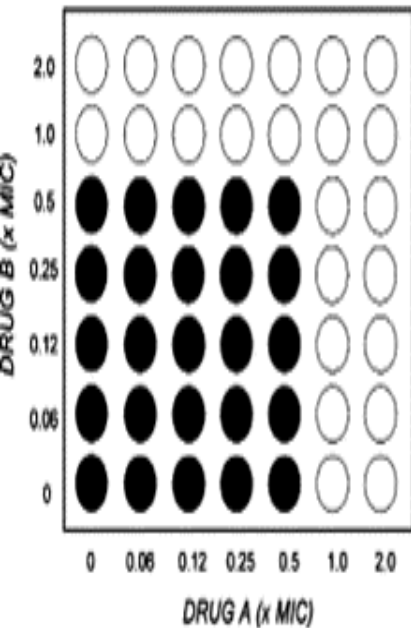


MIC of standard and selected phytochemicals

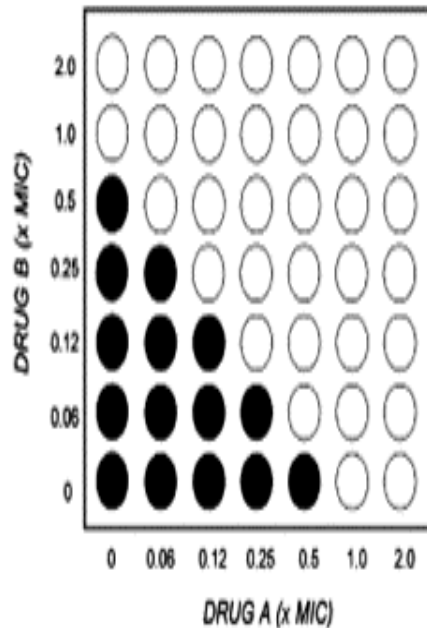
MRSA strains	MIC ($\mu\text{g/ml}$)					
	CEF	TET	ERY	CIP	VAN	TCIN ($\mu\text{l/L}$)
ATCC43300	16	2	≥ 64	≤ 0.5	1	312.5
HAMRSA1	≥ 64	16	≥ 64	64	1	312.5
HAMRSA2	≥ 64	≤ 0.5	≥ 64	≥ 64	1	312.5
HAMRSA3	32	≤ 0.5	≥ 64	32	0.5	312.5
HAMRSA4	≥ 64	1	≥ 64	≥ 64	1	312.5
HAMRSA5	32	≤ 0.5	≥ 64	≥ 64	1	312.5
HAMRSA6	≥ 64	≤ 0.5	32	32	1	312.5
CAMRSA1	32	1	≥ 64	16	1	312.5
CAMRSA2	16	4	≥ 64	≤ 0.5	2	312.5
CAMRSA3	32	≤ 0.5	≥ 64	≤ 0.5	1	312.5
CAMRSA4	32	1	≥ 64	≤ 0.5	1	312.5
CAMRSA5	16	32	≤ 0.5	≤ 0.5	1	312.5
CAMRSA6	16	≤ 0.5	32	≤ 0.5	2	312.5
ATCC29213	4	≤ 0.5	0.5	≤ 0.5	≤ 0.5	312.5

Checkerboard assay

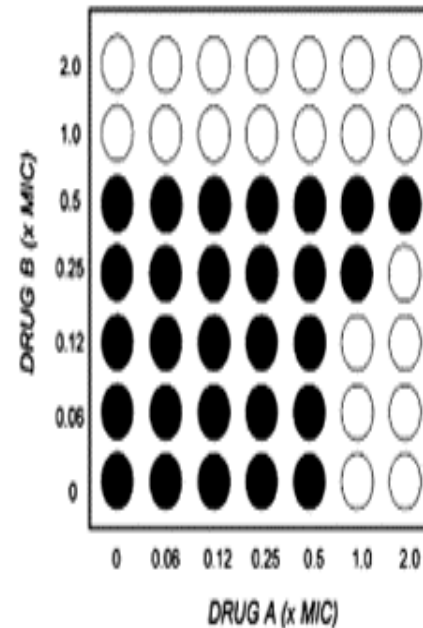
Null effect



Synergy



Antagonism



$$\frac{(A)}{(MIC_A)} + \frac{(B)}{(MIC_B)} = FIC_A + FIC_B = FIC \text{ Index}$$

FIC index:

- ≤0.5: Synergism
- 0.5-1.0: Additivity
- 1.0 to 4.0: Indifference
- ≥4.0: Antagonism

Response of MRSA to combination of phytochemicals and commercially available antimicrobials expressed as FIC index

MRSA Strains	Antimicrobials FIC		
	Cefoxitin	Tetracycline	Erythromycin
ATCC43300			
T-CIN	0.75	1.25	1.0
HAMRSA			
T-CIN	0.50	1.0	1.0
CAMRSA			
T-CIN	0.50	0.75	1.0

Conclusions

- Most of the phytochemical emulsions demonstrated antimicrobial activity against MRSA indicating their potential to treat MRSA infections.
- t-cinnamaldehyde combinations showed additive effect with commercial antimicrobials (cefoxitin, tetracycline and erythromycin) against MRSA suggesting their use as antimicrobial adjuvants.



Can phytochemicals serve as antimicrobial alternatives to inhibit *Salmonella Typhimurium*?

Will they work synergistically with each other?

MIC ($\mu\text{l/L}$) were lower in emulsions!!

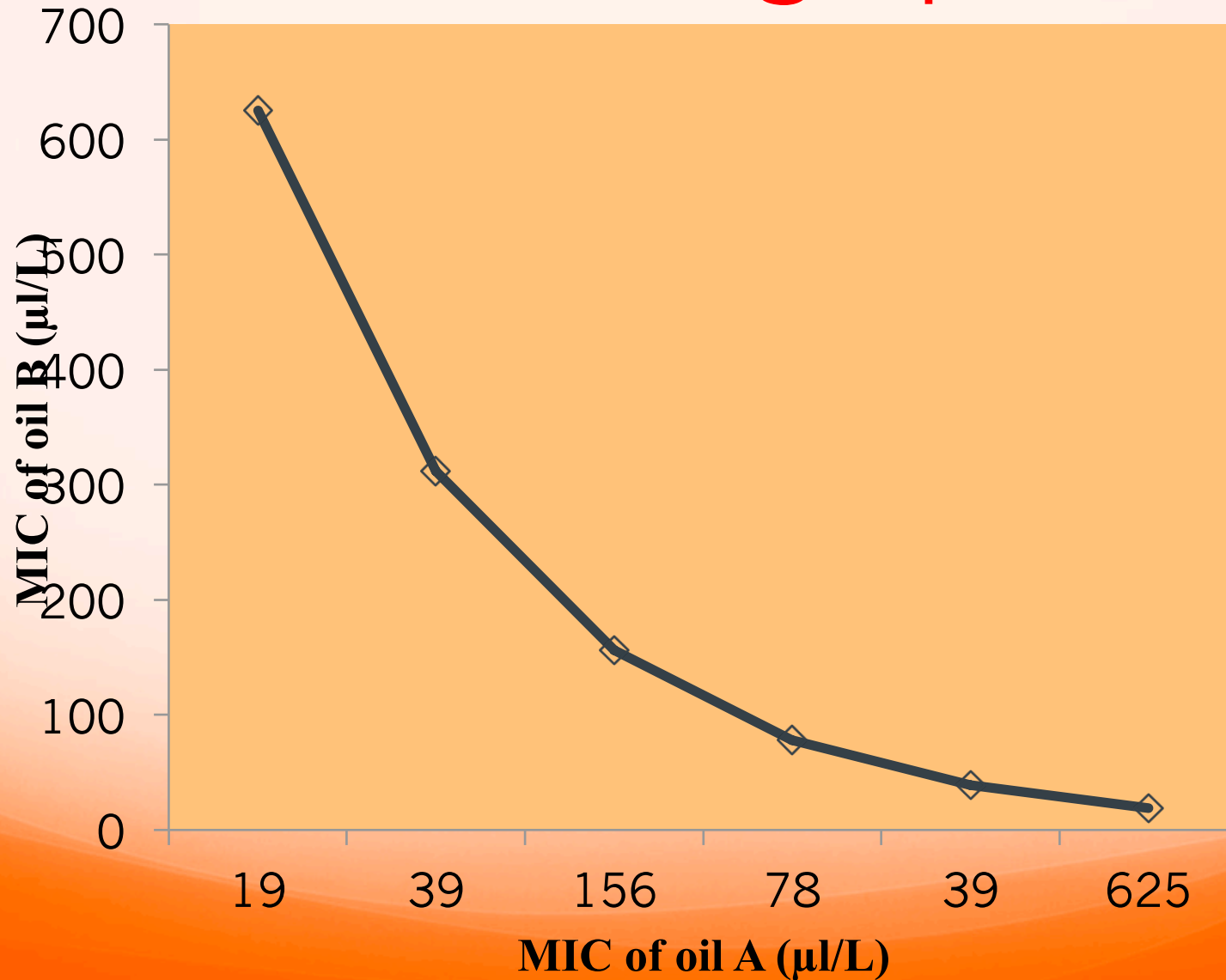
Essential oils	Oregano	Thyme	t-cinnamaldehyde	Eugenol	Basil	Nutmeg
Oils in 5% ethanol	1250	1250	1250	2500	>10,000	>10,000
Emulsion of oils	625	625	625	1250	10,000	10,000

*ATCC 19585

FIC Index for 15 Binary Combinations

	OR	TH	TC	EU	BA	NU
OR		0.062	0.062	0.075	0.281	0.125
TH			0.062	0.075	0.281	0.125
TC				0.075	0.281	0.125
EU					0.063	0.063
BA						0.007
NU						

Isobolograph



Conclusion

- Emulsions of essential oils offers a novel method for food based application of the antimicrobial essential oils.
- Synergistic effects were found between all binary combinations of essential oils emulsions. Through combination, the effective concentration of these oils could be significantly lowered than using alone. As a result, their impact on the organoleptic quality of foods could be minimized.
- Overall, combinations of essential oils emulsions have potential to be used as food additives for increased safety.

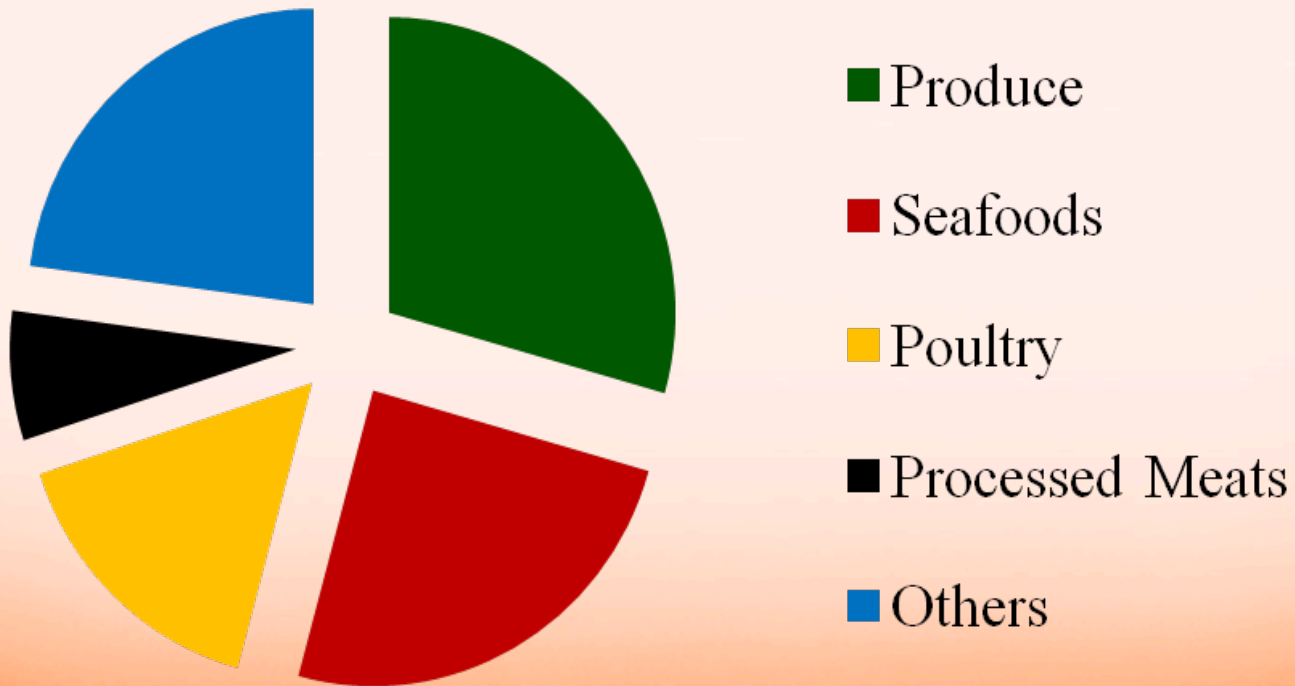


Can we utilize oregano oil emulsion as decontaminant for produce?

Will this emulsion inhibit food-borne pathogens on produce?

Foodborne Illnesses

Source?



Foodborne pathogens

Concern to Fresh Produce

- *Salmonella* spp.
- *Listeria monocytogenes*
- *E. coli* **O157:H7**



Solutions?

Chlorine (Liquid/hypochlorite): 50-200 ppm – SAFETY?

Essential Oils

Oregano (culinary herb)

- Well Known Antimicrobial
- GRAS Additive

Limited Application in Food Models?

- Insolubility in water
- Flavor/Aroma

Oil of Oregano



Nature's anti-biotic

Why Oregano oil?

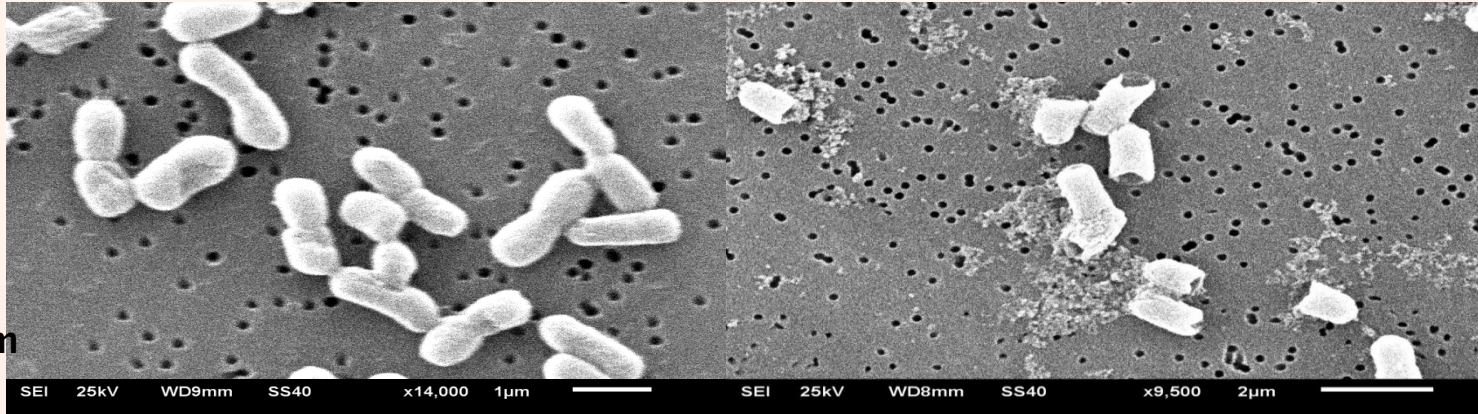
- **Consistent MIC for all pathogens**
- **Oregano is more compatible with salads with respect to flavor!**

**SCREENING OF 12 PHYTOCHEMICALS WERE
PERFORMED AGAINST THESE THREE MAJOR
PATHOGENS**

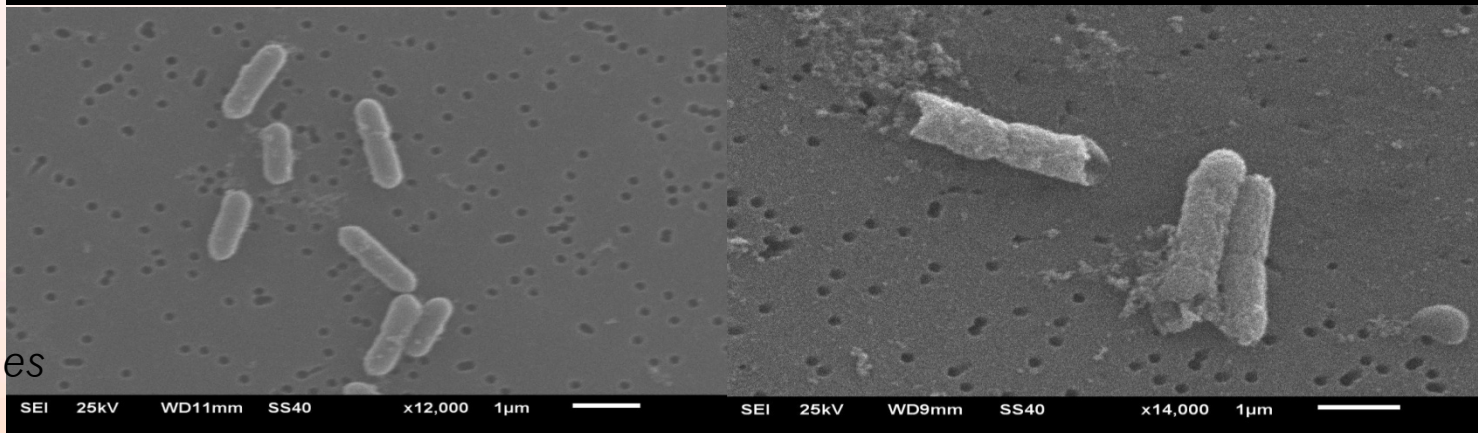
MIC and MBC's

M.O.	Minimum Inhibitory Concentration ($\mu\text{l/L}$)	Minimum Bactericidal Concentration ($\mu\text{l/L}$)
<i>Salmonella</i> Typhimurium ATCC 19585	625	625
<i>Listeria monocytogenes</i> ATCC 19115	625	625
<i>Escherichia coli</i> O157:H7 ATCC 700927	625	1250

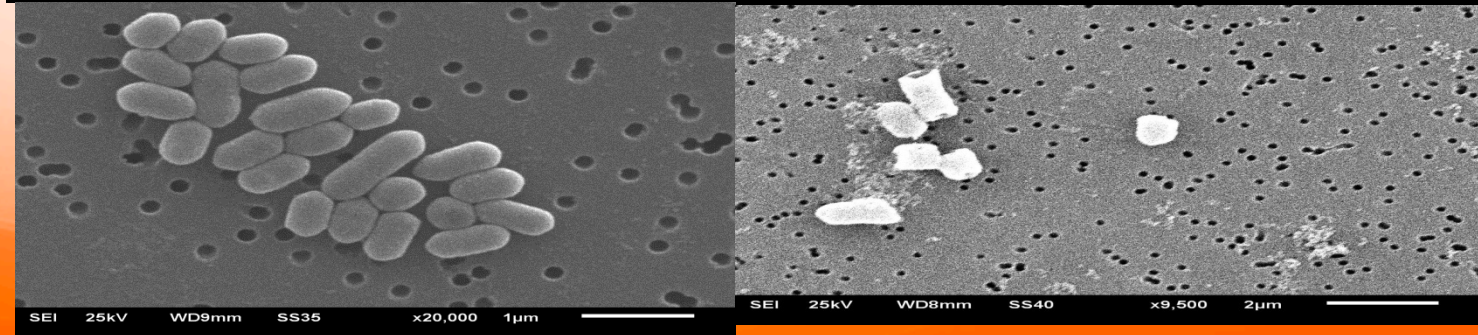
SEM Imaging



Salmonella Typhimurium



Listeria monocytogenes



E. Coli O157:H7

Methods

Antimicrobial efficacy in bagged lettuce model

Romaine
and Iceberg
Lettuce
(1:1)

Artificially
Innoculated
(10^7 CFU/
ml)
and dried

Dipped in

1. Water
2. 0.05%
Emulsion
3. 0.1%
Emulsion

Dried and
Stored at
4°C in
Ziploc Bags

Enumerate
d at 0, 1, 3
days on

1. XLD
2. PALCAM
3. Mc.
Conkey
Agar

Antimicrobial Efficacy

Log reductions?

3.0-3.6-log CFU/g

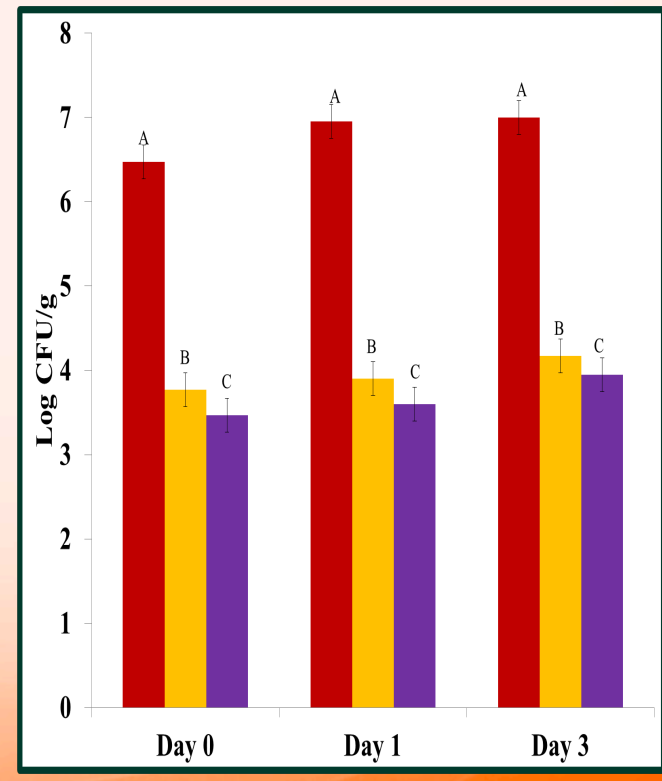
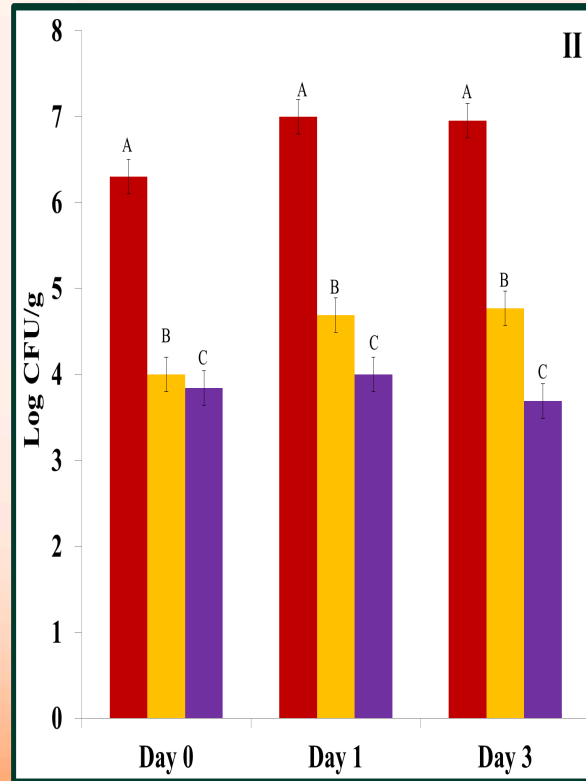
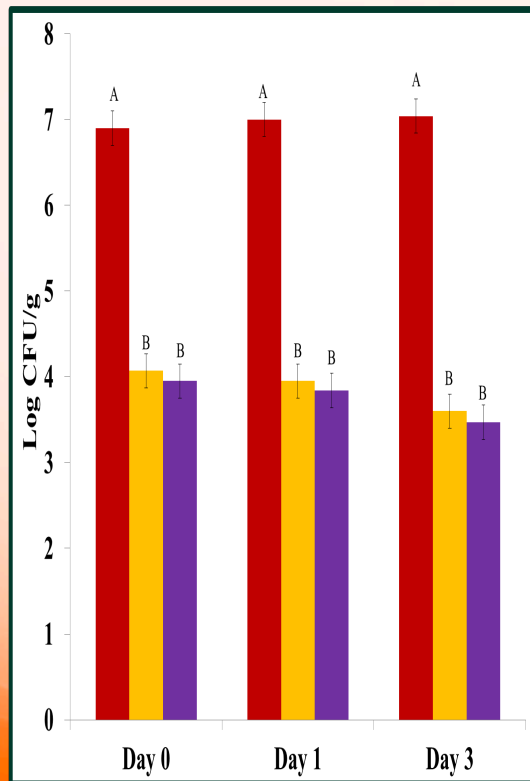
3.1-3.2-log CFU/g

2.0-2.4-log CFU/g

Listeria monocytogenes

Salmonella Typhimurium

Escherichia coli O157:H7



Control 0.05% oregano oil nano-emulsion 0.1% oregano oil nano-emulsion



OR



Conclusions

- Emulsions of oregano oil are effective in inhibiting foodborne pathogens
- Application of antimicrobial emulsion of oregano oil poses a simple and effective **preservation method** for ready to eat bagged lettuce or as a **produce wash**

Overall Conclusions!

- Emulsion based encapsulation system design is a valuable tool to improve antimicrobial activity in food model and better understand microbial growth in complex food model system

Future Work

- Other food models: Meat and Poultry, Coating on shelled eggs
- Does decreasing the particle size effect the antimicrobial efficacy?
- How treatment with emulsions effect the internalization of pathogens on produce?
- Lecithin based emulsions

Acknowledgments

- Dr. Yifan Zhang
- Dr. Sandro da Rocha
- Denise S. Conti
- Varun Tahlan

FUNDING SOURCE

Nell I. Mondy Fellowship



UCO Start-up Grant



Thanks!



Questions?

kbhargava@uco.edu

Emulsions, Microemulsions and Nanoemulsions

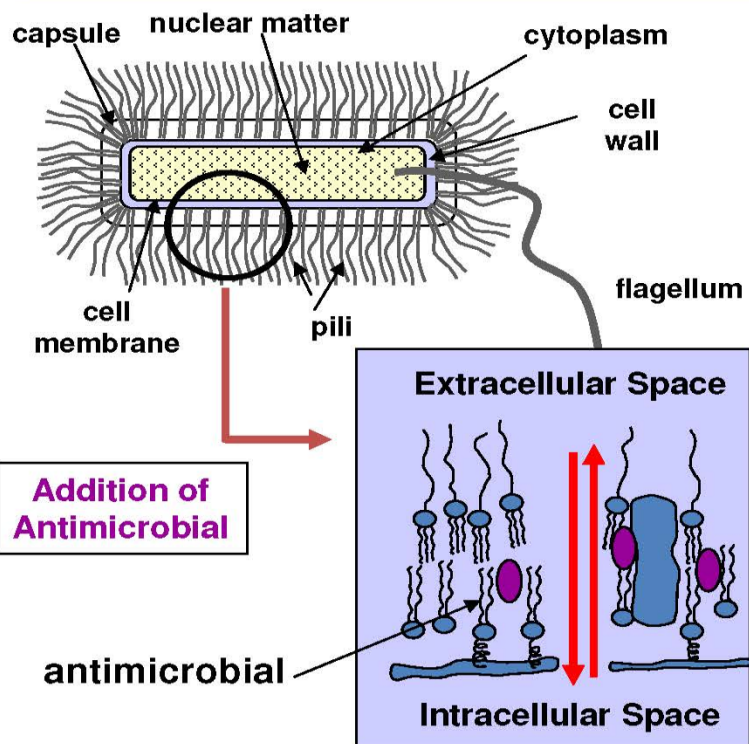
Name	Diameter	T/D Stability	Appearance	Surfactant: Oil
<i>Emulsion</i>	0.1 - 100 μm	No	Opaque	< 1:10
<i>Nano-emulsion</i>	10 - 100 Nm	No	Clear- Cloudy	\approx 1:1
<i>Micro-emulsion</i>	5 - 50 nm	Yes	Clear- Cloudy	> 1:1



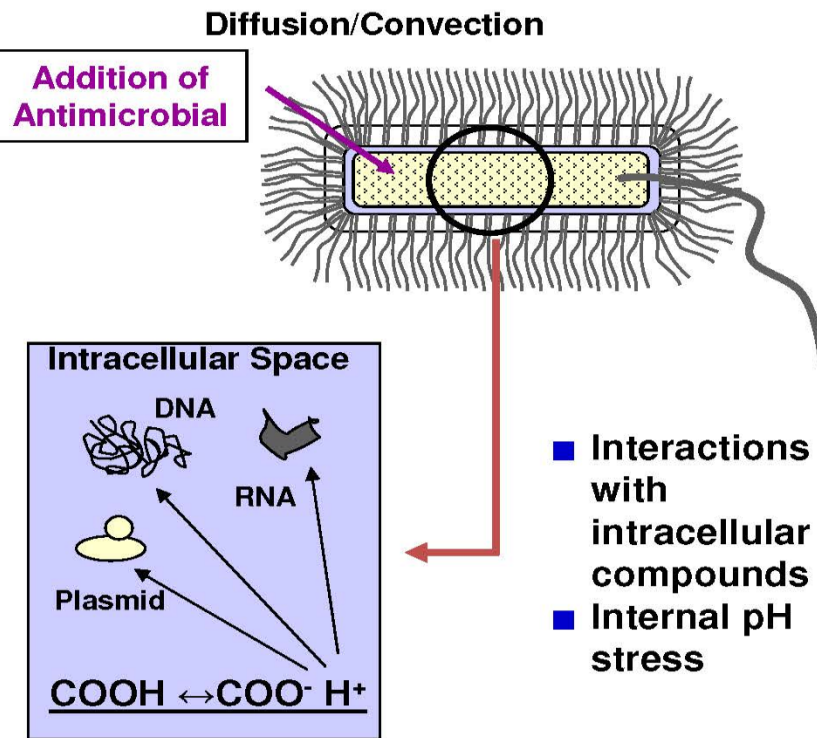
Food Antimicrobials

Two Basic Mechanisms of Action

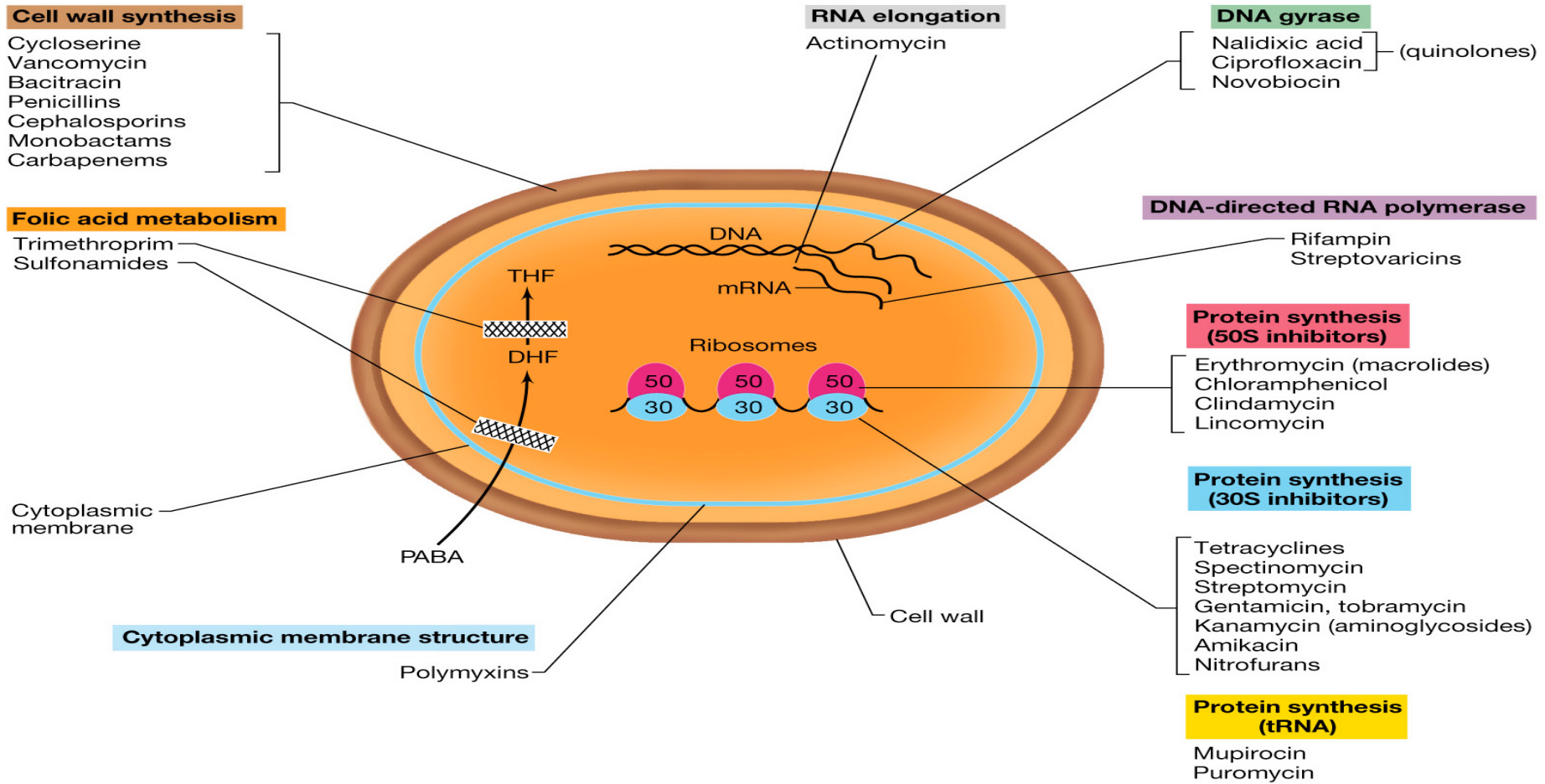
Membrane Perturbation and Disruption



Disruption of Proton Proton Transfer Dynamics

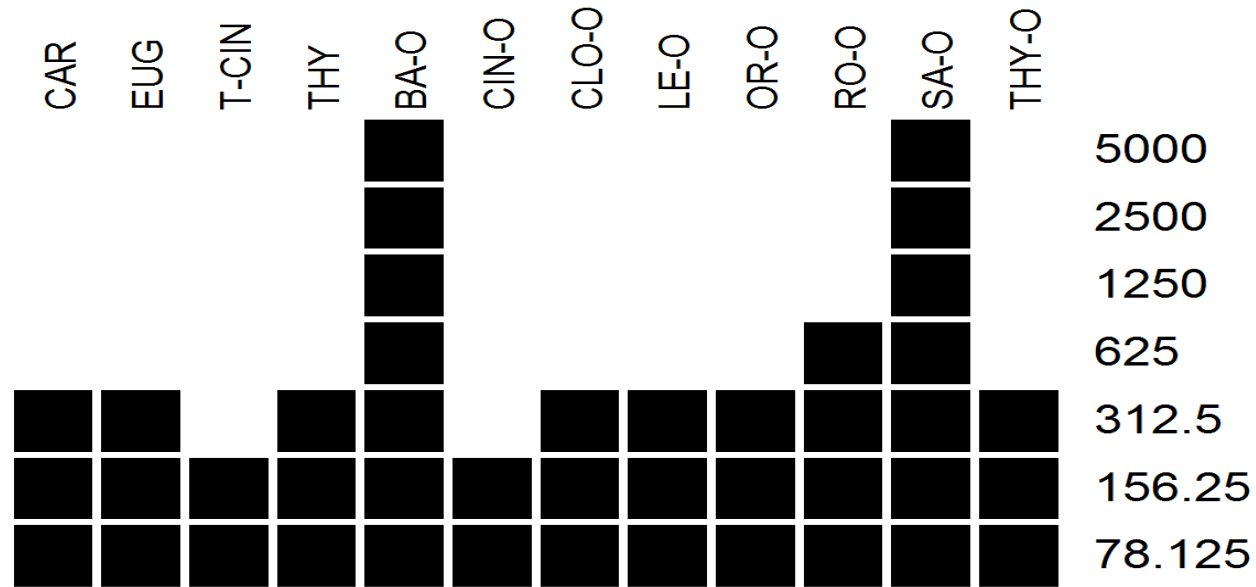


Mode of action of Antibiotics

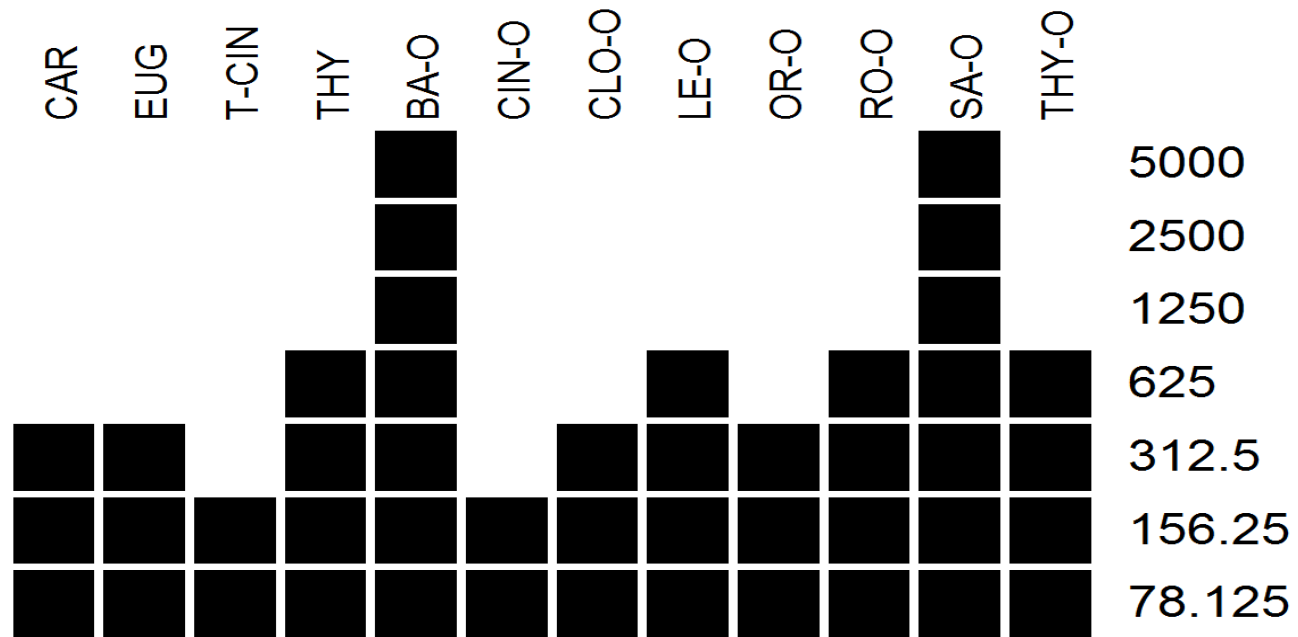


*Fig 20-15 Brock Biology of Microorganisms

Salmonella Typhimurium



Listeria monocytogenes



E. Coli O157:H7

