



Antimicrobial Activity of Insects Fats



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OUTLINE

- INSECTS FAT AS NATURAL ANTIMICROBICS
- ANTIMICROBIAL PROPERTIES OF FATS
- EXPERIMENTAL DESIGN
- RESULTS
- DISCUSSION

Insects and Antimicrobics (1)

- Epicuticular compounds (FLIES) :

Calliphora vicina pupae → 2,4 decadienal (1), PAA (1)

Sarcophaga carnaria/Calliphora vomitoria → PAA

Musca domestica → Tocopherol acetate (2)



1. Gram + (*Bacillus, Staph., Rhodococcus*); Gram – (*E.coli, Pseudomonas, Klebsiella*);
2. Gram + (*Bacillus, Rhodococcus*); Gram – (*Pseudomonas, Klebsiella*);

Golebiowski et al. 2013

Insects and Antimicrobics (2)

- Secretions:

Forcipomyia nigra larvae (secretory setae):

- Pelargonic acid (+++)
- Capric acid (++)
- Palmitoleic acid (+++)
- Lauric acid (+)
- Valeric acid (+)
- Caprylic acid (++)
- Enhantic acid (++)



B. cereus; B. subtilis; P. aeruginosa; C. freundii; Enterococcus faecium



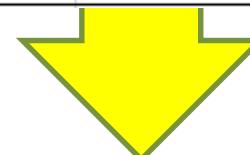
Urbaneck et al. 2012

FAs in Insects Fat/Oil



<i>S. carnaria</i>	<i>T. molitor</i>	<i>H. illucens</i>	<i>A. domesticus</i>	<i>Z. morio</i>	<i>G. assimilis</i>
Oleic	Oleic	Lauric	Linoleic	Palmitic	Linoleic
Palmitoleic	Palmitic	Myristic	Palmitic	Linoleic	Oleic
Linoleic	Linoleic	Palmitic	Oleic	Oleic	Palmitic
Palmitic	Stearic	Oleic	Stearic	Stearic	Stearic
Stearic	Myristic	Linoleic	Myristic	Myristic	Palmitoleic
Myristic	Palmitoleic	Palmitoleic	ALA	Arachidonic	Arachidonic
Arachidic	Lauric	Capric	Palmitoleic	Palmitoleic	Lauric
	Arachidonic	ALA	Arachidonic	ALA	ALA
		STD	Lauric	Lauric	Myristic

FAs order: most - least abundant
(green MUFA/PUFA; blue SFA)

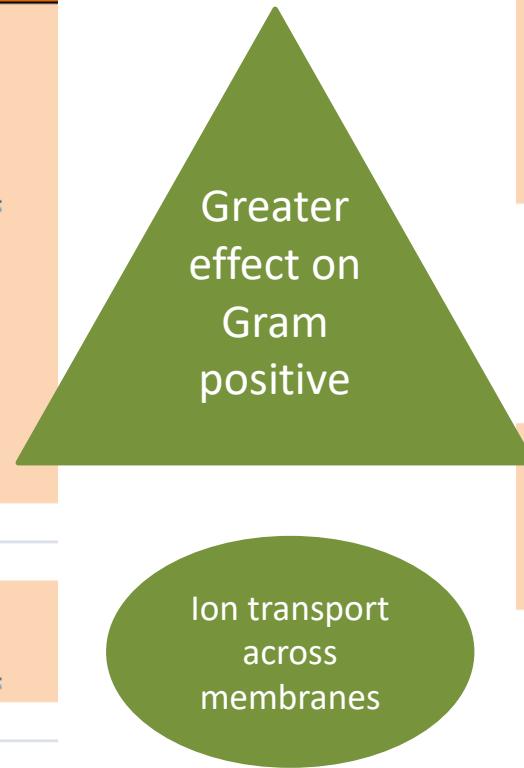


Concentrations vary: DIET AND DEVELOPMENT STAGE

Bondioli et al, 2013; Finke, 2015; Spranghers et al. 2016; Adamkova et al. 2017

Antimicrobial activity of FAs

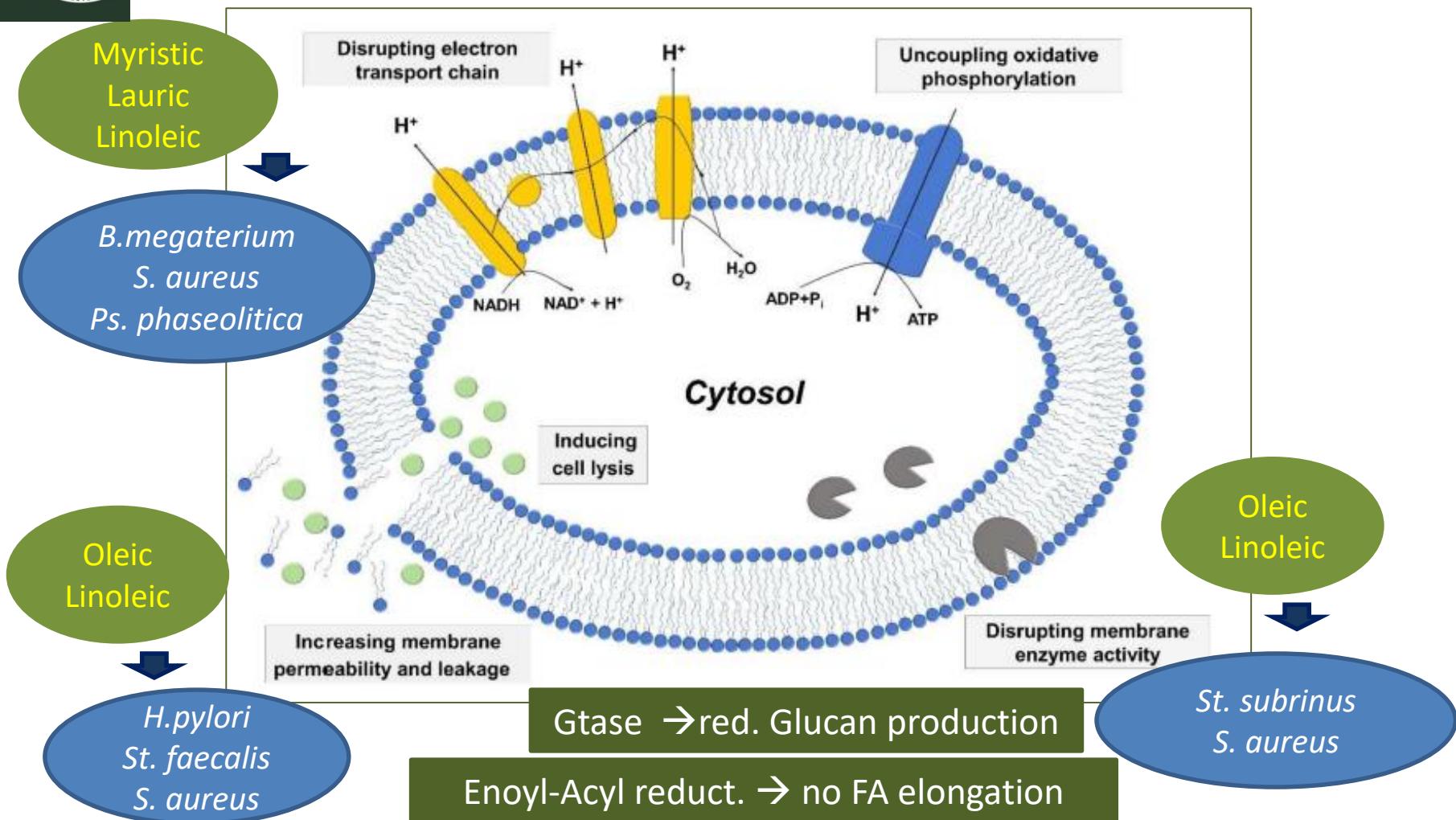
FAs	Inhibited Species
Lauric	<i>Bacillus spp.</i> <i>Staph. aureus</i> <i>Micrococcus</i> <i>Streptococcus spp</i> <i>Cl. butyricum/sporogenes</i> <i>Cl perfringens</i> <i>Corynebacterium</i> <i>L monocytogenes</i> <i>H. pylori</i> <i>N. gonorrhoeae</i> <i>Salmonella</i> <i>E. coli</i>
Linolenic	<i>B. cereus</i> <i>Staph. aureus</i>
Arachidonic	<i>Staph. aureus</i> <i>Bacillus megaterium</i> <i>Lactobacillus acidophilus</i> <i>Campylobacter jejuni</i>
Capric	<i>E. coli</i> <i>Salmonella</i>
Caprylic	<i>Campylobacter jejuni</i> <i>E. coli</i> <i>Cronobacter</i>



FAs	Inhibited Species
Linoleic	<i>Bacillus spp.</i> <i>Staph. aureus</i> <i>Micrococcus</i> <i>Streptococcus spp</i> <i>Cl. butyricum/sporogenes</i> <i>N. gonorrhoeae</i> <i>Streptococcus spp.</i> <i>Corynebacterium</i> <i>Pneumococcus</i> <i>Staphylococcus sp.</i> <i>Micrococcus</i> <i>Vibrio spp.</i> <i>L. garvæ</i> <i>Campylobacter</i> <i>L. monocytogenes</i> <i>Streptococcus Group A</i>
Palmitoleic	
Oleic	
Myristic	
Stearic	<i>Bacillus subtilis</i> <i>Bacillus cereus</i> <i>Mycobacterium fortuitum</i>

Xiangrong et al. 2002; Hinton and Ingram 2009; Anzacu et al. 2017; Anacarso et al. 2017; Yoon et al 2018

FAs' Antimicrobial Mechanisms



Yoon et al 2018

Practical Applications

- *Hermetia illucens* prepupae in diet of weaned piglets

- Extraction of Fat → Lauric acid ($\approx 60\%$)
 - In vitro effect on reducing the microbial load of *D-streptococci, Lactobacilli*
 - In vivo: reduced effect on gut *D-streptococci* (early absorption)



- *Aspongopus viduatus* (melon bug) oil

- Food preservation / Pharmaceutical use (Sudan)
 - High content of Oleic acid (47%)
 - In vitro: effect on *Staph. aureus; B. cereus /subtilis* and *Salmonella enterica*



Mustafa et al. 2007; Spranghers et al 2017

Aim

ANTIBACTERIAL ACTIVITY of oils from *Hermetia illucens* and *Tenebrio molitor*



- on
- *Listeria monocytogenes*
 - *Salmonella Tiphymurium*
 - *Salmonella Enteritidis*
 - *Pasteurella multocida*
 - *Yersinia enterocolitica*

Potential application in food/feed
preservation and animal health

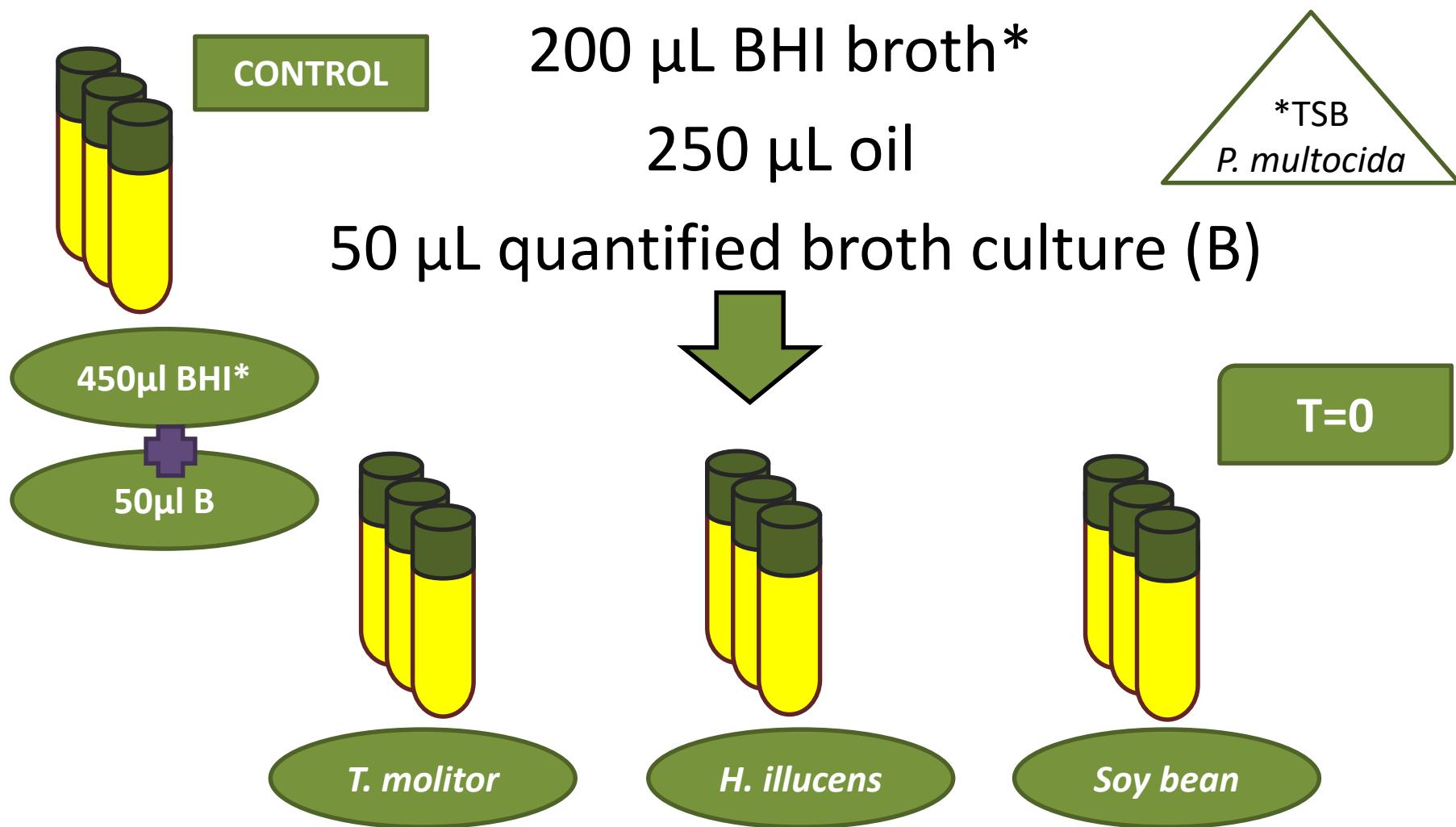
Experimental Design

- Bacterial reference strains were bought from the Pasteur Institute (selected pathogens)
- Insects' commercial oils were used and the composition in FAs was assessed by GC



Renna et al 2017

Trial preparation: TRIPLICATES

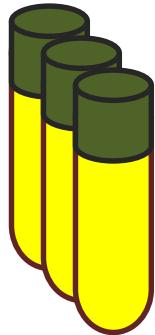


Triplicates over times

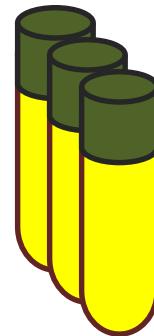
T = 4; T=6;
T=8; T=10;
T=12; T= 24



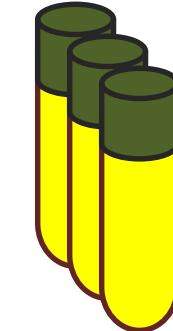
37°C



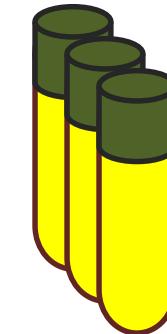
CONTROL



T. molitor

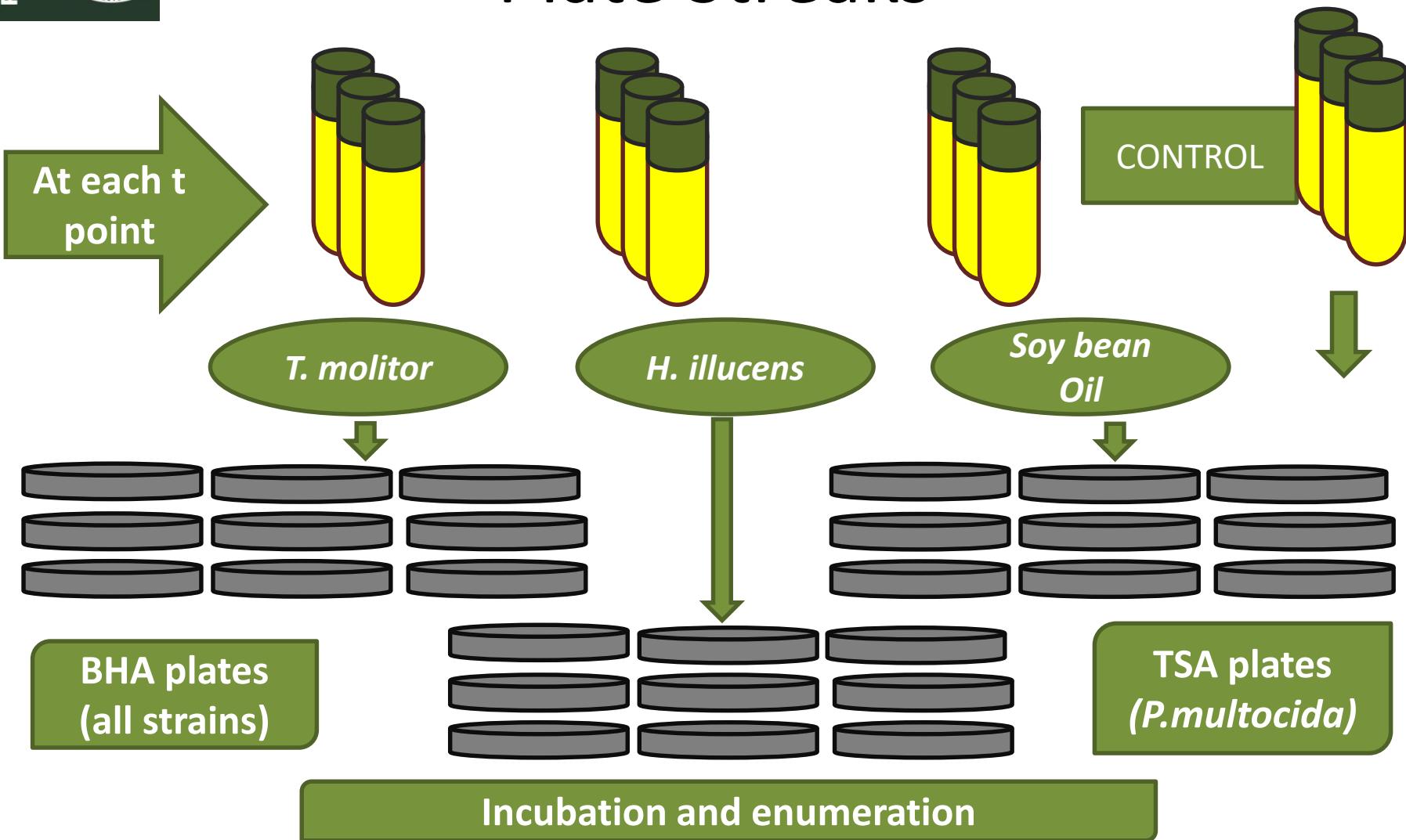


H. illucens

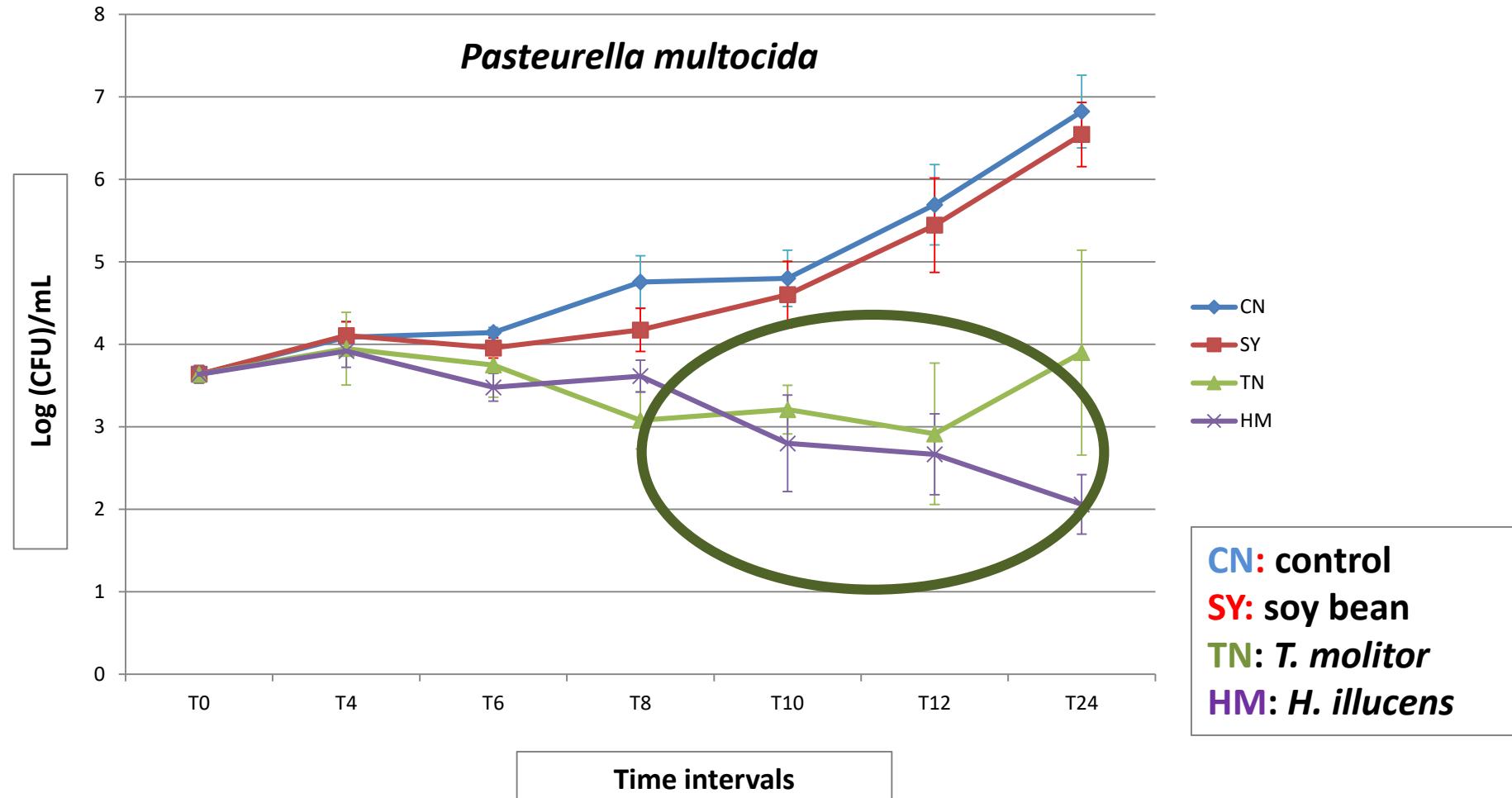


Soy bean
Oil

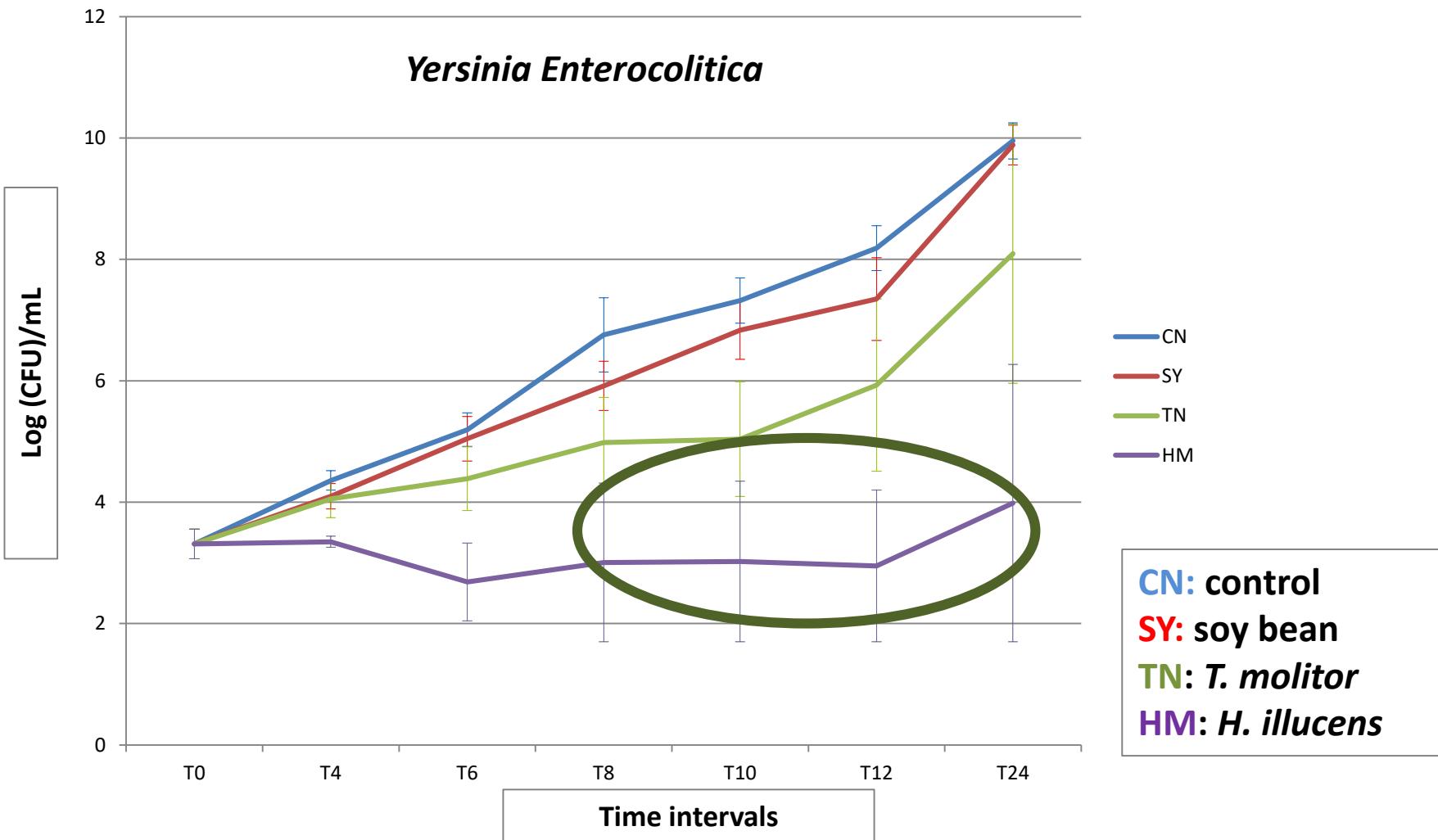
Plate Streaks



Results (1)

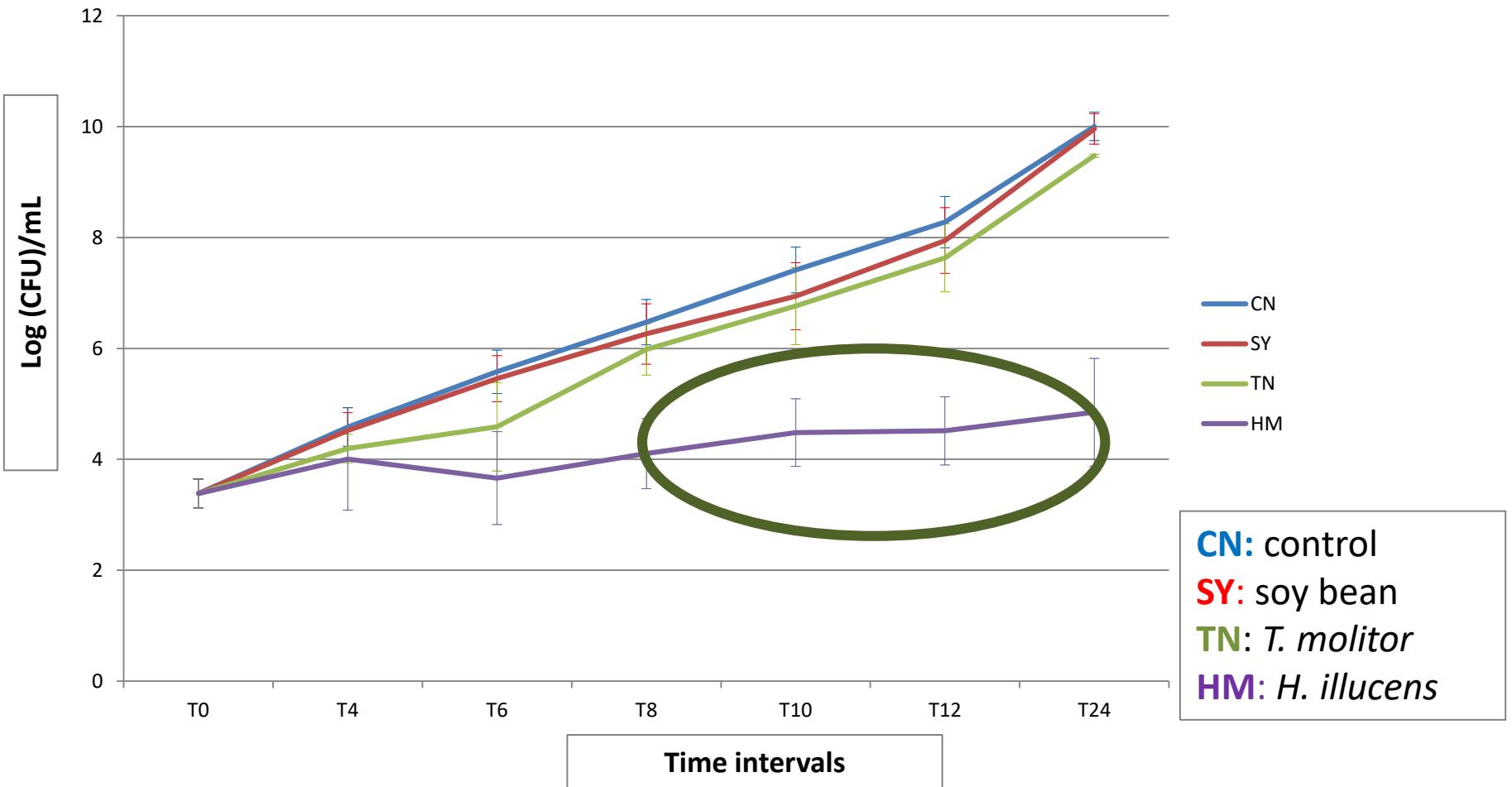


Results (2)



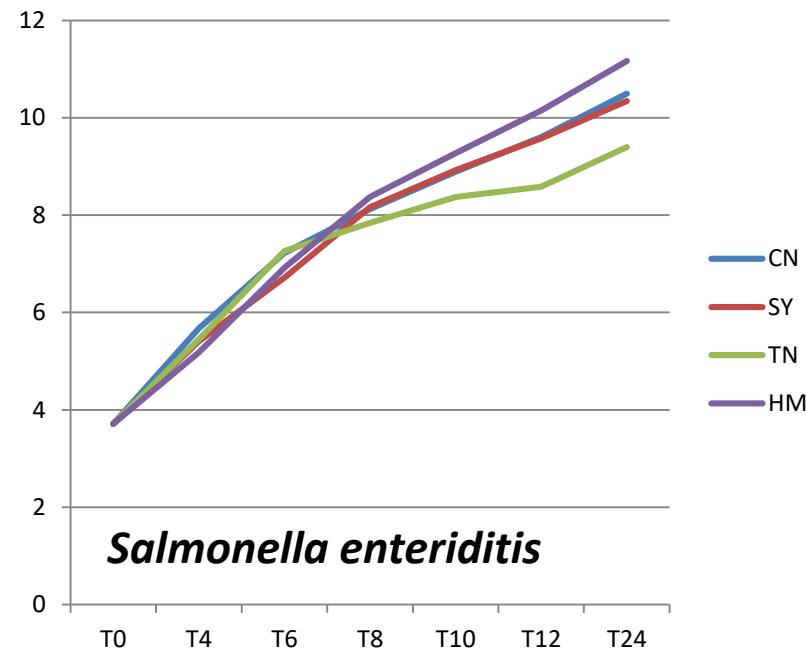
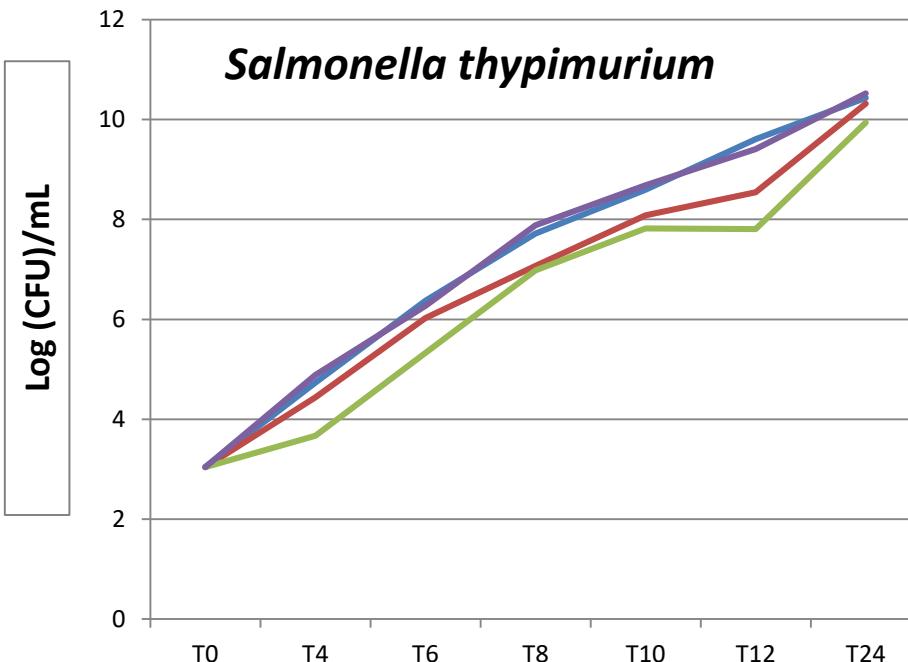
Results (3)

Listeria monocytogenes



CN: control
SY: soy bean
TN: *T. molitor*
HM: *H. illucens*

Results (4)



Time intervals

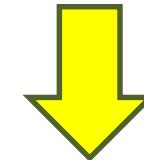
NO EFFECTS

CN: control
SY: soy bean
TN: *T. molitor*
HM: *H. illucens*

Discussion (1)

<i>H. illucens</i>	Fas	<i>T. molitor</i>	Soy
1,1	Ac. Capric	0,01	0
52,9	Ac. Lauric	0,2	0
10,4	Ac. Myristic	2,4	0,07
12,33	Ac. Palmitic	18,7	10,97
2,96	Ac. Palmitoleic	1,65	0,08
1,8	Ac. Stearic	2,32	4,39
7,88	Ac. Olieic	37,73	22,73
0,49	Ac. Vaccenic	0,65	1,61
7,82	Ac. Linoleic	33	52,58
0,79	ALA	1,63	6,67
79,11	SFA	24,7	16,05
11,82	MUFA	40,51	24,62
9,08	PUFA	34,74	59,33

On 3 /5 species tested
 CRUDE oils were
 effective →FAs
 Composition



Dilution + incubation →
 reduction of FA's
 concentration/activity

Soy > T.m > H. i

Discussion (2)

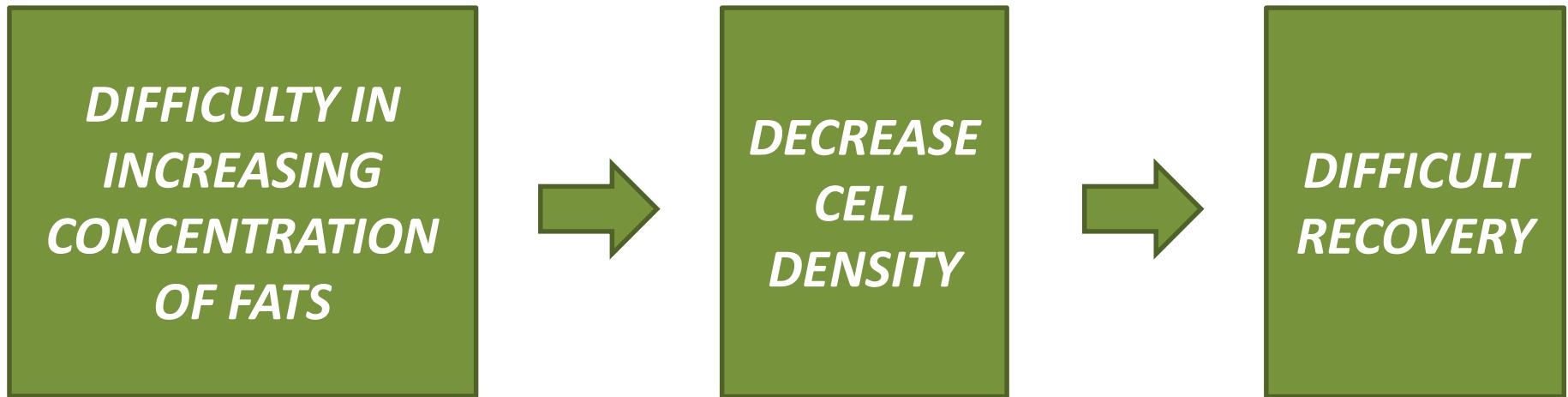
- The observed effects of *T. molitor* oil could be greater at room temperature → slower bacterial growth and reduced FAs oxidation
- *H. illucens* → high SFA (Lauric Ac.) → activity even at incubation T over times → shorter fatty chains (C10-C12-C14)



Application on feed/intestine

Discussion (3)

- Both oils showed effect on reduction of growth
 - cell repair mechanisms
 - dose dependent (?)



Yoon et al 2018

Discussion (4)

- P. multocida highly sensitive → more testing on wild strains together with in vivo studies (absorption of FAs → reduced activity)

Feed with insect oils may prevent growth and colonization (i.e. Rabbit)

- New analyses on free FAs by digesting oils with animal enzymes (lipases) → increased effect