

Antibacterial effect of plant oils rich in medium chain fatty acids and their possible interactions with antibiotics

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Introduction

Antibiotic resistance

Ban of using in-feed antibiotics in animal production

Search for new alternatives

Alternatives?

- Probiotics
- Prebiotics
- Bacteriocins
- Enzymes
- Bacteriophages
- Plant extracts
- Organic acids

WHY?

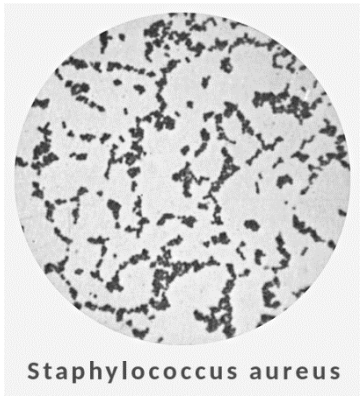
MCFA as a possible alternative to in-feed antibiotics?

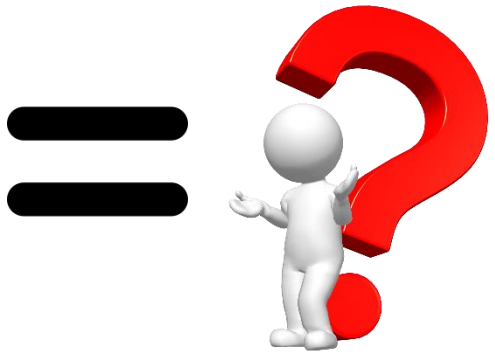
- Inhibitory activity *in vitro*
- Mechanism of action
- *In vivo testing*
  - The influence of production traits, GIT microbiota, toxicology,…
  - Experimental infections
  - Application form
  - Economy, combinations

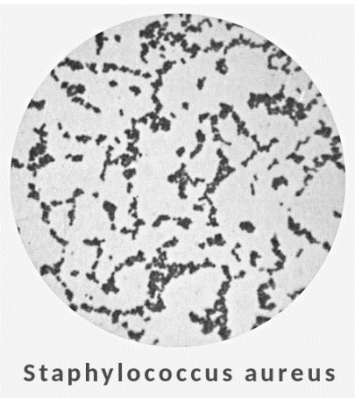
Aims

To determine the antibacterial effect of medium-chain fatty acids (MCFA) and oils rich in MCFA

To analyse MCFA contents of the tested oils





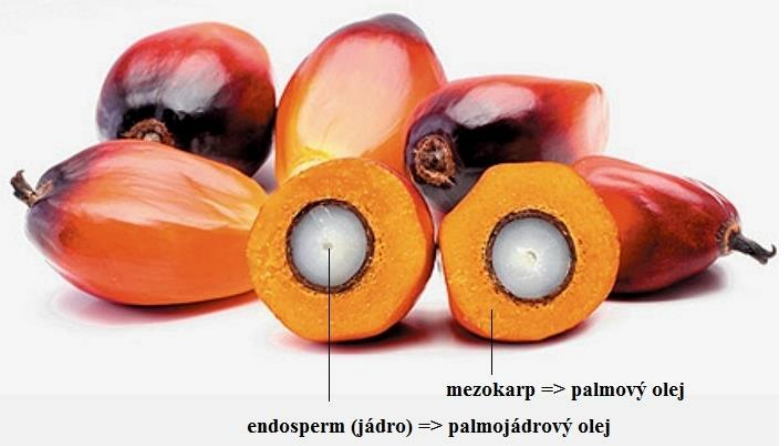


MCFA & oils

Oil	Source
Coconut oil (*Cocos nucifera*)	Sigma-Aldrich, ČR
Palm kernel oil (*Elais guineensis*)	Sigma-Aldrich, ČR
***Cuphea* oil** (*C. lanceolata* a *C. ignea*)	USDA-ARS, USA
Palm oil(*Elais guineensis*)	SNB, USA
Palm red oil (*Elais guineensis)*	SNB, USA
Babassu oil (*Attalea speciosa*)	SNB, USA
Tucuma oil (*Astrocaryum vulgare*)	SNB, USA
Muru-muru oil (*Astrocaryum murumuru*)	SNB, USA

Free MCFA: C_6 – C_{12}

- dissolved in DMSO + Tween 80, diluted in growth medium for a certain bacterium
- with or without pancreatic lipase
- 37°C dissolved and/or hydrolyzed



Bacterial strains

Bacterium	Strain
Escherichia coli	ATCC 29522 C6
Enterococcus cecorum	CCM 3659 ^{T} CCM 4285
Campylobacter jejuni	CCM 6189 CAMP/VFU 612/21
Clostridium perfringens	CIP 105178 CNCTC 5454 UGent 56
Listeria monocytogenes	ATCC 7644

Bacterium	Strain
Salmonella enteritidis	ATCC 13076
Salmonella infantis	K2
Salmonella typhimurium	K3
Staphylococcus aureus	ATCC 25923
Bifidobacterium animalis	CCM 4988 MA5
Bifidobacterium longum	CCM 4990 TP1
Lactobacillus acidophilus	CCM 4833
Lactobacillus fermentum	CCM 91

• *In vitro* determination of MIC

- Broth microdilution method in 96-well plate
- 24/48 h **incubation** in aerobic/microaerophilic/anaerobic conditions at 37 °C

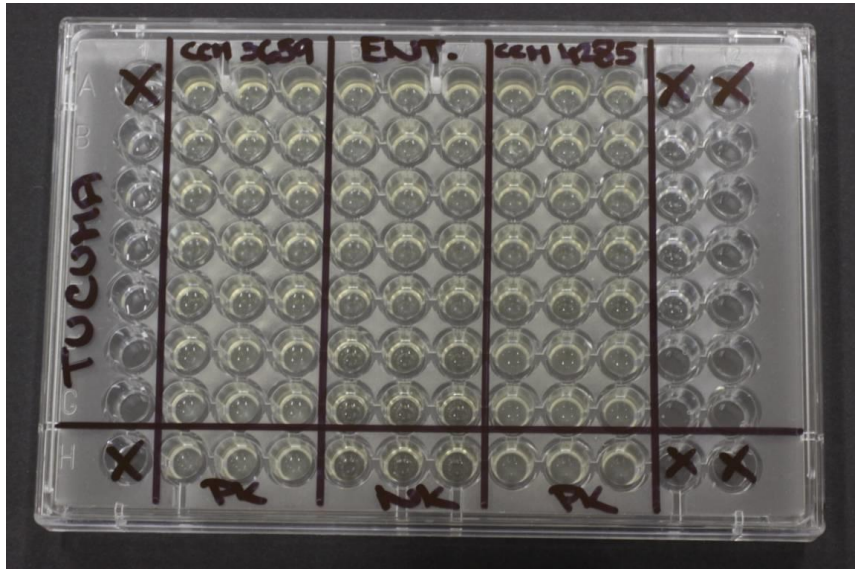
96-well plate design (according to Cos *et al.*, 2006; Hecht *et al.*, 2007)

	X	Strain 1			DK			Strain 2			X	X
	1	2	3	4	5	6	7	8	9	10	11	12
A	M	4,5	4,5	4,5	4,5	4,5	4,5	4,5	4,5	4,5	M	M
B	M	2,25	2,25	2,25	2,25	2,25	2,25	2,25	2,25	2,25	M	M
C	M	1,125	1,125	1,125	1,125	1,125	1,125	1,125	1,125	1,125	M	M
D	M	0,5625	0,5625	0,5625	0,5625	0,5625	0,5625	0,5625	0,5625	0,5625	M	M
E	M	0,2812	0,2812	0,2812	0,2812	0,2812	0,2812	0,2812	0,2812	0,2812	M	M
F	M	0,1406	0,1406	0,1406	0,1406	0,1406	0,1406	0,1406	0,1406	0,1406	M	M
G	M	0,0703	0,0703	0,0703	0,0703	0,0703	0,0703	0,0703	0,0703	0,0703	M	M
H	M	PK1	PK1	PK1	NK	NK	NK	PK2	PK2	PK2	M	M

legenda:
DK – dilution control
PK – positive control
NK – negative control
M – pure medium

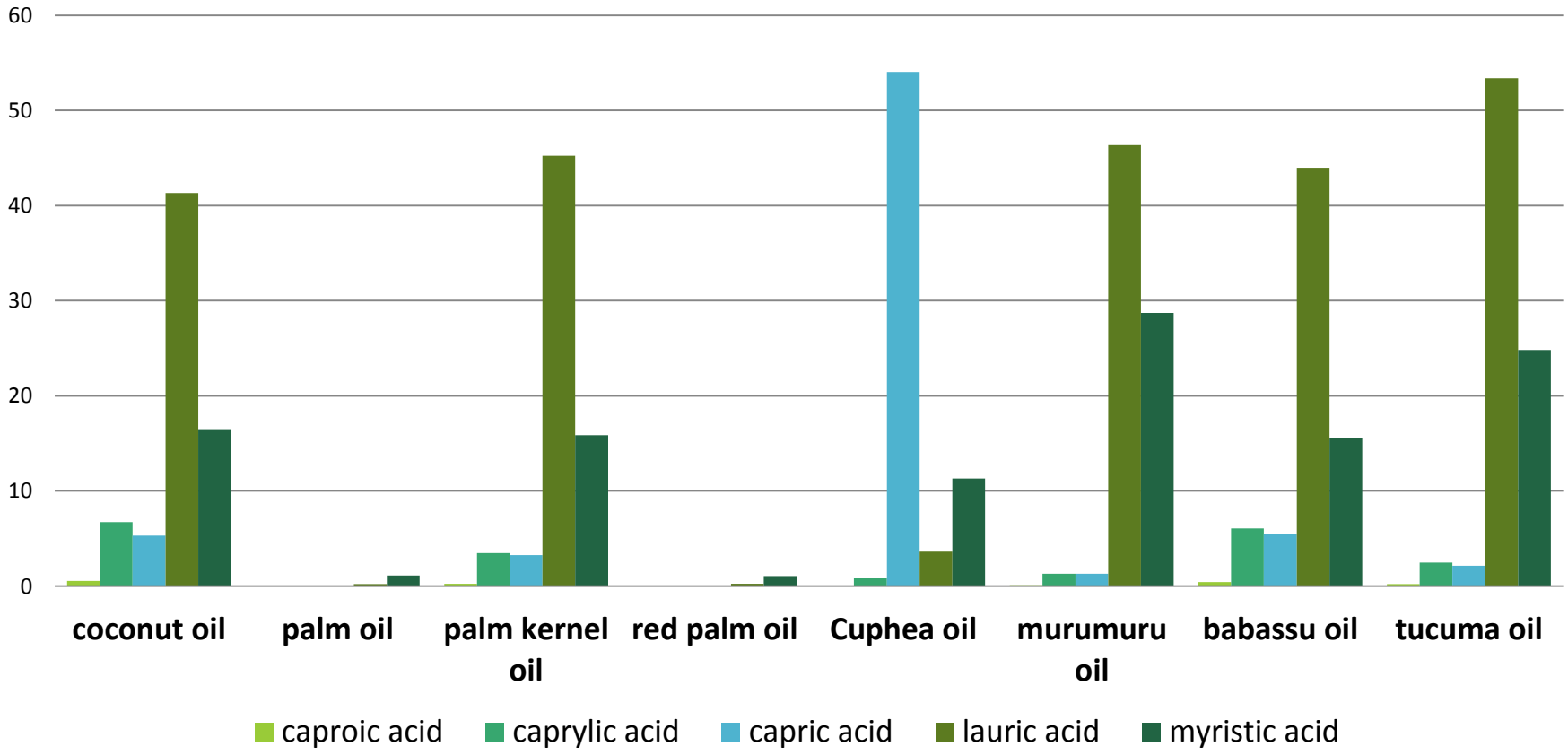
In vitro determination of MIC

- After the incubation: absorbance at 405 nm (Infinite ® 200 PRO Microplate Reader; Tecan)
  - Pipetting performed by the automatic pipetting station Freedom EVO 100 (Tecan)
- MIC = minimal concentration, where **at least 80% cell growth reduction occured** (vs. positive control)



MCFA contents of oils: gas chromatography (GC-FID) according to ISO 5509 (2001)

Results: MCFA profile of selected oils (mg/kg)



Results: MIC (mg/mL)

	BA		BL		CJ		CP			ECe		ECo		LA	LF	LM	SE	SI	ST	SA
Oil origin	CCM 4988	MA5	CCM 4990	TP1	CAMP/VFU 612/21	CCM 6189	CNCTC 3659	UGent 56	CIP 105178	CCM 3659	CCM 4285	ATCC 29522	C6	CCM 4833	CCM 91	ATCC 7644	ATCC 13076	K2	K3	ATCC 25923
coconut																				
palm																				
red palm																				
palm kernel																				
Cuphea																				
muru-muru																				
tucuma																				
babassu																				

BA – *B. animalis*, **BL** – *B. longum*, **CJ** – *C. jejuni*, **CP** – *C. perfringens*, **ECe** – *E. cecorum*, **ECo** – *E. coli*, **LA** – *L. acidophilus*, **LF** – *L. fermentum*, **LM** – *L. monocytogenes*, **SE** – *S. enteritidis*, **SI** – *S. infantis*, **ST** – *S. typhimurium*, **SA** – *S. aureus*

MIC (mg/mL):

0,14	0,28	0,56	1,12	2,25	4,5	>4,5

Conclusion

- **Gram-positive bacteria** were sensitive **after lipase digestion**
  - Free MCFA or monoglycerides
- **Cuphea oil**
  - the widest effect (7 bacterial strains)
- **Tucuma oil**
  - the strongest effect (MIC 0.14 mg/mL)
- **No effect on benefitial bacteria!**

Thank you!

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