Lactic acid bacteria and their role in the meat processing

Kameník, J., Dušková, M., Lačanin, I., Šedo, O., Zdráhal, Z, Saláková, A., Borkovcová, I.

Lactic Acid Bacteria

Lactic Acid Bacteria

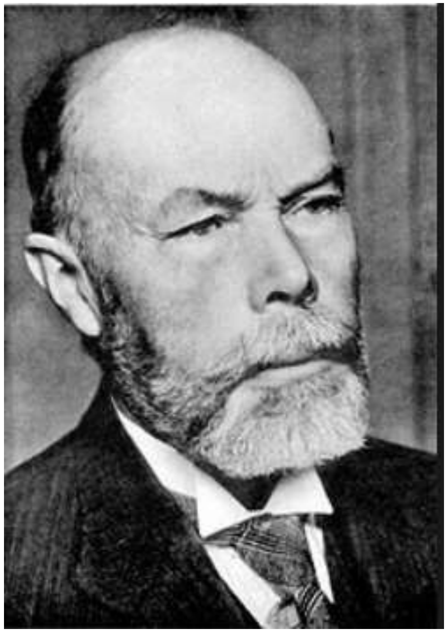
- in the turn from 19th to 20th century – specification of microorganisms that cause **milk fermentation**
- **Orla-Jensen** in year 1919 proposed today's classification of LAB
- only four genus:

Lactobacillus

Leuconostoc

Pediococcus

Streptococcus



Lactic Acid Bacteria

- energy phosphorylation of suitable substrates
- two basic fermentation pathway

  - **homofermenatitive**:
    - hydrolysis of 1 mol of glucose releases 2 moles of ATP
    - Embden-Meyerhof-Parnas Pathway => lactic acid
    - out of hexoses > 85% lactic acid

  - **heterofermenatitive**
    - yield 1 mole of ATP (acetyl phosphate is reduced to ethanol)
    - yield 2 moles of ATP (acetyl-phosphate is converted to acetic acid)

Lactic Acid Bacteria

- central role in fermentation of carbohydrates plays

  pyruvic acid

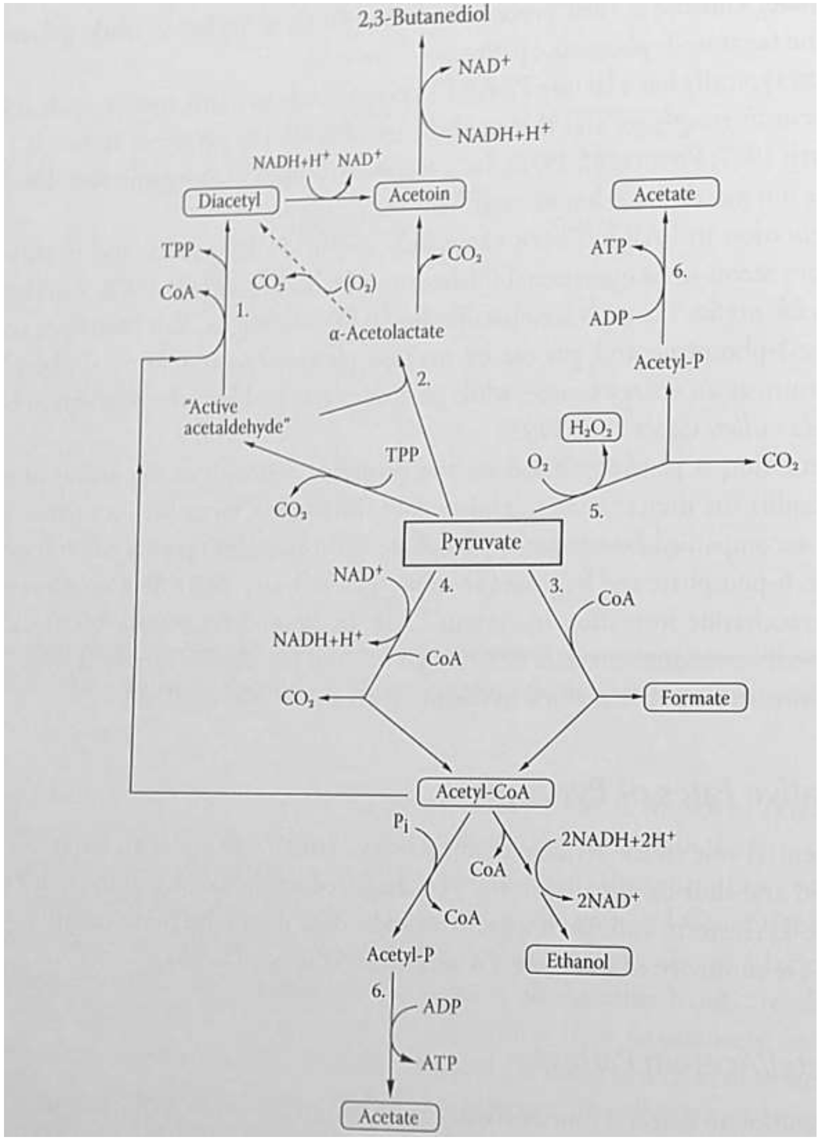
- electron acceptor => lactic acid

- helps keeping the redox balance of the cell

- also alternative pathway exists to use the pyruvate

Lactic Acid Bacteria

(Wright and Axelsson, 2012)



Lactic Acid Bacteria in the field of meat processing

- **positive effect:**
  - **starter cultures** in fermented meat products (GRAS/QPS)
    - formation of lactic acid
    - antagonistic effect on the competitive microflora
      - competition for nutrients
      - formation of substances with antimicrobial activity (bacteriocins, hydrogen peroxide)
  - **protective cultures** (growth prevention of *Listeria monocytogenes*)

Effect of SafePro®ImPorus at the reduction of pores

Cocktail 055 (~ 1.0E+02 CfU/g in meat)

Cocktail 055 (~ 1.0E+05 CfU/g in meat)

Cocktail 055 (~ 1.0E+02 CfU/g in meat) + S+ ImPorous

Cocktail 055 (~ 1.0E+05 CfU/g in meat) + S+ ImPorous

Aplikace SafePro ImPorous signifikantně redukuje množství pórů a to i při vysoké kontaminaci heterofermentativní bakterií 1.0E+05KTJ/g

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Improving food & health

Lactic Acid Bacteria in the field of meat processing

- **negative effect**:
  - spoilage of meat and meat products
  - formation of biogenic amines
  - resistance genes to antibiotics

LAB as a spoilage agents of meat

Aerobic conditions:

- rapid growth of bacteria of genus *Pseudomonas*
- 10^7-10^8 CFU.g-1 induces slime production on meat and causing off-odour
- spoilage contributing bacteria of the genera *Serratia, Enterobacter, Pantoea, Klebsiella, Proteus* and *Hafnia*

LAB as a spoilage agents of meat

Microaerophilic to **anaerobic conditions** (vacuum packing or MAP):

- inhibits the growth of bacteria of genus *Pseudomonas*
- **psychrotrophic LAB** and ***Brochothrix thermosphacta***

LAB as a spoilage agents of meat

Microaerophilic to **anaerobic conditions** (vacuum packing or MAP):

- **effect of LAB: spoilage or stabilization?**
  - suppression / slowdown the growth of Gram-negative bacteria
  - suppressing of the foodborne diseases agents

LAB as a spoilage agents of meat

- **Which species?**
  - *Lactobacillus sakei*
  - *Lb. curvatus*
  - *Leuconostoc mesenteroides*
  - *Leuc. carnosum*
  - *Carnobacterium divergens*

- **Why they?**
  - temperature, length and type of storage, type of meat....

LAB as a spoilage agents of meat

Vacuum packaging:

- highly selective to the growth of LAB
- longer shelf-life
- *Lactobacillus* spp.

Modified atmosphere:

- allowed the growth of other bacterial groups (O_2)
- shorter shelf-life
- *Leuconostoc* spp., *B. thermosphacta, Enterobacteria* ...

LAB as a spoilage agents of meat

Group of MO	Day of sampling	CVP	VSP	MAP
LAB	0	0.00 ± 0.00		
PSY	0	1.33 ± 0.58		

LAB as a spoilage agents of meat

Group of MO	Day of collection	CVP	VSP	MAP
LAB	0		0.00 ± 0.00	
	7	0.00 ± 0.00	0.41 ± 0.65	0.05 ± 0.12
PSY	0		1.33 ± 0.58	
	7	3.62 ± 0.18	2.61 ± 0.97	3.26 ± 0.94
	14			
	21			
	35			

LAB as a spoilage agents of meat

Group of MO	Day of sampling	CVP	VSP	MAP
LAB	0		0.00 ± 0.00	
	7	0.00 ± 0.00	0.41 ± 0.65	0.05 ± 0.12
	14	2.50 ± 0.65	2.51 ± 0.49	0.34 ± 0.68
PSY	0		1.33 ± 0.58	
	7	3.62 ± 0.18	2.61 ± 0.97	3.26 ± 0.94
	14	3.24 ± 0.77	1.60 ± 0.55	3.81 ± 0.39

LAB as a spoilage agents of meat

Group of MO	Day of sampling	CVP	VSP	MAP
LAB	0		0.00 ± 0.00	
	7	0.00 ± 0.00	0.41 ± 0.65	0.05 ± 0.12
	14	2.50 ± 0.65	2.51 ± 0.49	0.34 ± 0.68
	21	4.30 ± 0.88	5.01 ± 0.92	2.28 ± 0.98
PSY	0		1.33 ± 0.58	
	7	3.62 ± 0.18	2.61 ± 0.97	3.26 ± 0.94
	14	3.24 ± 0.77	1.60 ± 0.55	3.81 ± 0.39
	21	6.02 ± 0.56	5.70 ± 0.42	6.22 ± 0.40

LAB as a spoilage agents of meat

Group of MO	Day of sampling	CVP	VSP	MAP
LAB	0		0.00 ± 0.00	
	7	0.00 ± 0.00	0.41 ± 0.65	0.05 ± 0.12
	14	2.50 ± 0.65	2.51 ± 0.49	0.34 ± 0.68
	21	4.30 ± 0.88	5.01 ± 0.92	2.28 ± 0.98
	35	4.94 ± 0.68	5.14 ± 0.75	1.97 ± 0.82
PSY	0		1.33 ± 0.58	
	7	3.62 ± 0.18	2.61 ± 0.97	3.26 ± 0.94
	14	3.24 ± 0.77	1.60 ± 0.55	3.81 ± 0.39
	21	6.02 ± 0.56	5.70 ± 0.42	6.22 ± 0.40
	35	7.16 ± 0.22	7.11 ± 0.27	7.10 ± 0.51

LAB as a spoilage agents of meat

Group of MO	Day of sampling	CVP	VSP	MAP
PSE	0		0.33 ± 0.58	
	7			
	14			
	21			
	35			
BRO	0		0.00 ± 0.00	
	7			
	14			
	21			
	35			

LAB as a spoilage agents of meat

Group of MO	Day of sampling	CVP	VSP	MAP
PSE	0		0.33 ± 0.58	
	7	0.63 ± 0.88	0.55 ± 0.76	2.02 ± 0.23
	14			
	21			
	35			
BRO	0		0.00 ± 0.00	
	7	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	14			
	21			
	35			

LAB as a spoilage agents of meat

Group of MO	Day of sampling	CVP	VSP	MAP
PSE	0		0.33 ± 0.58	
	7	0.63 ± 0.88	0.55 ± 0.76	2.02 ± 0.23
	14	0.74 ± 1.02	0.00 ± 0.00	2.09 ± 0.37
	21			
	35			
BRO	0		0.00 ± 0.00	
	7	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	14	0.00 ± 0.00	0.64 ± 0.63	0.96 ± 0.60
	21			
	35			

LAB as a spoilage agents of meat

Group of MO	Day of sampling	CVP	VSP	MAP
PSE	0		0.33 ± 0.58	
	7	0.63 ± 0.88	0.55 ± 0.76	2.02 ± 0.23
	14	0.74 ± 1.02	0.00 ± 0.00	2.09 ± 0.37
	21	1.46 ± 0.41	0.00 ± 0.00	2.04 ± 0.47
	35			
BRO	0		0.00 ± 0.00	
	7	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	14	0.00 ± 0.00	0.64 ± 0.63	0.96 ± 0.60
	21	1.13 ± 0.18	0.42 ± 0.58	1.81 ± 0.19
	35			

LAB as a spoilage agents of meat

Group of MO	Day of sampling	CVP	VSP	MAP
PSE	0		0.33 ± 0.58	
	7	0.63 ± 0.88	0.55 ± 0.76	2.02 ± 0.23
	14	0.74 ± 1.02	0.00 ± 0.00	2.09 ± 0.37
	21	1.46 ± 0.41	0.00 ± 0.00	2.04 ± 0.47
	35	1.85 ± 0.36	0.54 ± 0.89	5.01 ± 0.97
BRO	0		0.00 ± 0.00	
	7	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	14	0.00 ± 0.00	0.64 ± 0.63	0.96 ± 0.60
	21	1.13 ± 0.18	0.42 ± 0.58	1.81 ± 0.19
	35	1.14 ± 0.66	0.00 ± 0.00	2.53 ± 0.73

LAB as spoilage agents of meat products

- **psychrotrophic** (growth at $T < 7$ °C)
- tolerant to **lower a_w value** ($a_w < 0.98$)
- **growth at higher level of NaCl** > 2%; NO_2^-
- **heat resistant**
- **microaerophilic** (growth at a lower O_2 concentration)

LAB in the production of cooked hams

Environment	Sample	n	Number of CFU/g	positive/ 15 °C	positive/ 30 °C
Slicing and packaging	**ham after heat treatment**	4	1,3 x 10^2 (1/4)	4	0

LAB in the production of cooked hams

Environment	Sample	n	Number of CFU/g	positive/ 15 °C	positive/ 30 °C
Slicing and packaging	ham after HT	4	1,3 x 10^2 (1/4)	4	0
	slices 0. day	**10**	**1,5 x 10^2 (2/10)**	**10**	**0**

LAB in the production of cooked hams

Environment	Sample	n	Number of CFU/g	positive/ 15 °C	positive/ 30 °C
Slicing and packaging	ham after HT	4	$1,3 \times 10^2$ (1/4)	4	0
	slices 0. day	10	$1,5 \times 10^2$ (2/10)	10	0
	slices 7. day	**10**	**$1,0 \times 10^4$**	**10**	**6**

LAB in the production of cooked hams

Environment	Sample	n	Number of CFU/g	positive/ 15 °C	positive/ 30 °C
Slicing and packaging	ham after HT	4	$1{,}3 \times 10^2$ (1/4)	4	0
	slices 0. day	10	$1{,}5 \times 10^2$ (2/10)	10	0
	slices 7. day	10	$1{,}0 \times 10^4$	10	6
	slices 14. day	**10**	**$1{,}0 \times 10^6$**	**10**	**10**

LAB in the production of cooked hams

Environment	Sample	n	Number of CFU/g	positive/ 15 °C	positive/ 30 °C
Slicing and packaging	ham after HT	4	$1{,}3 \times 10^2$ (1/4)	4	0
	slices 0. day	10	$1{,}5 \times 10^2$ (2/10)	10	0
	slices 7. day	10	$1{,}0\times 10^4$	10	6
	slices 14. day	10	$1{,}0\times 10^6$	10	10
	slices 21. day	**10**	**$1{,}0 \times 10^7$**	**10**	**10**

LAB in the production of cooked hams

Environment	Sample	n	Number of CFU/g	positive/ 15 °C	positive/ 30 °C
Slicing and packaging	ham after HT	4	$1{,}3 \times 10^{2}$ (1/4)	4	0
	slices 0. day	10	$1{,}5 \times 10^{2}$ (2/10)	10	0
	slices 7. day	10	$1{,}0 \times 10^{4}$	10	6
	slices 14. day	10	$1{,}0 \times 10^{6}$	10	10
	slices 21. day	10	$1{,}0 \times 10^{7}$	10	10
	slices 28. day	**10**	$\mathbf{1{,}0 \times 10^{8}}$	**10**	**10**
	slices 35. day	**10**	$\mathbf{1{,}0 \times 10^{8}}$	**10**	**10**

LAB in the plant environment (cooked hams)

Environment	Number of LAB (CFU)	Species of LAB	Note
pork carcasses	**$< 5.10^{1}$**	*Leuconostoc carnosum, L. pseudomesenteroides, Lactococcus garvieae*	only after enrichment n = 10

LAB in the plant environment (cooked hams)

Environment	Number of LAB (CFU)	Species of LAB	Note
pork carcasses	$< 5.10^1$	*Leuconostoc carnosum, L. pseudomesenteroides, Lactococcus garvieeae*	only after enrichment n = 10
raw pork leg (boning room)	**$< 5.10^1$**	*Lact.garvieeae, L.piscium, Leuc.pseudomesenteroides, (Brochothrix thermosphacta)*	only after enrichment n = 10

LAB in the plant environment (cooked hams)

Environment	Number of LAB (CFU)	Species of LAB	Note
pork carcasses	$< 5.10^1$	*Leuconostoc carnosum, L. pseudomesenteroides, Lactococcus garvieae*	only after enrichment n = 10
raw pork leg (boning room)	$< 5.10^1$	*Lact.garvieae, L.piscium, Leuc.pseudomesenteroides, (Brochothrix thermosphacta)*	only after enrichment n = 10
boning room	**$< 5.10^1$**	*Lactobacillus sakei, Leuc. gelidum, Lact.lactis, (B.thermosphacta)*	gloves, tables

LAB in the plant environment (cooked hams)

Environment	Number of LAB (CFU)	Species of LAB	Note
brine	**$5,4.10^1$**	*(B.thermosphacta)*	before and after injection
	$2,1.10^3$	*Leuc.mesenteroides, Enterococcus gilvus, Lb.* spp., *(B. thermosphacta)*	
raw pork ham after injection	**$9,2.10^2$**	*Leuc.mesenteroides, Leuc.gelidum, (B.thermosphacta)*	n = 7; max. $2,4.10^3$

LAB in the plant environment (cooked hams)

Environment	Number of LAB (CFU)	Species of LAB	Note
pork ham after tumbling	**$4{,}3.10^4$**	*Leuc.carnosum, Lbc. sakei, Lbc.curvatus, Lact.lactis*	n=7; max. $1{,}1.10^5$
tumbler machine	**$4{,}4.10^5$**	*Leuc.carnosum, Leuc.mesenteroides, Lact.lactis, W.viridescens*	n=5; min. 5.10^3; max. $2{,}1.10^6$

Sausage Vysočina – FVHE VFU Brno

dose	amount *W. viridescens* (log CFU.g^{-1})				
			ripening		storage - VP
	before HT	after HT	after 1 week	after 2 weeks	after 4 weeks
W.v./Av	**4,41**				
W.v./Av	**4,91**				
W.v./Hm	**4,52**				
W.v./Hm	**4,45**				
Lb.sakei	**4,57**				
Lb.sakei	**4,40**				
W.v.&Lb.s.	**4,73**				
W.v.&Lb.s.	**4,76**				
W.v./Av	**4,65**				

Experiment II – FVHE VFU Brno

dose	amount *W. viridescens* (log CFU.g^{-1})				
			ripening		storage - VP
	before HT	after HT	**after 1 week**	after 2 weeks	after 4 weeks
control	4,41	nd	**nd**		
W.v./Av	4,91	nd	**6,43**		
W.v./Av	4,52	nd	**6,48**		
W.v./Hm	4,45	nd	**nd**		
W.v./Hm	4,57	nd	**nd**		
Lb.sakei	4,40	nd	**nd**		
Lb.sakei	4,73	nd	**nd**		
W.v.&Lb.s.	4,76	nd	**nd**		
W.v.&Lb.s.	4,65	nd	**nd**		

Experiment II – FVHE VFU Brno

dose	amount *W. viridescens* (log CFU.g^{-1})				
			ripening		storage - VP
	before HT	after HT	after 1 week	**after 2 weeks**	after 4 weeks
control	4,41	nd	nd	**nd**	
W.v./Av	4,91	nd	6,43	**6,15**	
W.v./Av	4,52	nd	6,48	**6,41**	
W.v./Hm	4,45	nd	nd	**nd**	
W.v./Hm	4,57	nd	nd	**nd**	
Lb.sakei	4,40	nd	nd	**nd**	
Lb.sakei	4,73	nd	nd	**nd**	
W.v.&Lb.s.	4,76	nd	nd	**nd**	
W.v.&Lb.s.	4,65	nd	nd	**nd**	

Experiment II– FVHE UVPS Brno

dose	amount *W. viridescens* (log CFU.g^{-1})				
			ripening		**storage - VP**
	before HT	after HT	after 1 week	after 2 weeks	**after 4 weeks**
control	4,41	nd	nd	nd	**6,85**
W.v./Av	4,91	nd	6,43	6,15	**7,75**
W.v./Av	4,52	nd	6,48	6,41	**7,97**
W.v./Hm	4,45	nd	nd	nd	**nd**
W.v./Hm	4,57	nd	nd	nd	**nd**
Lb.sakei	4,40	nd	nd	nd	**nd**
Lb.sakei	4,73	nd	nd	nd	**nd**
W.v.&Lb.s.	4,76	nd	nd	nd	**nd**
W.v.&Lb.s.	4,65	nd	nd	nd	**nd**

LAB and biogenic amines (BA)

- BA are formed primarily during **decarboxylases of free amino acids**
- **sausages fermentation** creates favorable conditions for the BA formation

LAB and biogenic amines (BA)

- **increased proteolytic activity** caused by surface's mold => increased amount of amino acids

- ???

LAB and biogenic amines (BA)

- increased proteolytic activity caused by surface’s mold => increased amount of amino acids
- microorganisms amine activity is promoted by **organic acids**
- bacteria releases BA as a defense agent against acidic environment

LAB and biogenic amines (BA)

	pH	lactic acid ($\mu mol.g^{-1}$ dry matter)	BA ($mg\text{-}kg^{-1}$)
meat dough	**5,64-5,77**	**114,2-132,00**	**10,5-12,2**
salami with $molds_{35}$			
salami without $molds_{35}$			
salami with $molds_{65}$			
salami without $molds_{65}$			

LAB and biogenic amines (BA)

	pH	lactic acid ($\mu mol.g^{-1}$ dry matter)	BA ($mg\text{-}kg^{-1}$)
meat dough	**5,64-5,77**	**114,2-132,00**	**10,5-12,2**
salami with molds$_{35}$	**6,11-6,40**	**32,43-38,37**	**53,2-62,7**
salami without molds$_{35}$			
salami with molds$_{65}$			
salami without molds$_{65}$			

LAB and biogenic amines (BA)

	pH	lactic acid ($\mu mol.g^{-1}$ dry matter)	BA ($mg\text{-}kg^{-1}$)
meat dough	**5,64-5,77**	**114,2-132,00**	**10,5-12,2**
salami with $molds_{35}$	**6,11-6,40**	**32,43-38,37**	**53,2-62,7**
salami without $molds_{35}$	**4,93-4,97**	**241,89-268,98**	**22,8-47,7**
salami with $molds_{65}$			
salami without $molds_{65}$			

LAB and biogenic amines (BA)

	pH	lactic acid (µmol.g^{-1} dry matter)	BA (mg-kg^{-1})
meat dough	**5,64-5,77**	**114,2-132,00**	**10,5-12,2**
salami with $molds_{35}$	**6,11-6,40**	**32,43-38,37**	**53,2-62,7**
salami without $molds_{35}$	**4,93-4,97**	**241,89-268,98**	**22,8-47,7**
salami with $molds_{65}$			**58,8-94,4**
salami without $molds_{65}$			**19,1-51,2**

LAB in salami with molds

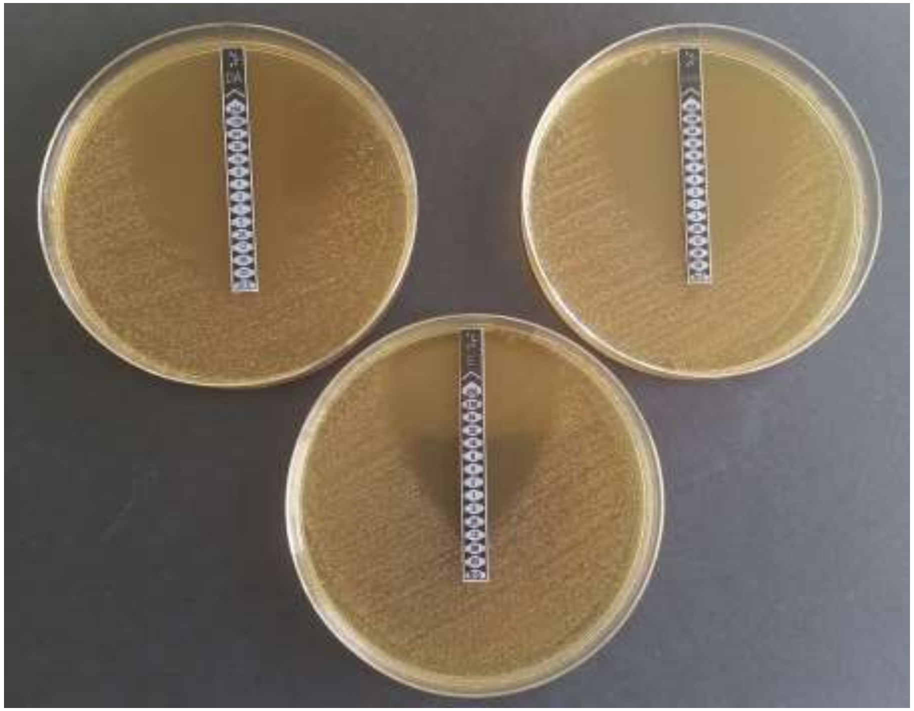
- *Lactobacillus sakei*
- *Lb. plantarum**
- *Lb. brevis**
- *Lb. coryniformis*
- *Enterococcus faecalis**

LAB and resistance to antibiotics

- Toomey *et al.*(2010):
  - 37 strains of LAB from environment of Irish's slaughterhouses
  - 6 antibiotics (AM, CL, E, SM, TC, VM)
  - 33 strains were positive on 1 or more antibiotics
  - all strains were sensitive on AM and CL
  - resistance to SM showed all strains (n=8) of *Streptococcus* spp.
  - resistance to VM showed (4/10) *E. faecium* strains
  - transport of *tet*(M) conjugation between *L. plantarum* and *L. lactis* and *E. faecalis*

LAB and resistance to antibiotics

- 20 strains *Weissella viridescens* (Lačanin *et al.*, 2015)
  - 7 antibiotics (AM, GM, SM, E, CM, TC, CL)
  - sensitivity on:
    - AM
    - GM
    - E
  - rezistance on:
    - SM (10 %)
    - CM (5 %)
    - TC (35 %)
    - CL (10 %)



Thank You for Your attention !!!