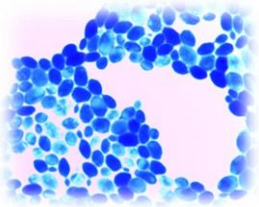
Isolation and identification of lactic acid bacteria and yeasts in type I sourdoughs with focus on traditional Austrian sourdough

Vera Fraberger
Klara Cerk
Christine Lang
Christian Kummer
Konrad J. Domig

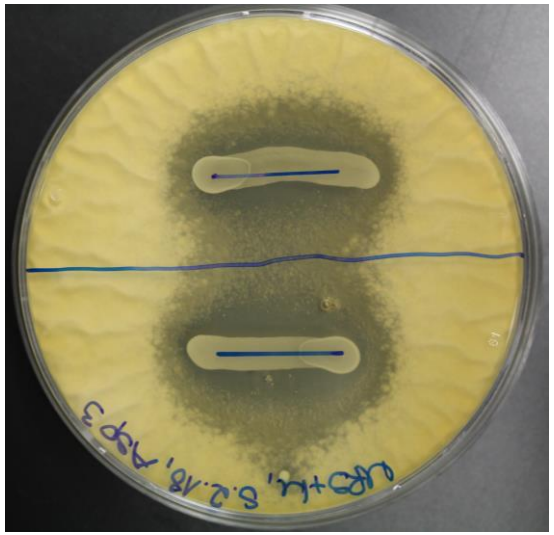
Natural sourdough

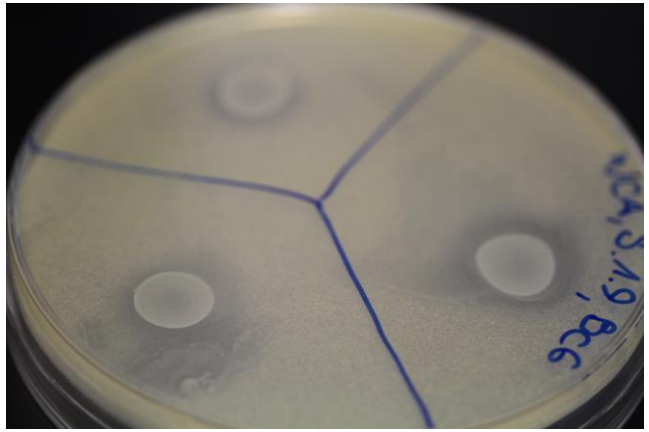
- Mixture of flour and water, fermented by Lactic Acid Bacteria (LAB) and Yeasts
- Spontaneous sourdough without starter strains
- Microbiota
  - Predominant LAB microbiota (> 8 log(cfu)/g)
  - heterofermentative lactobacilli
  - Lower Yeast count levels (< 7 log(cfu)/g)
  - *Saccharomyces* and *Candida*



Natural sourdough - advantages

- Technological properties
- Nutritional properties
- Superior sensory quality
- Prolonged shelf-life

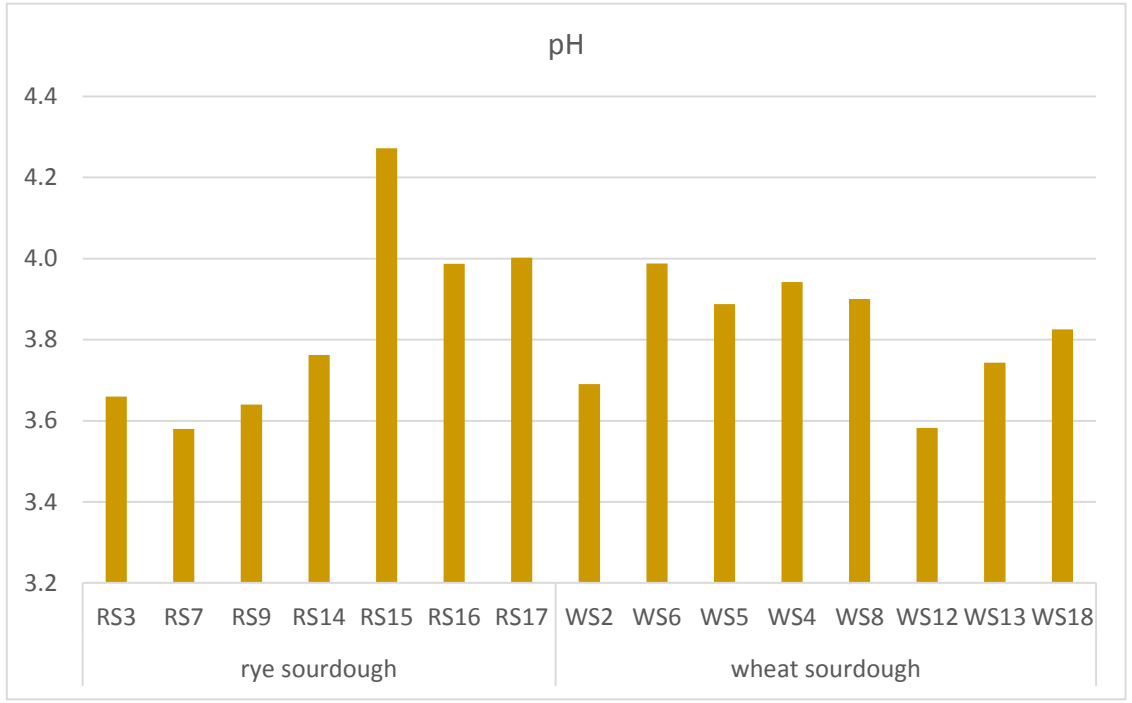


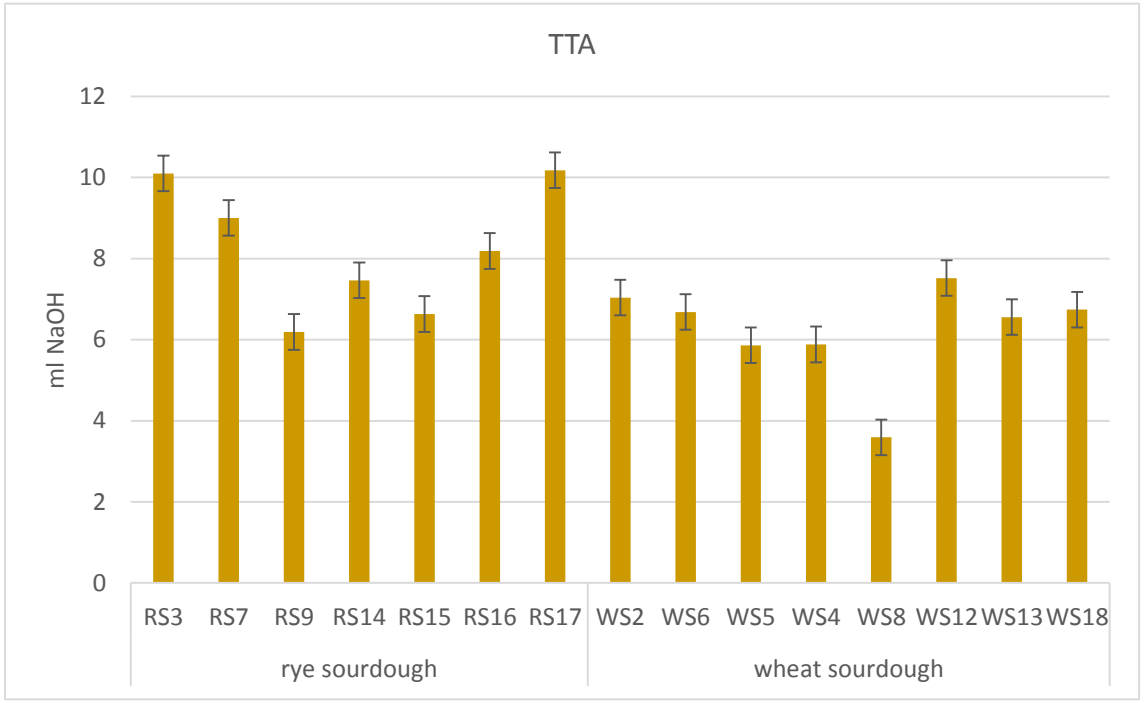


Analysis

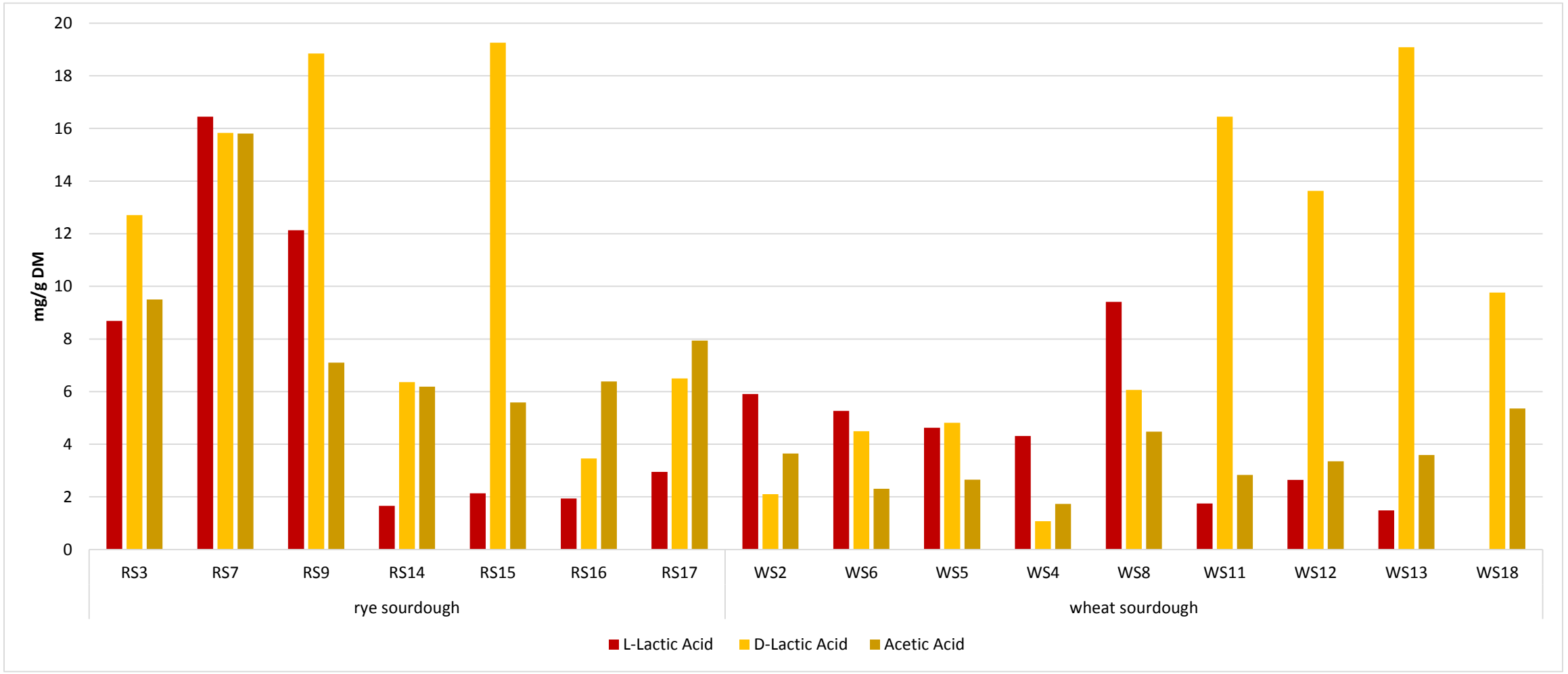
- Samples: 8 rye and 7 wheat sourdoughs
- Dough property measurements
  - pH, TTA
  - D-/L-Lactic Acid, L-Lactic Acid, Acetic Acid
- Cultivation of LAB and yeasts
  - Colony counts by plating on different media
  - Identification of LAB and yeasts:
    - Sequencing
    - MALDI-TOF MS
    - Species-specific PCR acc. to Torriani, Felis et al. 2001
    - Typing

Results - dough properties



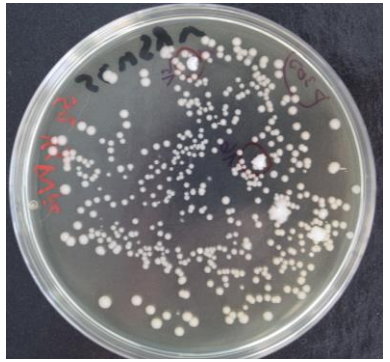


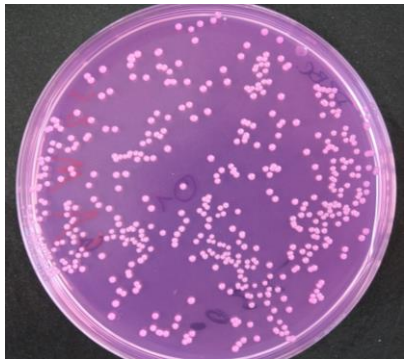
Acid measurements

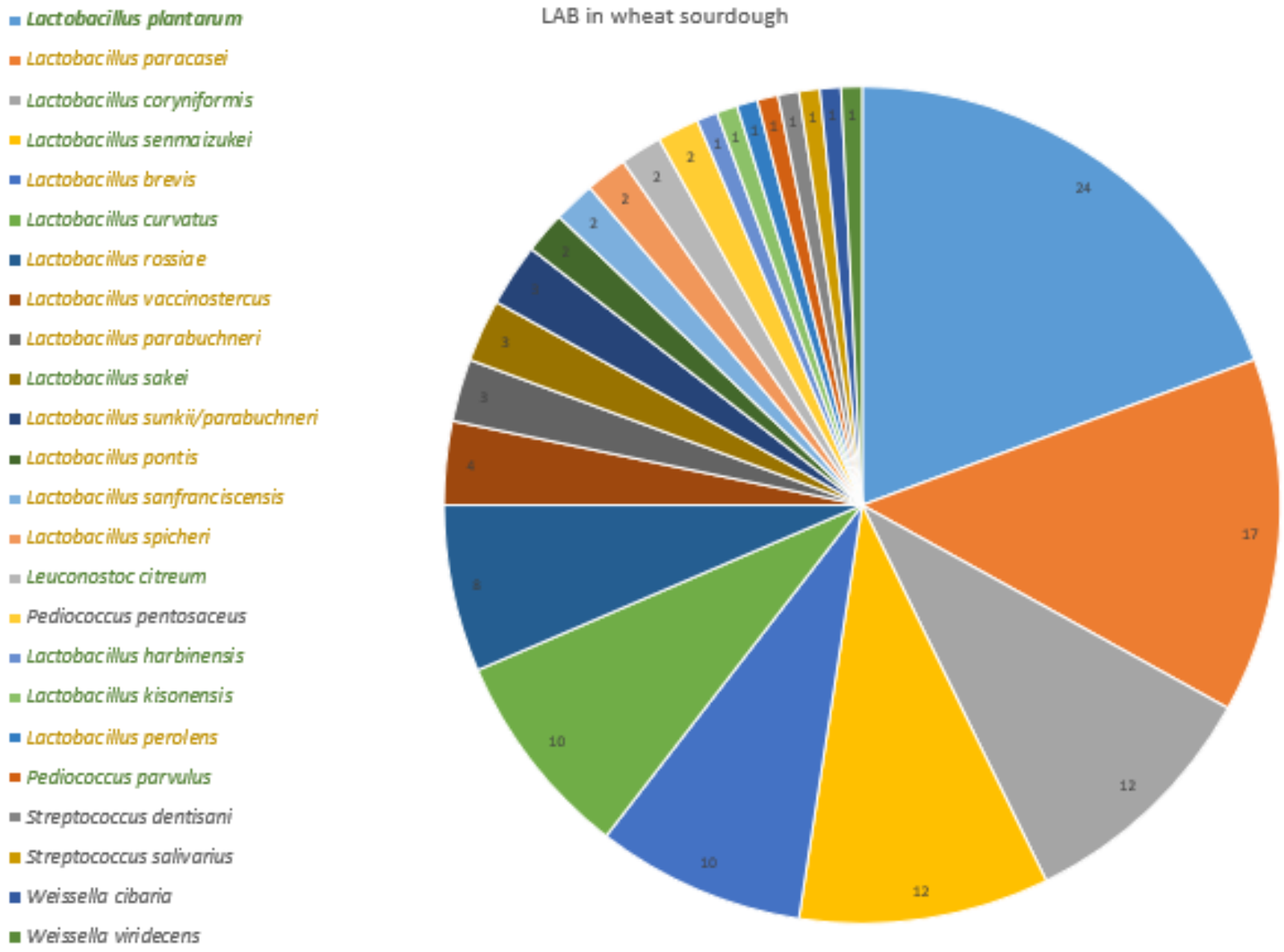


Results – average colony count

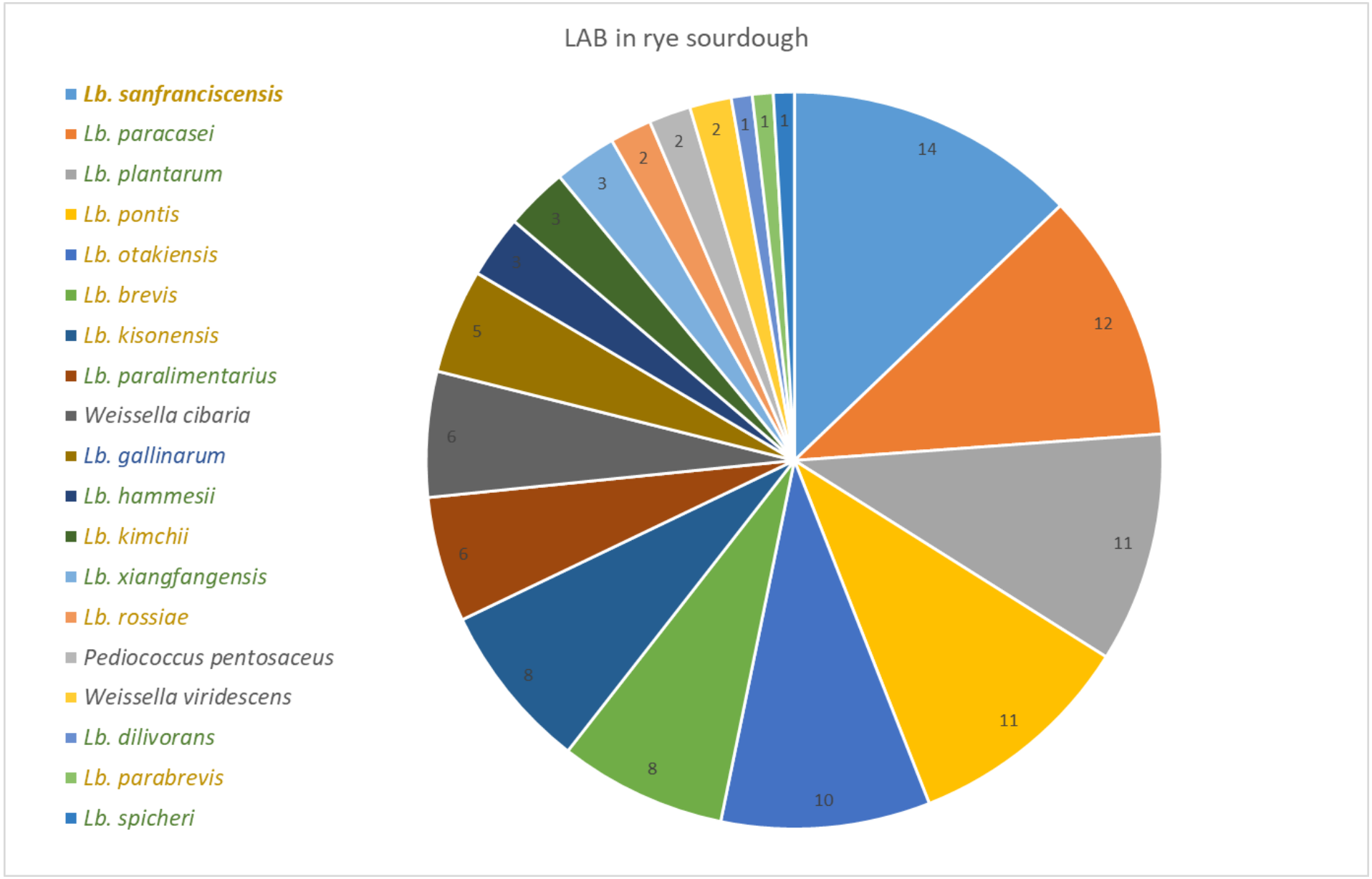
- Wheat sourdough
  - LAB: 10^9 cfu/g
  - Yeasts: 10^6 cfu/g
- Rye sourdough
  - LAB: 10^9 cfu/g
  - Yeasts: 10^7 cfu/g



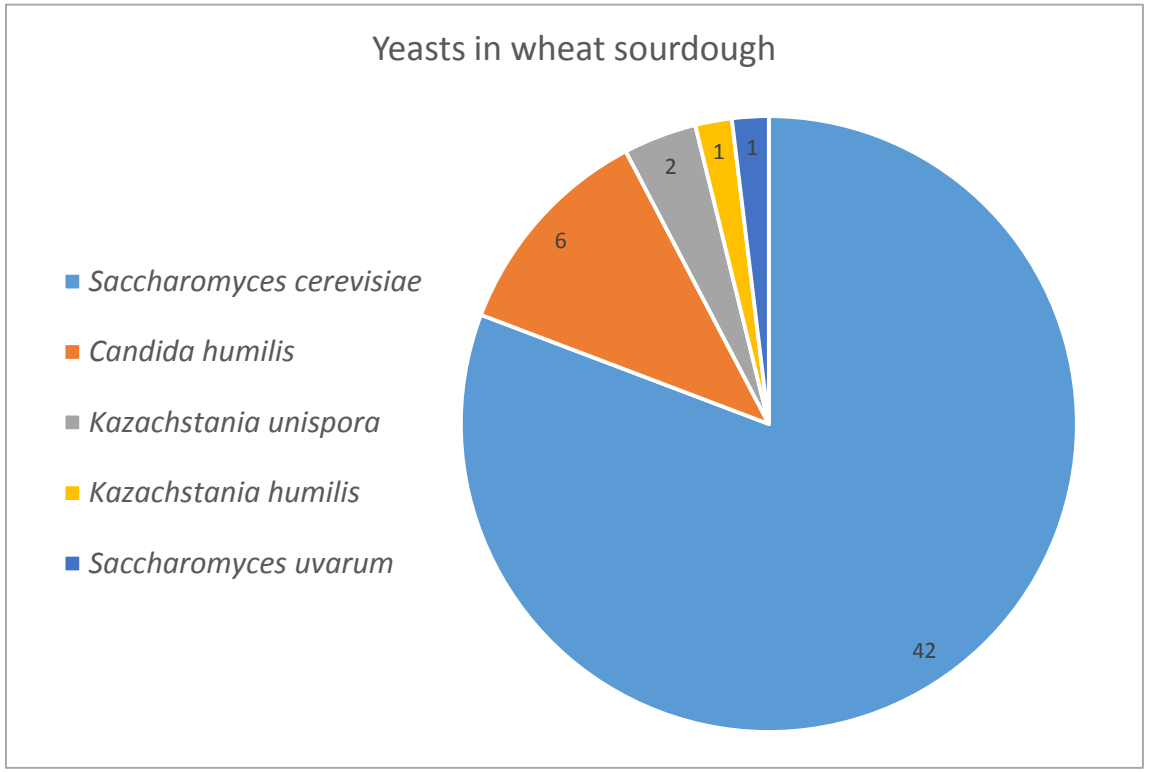


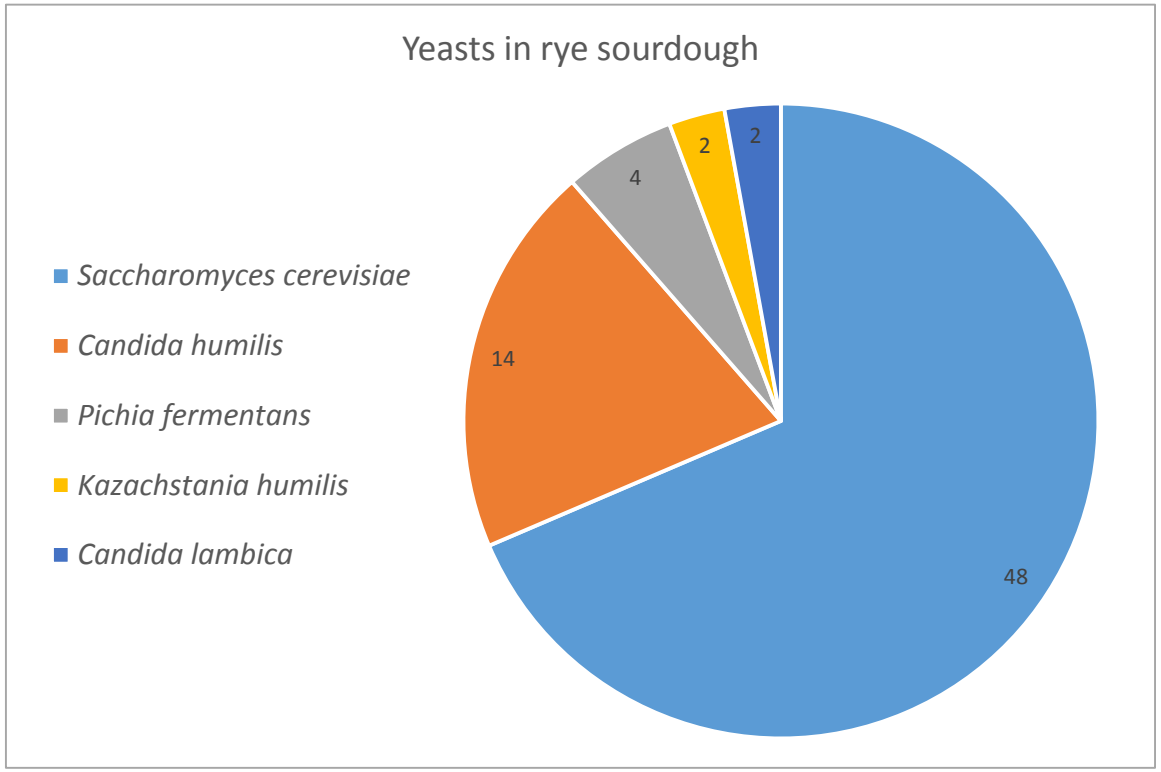


Green – facultative heterofermentative lactobacilli
Yellow – heterofermentative lactobacilli



Green – facultative heterofermentative lactobacilli
Yellow – heterofermentative lactobacilli
Blue – homofermentative lactobacilli


Yeasts in wheat sourdough
Saccharomyces cerevisiae
Candida humilis
Kazachstania unispora
Kazachstania humilis
Saccharomyces uvarum
42
6
2
1
1


Yeasts in rye sourdough
Saccharomyces cerevisiae
Candida humilis
Pichia fermentans
Kazachstania humilis
Candida lambica
48
14
4
2
2

Conclusion

- Ratio up to 1:1000 (yeasts:LAB)
- Examination of 8 rye and 7 wheat sourdoughs
- Broad spectrum of Lactic Acid Bacteria was identified:
  - 6 different genera
  - 31 different species
- Differences on subspecies/strain level

Thank you for your attention!

	MRS	MRS+M	M17	M17+G	SDM	WCA
Enzymatic Digest of Casein	10	10				
Casein peptone, typtic digest					6	
Tryptone						10
Gelatin peptone						10
Peptone from soymeal			5	5		
Peptone from meat			2,5	2,5		
Peptone from casein			2,5	2,5		
Meat Extract	10	10	5	5		
Yeast Extract	4	4	2,5	2,5	3	5
Fresh Yeasts Extract					15 mL	
D(+)-Glucose	20	20		5		1
Lactose monohydrate			5	5		
Maltose		20			20	
Tween 80	1,08	1,08			0,3	1
Dipotassium Hydrogen Phosphate	20	20				
Di-Ammonium Hydrogen Citrate	2	2				
Sodium Acetate	5	5				
Sodium Chloride						5
Sodium Pyruvate						1
Magnesium Sulfate Heptahydrate	0,2	0,2	0,25	0,25		
Manganese Suflate Monohydrate	0,04	0,04				
Ascorbic acid			0,5	0,5		
Sodium b-glycerophosphate			19	19		
L-Arginine						1
Menadione						0,0005
Haemin						0,005
Nalidixic Acid						30 mg/ml
Defibrinated sheep blood						50 mL
Agar-Agar	14	14	12,75	12,75	14	10
pH	5,6-5,9		7,2		5,6	7,1

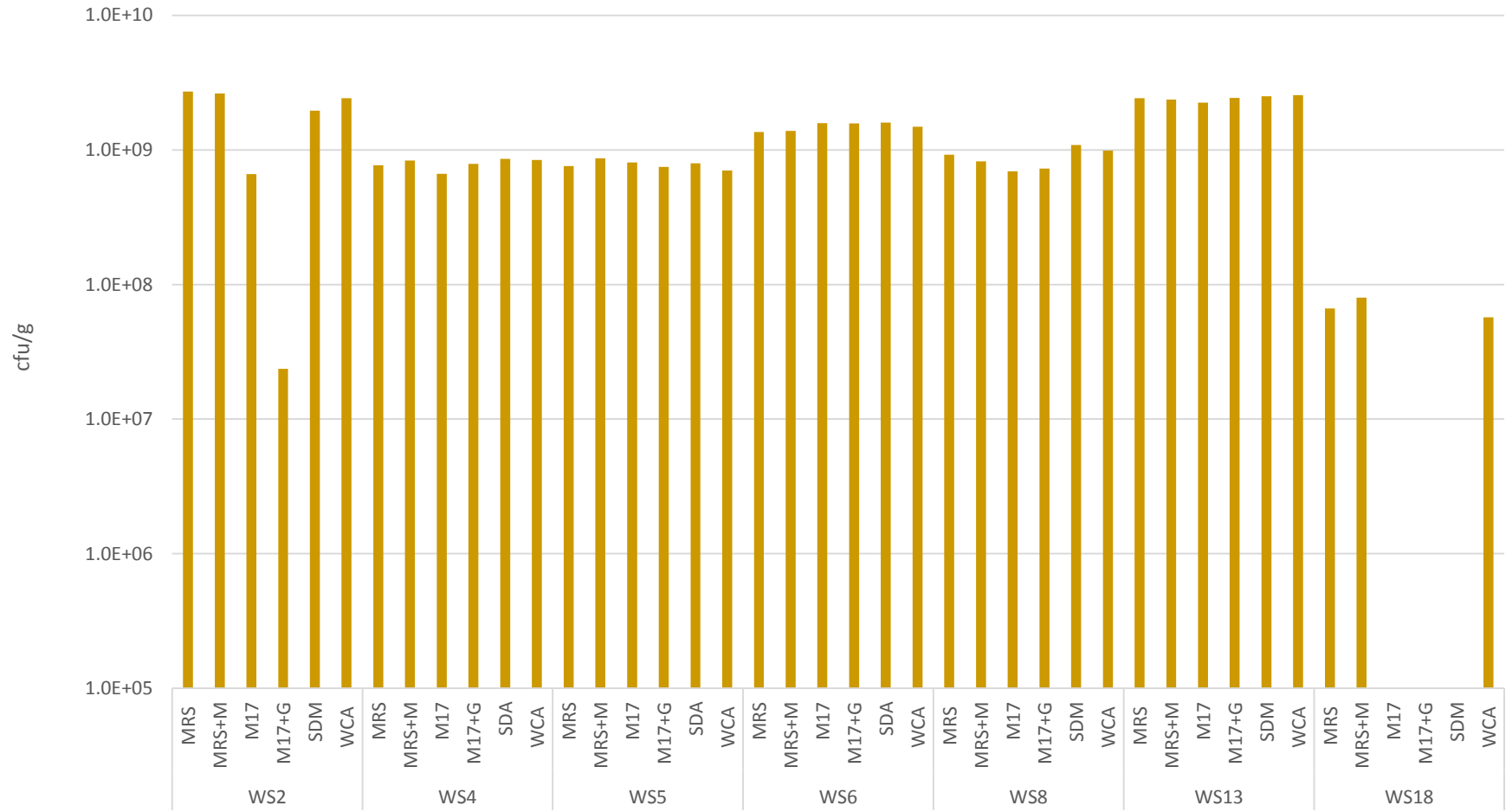
	YGC	DRBC	MEA + G	MEA + Y
Peptone aus Gelatine			0,78	0,78
Bacteriolocigal Peptone		5		
Malt Extract			12,75	12,75
Yeast Extract	5			10
D-Glucose	20	10	20	
Dextrin			2,75	2,75
KH2PO4		1		
MgSO4		0,5		
Dichloran		0,002		
Rose Bengal		0,025		
Glycerin			2,35	2,35
Chloramphenicol	0,1	0,1	0,1	0,1
Agar	14,9	15		
pH	6,6	5,6		

	RS1 (n=17)	WS2 (n=20)	RS3 (n=16)	WS4 (n=29)	WS5 (n=35)	WS6 (n=23)	RS7 (n=14)	WS8 (n=24)	RS9 (n=24)	WS13 (n=19)	RS14 (n=17)	RS15 (n=17)	RS16 (n=5)	RS17 (n=9)	WS18 (n=7)
Lb. brevis			1	3	5	2				3	7				
Lb. coryniformis				7	3	2									
Lb. curvatus				4	4	2									
Lb. diolivorans			1												
Lb. gallinarum	2											3			
Lb. hammesii										2	3				
Lb. harbinensis								1							
Lb. kimchii							1		2						
Lb. kisonensis			3					1	5						
Lb. otakiensis			10												
Lb. parabrevis									1						
Lb. parabuchneri		3						3							
Lb. paracasei	1	3						14	10					1	
Lb. paralimentarius							6								
Lb. perolens								1							
Lb. plantarum				9	6	5	1	2	3	8	6			1	
Lb. pontis	2	2										9			
Lb. rossiae	2	8													
Lb. sakei				3											
Lb. sanfranciscensis		2					4		2				2	6	5
Lb. senmaizukei			1	2	6	4									
Lb. spicheri				1		1				6	1				
Lb. vaccinostercus						4									
Lb. xiangfangensis							2		1						
Leuconostoc citreum								2							
Pediococcus parvulus					1										
Pediococcus pentosaceus	2				2										
Streptococcus dentisani					1										
Streptococcus salivarius						1									
Weissella cibaria	6	1													
Weissella viridescens	2	1													

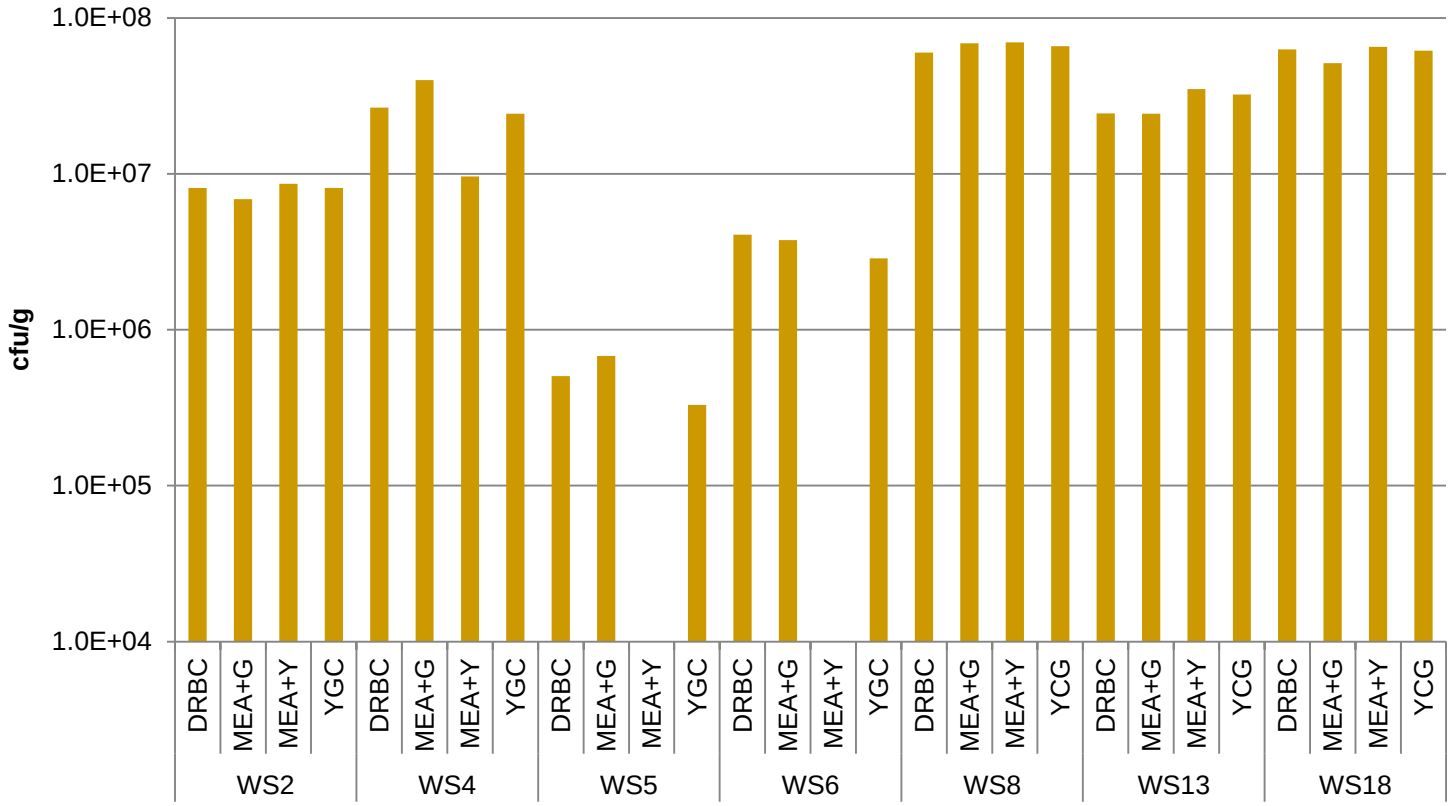
Homofermentativ	Facultative heterofermentativ	Heterofermentativ
Lb. gallinarum (R)	*Lb. coryniformis (W)*	*Lb. brevis*
	Lb. curvatus (W)	*Lb. kimchii (R)*
	Lb. diolivorans (R)	*Lb. kisonensis*
	Lb. hammesii	*Lb. otakiensis (R)*
	Lb. harbinensis (W)	*Lb. parabrevis (R)*
	Lb. paracasei	*Lb. parabuchneri*
	Lb. paralimentarius (R)	*Lb. pontis*
	Lb. perolens (W)	*Lb. rossiae*
	Lb. plantarum	*Lb. sanfranciscensis*
	Lb. sakei (W)	*Lb. vaccinostercus (W)*
	Lb. senmaizukei (W)	*Lb. sunkii*
	Lb. spicheri	
	Lb. xiangfangensis (R)	

	RS1 (n=9)	RS3 (n=8)	RS7 (n=10)	RS9 (n=8)	RS14 (n=8)	RS15 (n=8)	RS16 (n=10)	RS17 (n=9)	WS2 (n=8)	WS4 (n=8)	WS5 (n=9)	WS6 (n=9)	WS8 (n=8)	WS13 (n=8)	WS18 (n=)
Candida humilis				+		+		+	+						+
Candida lambica							+								
Kazachstania humilis								+							+
Kazachstania unispora										+					
Pichia fermentans			+				+								
S. cerevisiae	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
S. uvarum												+			

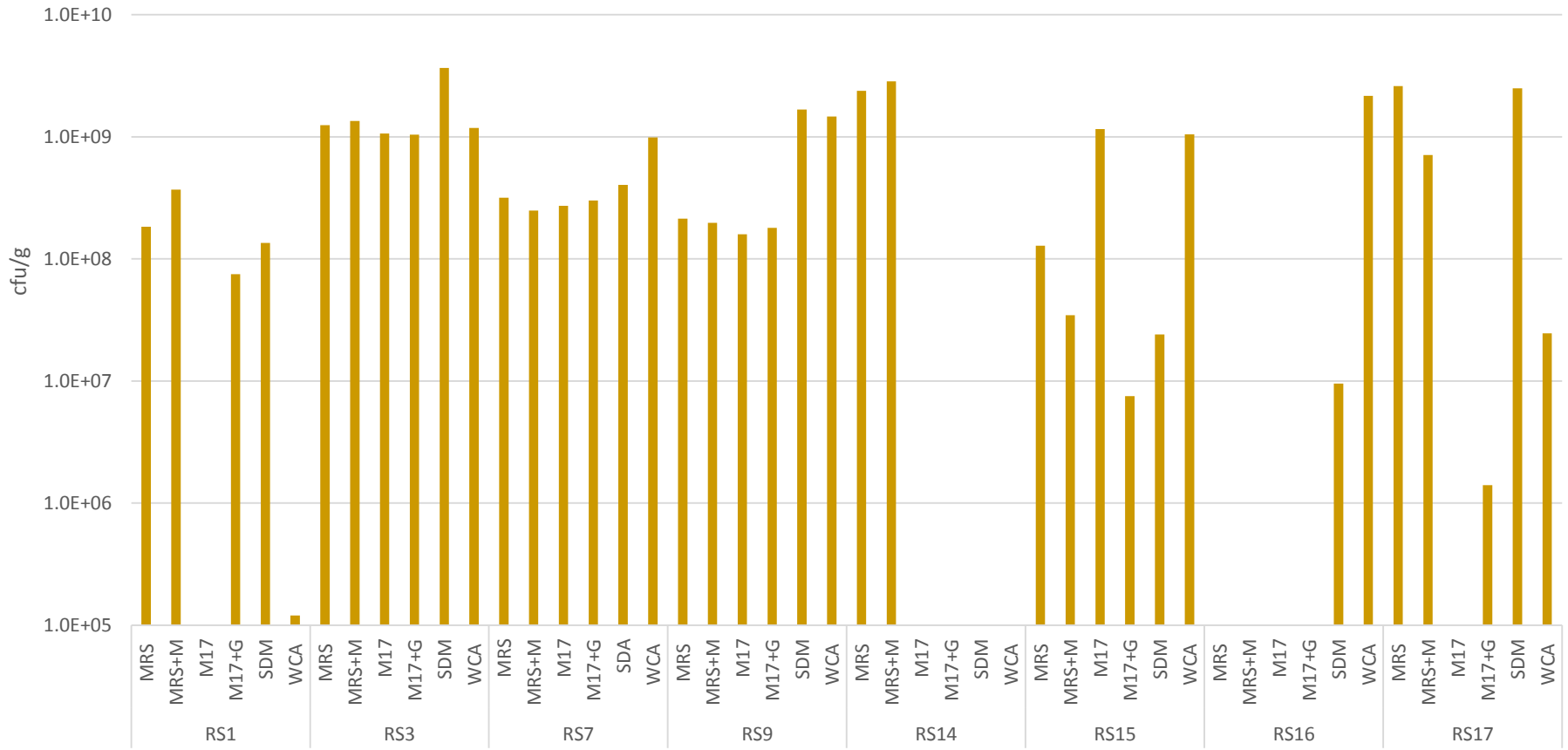
LAB counts in wheat sourdough



Yeast counts in wheat sourdough



LAB counts in rye sourdough



Yeast counts in rye sourdough