

# Yeast–Yeast Interactions: Mechanisms, Methodologies and Impact on Composition

Fanny Bordet <sup>1,2,†</sup>, Alexis Joran<sup>1,†</sup>, Géraldine Klein<sup>1</sup>, Chloé Roullier-Gall<sup>1</sup>, Hervé Alexandre <sup>1,\*</sup>

<sup>1</sup> Univ. Bourgogne Franche-Comté, AgroSup Dijon, PAM UMR A 02.102, F-21000 Dijon, France-IUVV Equipe VAIMiS, rue Claude Ladrey, CEDEX BP 27877, 21078 Dijon, France; bordetfanny@gmail.com (FB); alexis.joran@gmail.com (AJ); geraldine.klein@u-bourgogne.fr (GK.); Chloe.Roullier-Gall@u-bourgogne.fr(CRG)

<sup>2</sup> Lallemand SAS, 19, rue des Briquetiers, CEDEX BP 59, 31702 Blagnac, France

\* Correspondence: rvalex@u-bourgogne.fr (HA); Tel.: +33-380-396-393

† These authors contributed equally to this work.

Table S1: Diversity of methodologies and results in yeast interaction experiments

Species	<i>Saccharomyces cerevisiae</i> / <i>Metschnikowia pulcherrima</i>									
Matrix	Synthetic Medium						Grape Juice / Must (unspecified color)			
	SGJ	SGJ				SGJ	Grape must			
Sugar Initial Concentration (g/L)	200 (Glc 100 Fru 100)	200 (Glc 100 Fru 100)				200 (Glc 100 Fru 100)	231			
YAN / PAN (mg/L)	YAN 410					200	PAN 154 / NH <sub>3</sub> 22			
Temperature (°C)	25	25				20	25			
Oxygenation	++	-	+	++	+++	+	+			
Inoculation delay between 2 strains (h)	0	0					0	0		
Ratio Sc/NS	1:1	1:10				1:10	1:1	1:100	1:10000	
Sc population	Max	1	3	3	3	3	4	3	2	3
	Dominance		+					2	2	2
	Decrease	-	-	+	+	+	+	-	-	-
NS population	Max	1	1	1	1	1	1	7	1	3
	Decrease	2 or 1	1	3	3	3	-	7	3	3
	Detectable End	No 2 or 4						No 22	No 7	No 7
Fermentation completion						+	+	+	+	
Ethanol	=	=	--	--	--	--	-	--	--	--
Glycerol		++	--	--	--	--	+	++	++	++
Organic acids	TA							--	--	--
	VA							--	--	--
	LA									
	AA									
Impacting Factors	SPE / NS	OX / SPE				SPE	RAT / SPE			
Interaction Mechanisms	TOX	COMP				COMP / TOX				
Reference	[65]	[29]				[95]	[32]			

Species	<i>Saccharomyces cerevisiae</i> / <i>Metschnikowia pulcherrima</i>											
Matrix	Red Grape Juice / Must					White Grape Juice / Must						
	Shiraz	Merlot	Shiraz	Tempranillo	Pinot Grigio	Sauvignon blanc	Riesling	Sauvignon blanc	Chardonnay	Muscat commercial grape juice	Sauvignon blanc	Riesling
Sugar Initial Concentration (g/L)	210	240	280 (Glc 140 Fru 140)	245	236	219 (Glc 110 Fru 109)	237	217	240 (Glc 120 Fru 120)	200 (supp)	221 (Glc 112 Fru 109)	225
YAN / PAN (mg/L)	200	190	YAN 170 (supp)	YAN 181	YAN 236	YAN 588	PAN 147	YAN 170 (supp)	260		YAN 378	YAN 250 (supp)
Temperature (°C)	22	22	25		22	20	20	15	22	20	20	20
Oxygenation	+	++	++	+	+	+	+	+	+	+	+	+
Inoculation delay between 2 strains (h)	24 days	0	96	72	48	48	24	168	24 days	72	48	48
Ratio Sc/NS	1:1	1:10	1:1	1:1	1:1	1:10	10:1	1:1	1:1	1:5	1:10	1:1
Sc population	Max						4			5	3	4
	Dominance		+	+								
	Decrease		-				-	-		-	-	-
NS population	Max											0
	Decrease	+	2	+	+	+	+	+	15	5	4	2
	Detectable End			No 7	No 4-5		No 8	No 15	No 12	No 25	Low 13	No 8
Fermentation completion	+		17		9	8	+	19	40	10	10	+
Ethanol	--	-	=	--	-	-	--	=	-		-	=
Glycerol	-	++		++	++		++		+		++	++
Organic acids	TA					--		+				--
	VA	+		=	--			++	++			
	LA											
	AA	--	=			=	--	=	++	--	--	=
Impacting Factors	GRA		SPE	SPE	SPE / NS	SPE	SPE	SPE	GRA	SPE / TEMP / DEL / MED	DEL	DEL
Interaction Mechanisms										COMP	COMP	
Reference	[155]	[153]	[36]	[37]	[14]	[47]	[38]	[41]	[155]	[55]	[87]	[44]

Species		<i>Saccharomyces cerevisiae</i> / <i>Starmerella bacillaris</i>										
Matrix		Synthetic Medium	Grape Juice / Must (unspecified color)			White Grape Juice / Must						
		SGJ	Grape must			Macabeo	Erbaluce / dried grape must		Sauvignon blanc	Sauvignon blanc	Muscat (commercial grape juice)	Chardonnay / Muscat / Riesling / Sauvignon blanc
Sugar Initial Concentration (g/L)		200 (Glc 100 Fru 100)	231			180	403 (Glc 210 Fru 193)		219 (Glc 110 Fru 109)	217	200 (supp)	245
YAN / PAN (mg/L)		YAN 410	PAN 154 / NH <sub>3</sub> 22			YAN 115			YAN 588	YAN 170 (supp)		YAN 180 adj
Temperature (°C)		25	25			20	25		20	15	20 / 28	20
Oxygenation		++	+			+	+		+	+	+	+
Inoculation delay between 2 strains (h)		0	0			0	0 48		24	144	72	48
Ratio Sc/NS		1:1	1:1	1:100	1:10000	1:9	1:1		1:10	1:1	1:5	1:1
Sc population	Max		2	2	10				3		=	
	Dominance	-	-	-	-	-	-		-		-	=
	Decrease		1	3	10	- +						-
NS population	Max		1	2	2				1			
	Decrease	4	7	10	15	+	6 6/12		-	+	-	7
	Detectable End	No 5 / Yes	No 15	No 22	No 22	Low 10				No 14		No 14
Fermentation completion			+	+	+	10			7	24		14
Ethanol			--	--	--		-- --		-	=	=	-
Glycerol			++	++	++	++	++ ++					++
Organic acids	TA		--	--	--				+			++
	VA		--	--	--							
	LA											
	AA					++	= / -- = / --			++		
Impacting Factors		SPE / NS	RAT / SPE			SPE	DEL / SC / NS		SPE	SPE	SPE / TEMP / DEL / MED	GRA / TEMP
Interaction Mechanisms		TOX				COMP					COMP	
Reference		[65]	[32]			[48]	[23]		[47]	[41]	[55]	[79]

Species	<i>Saccharomyces cerevisiae</i> / <i>Starmerella bacillaris</i>																	
Matrix	Red Grape Juice / Must																	
	Merlot			Barbera		Shiraz	Barbera		Cabernet sauvignon / Merlot / Pinot noir / Shiraz		Kotsifali - Manilari 3:1			Barbera	Nebbiolo	Pinot Grigio		
Sugar Initial Concentration (g/L)	240			244		280 (Glc 140 Fru 140)	246		250		220 214			234 (Glu 118 Fru 116)	234 (Glu 115 Fru 120)	236		
YAN / PAN (mg/L)						YAN 170 (supp)	YAN 180 adj		YAN 180 adj		YAN 240 YAN 71 + suppl		YAN 180 adj	YAN 180 adj		YAN 236		
Temperature (°C)	24			25		25	25		25		25 25			25	25	22		
Oxygenation	+			+		++	+ ++		+		+ +			+	+	+		
Inoculation delay between 2 strains (h)	0	24	48	0	48	96	48		24	48	0	30	0	30	48	48	48	
Ratio Sc/NS	1:5			1:1		1:1	1:1		1:1		1:1			1:1	1:1	1:1		
Sc population	Max																	
	Dominance		-			-				= / + -		+ -			-		-	
	Decrease					+		- -		- -		- - -			10		- +	
NS population	Max		4															
	Decrease					+		+ 4 4		4 4/7		3 8 2 2			7		7 +	
	Detectable End		No 21 Yes 21		No 7		No 14 / 21 No 14 / Yes 21				Low 5 Low 6 Low 7			No 10 / 14		No 9 / Yes 14		Low 5
Fermentation completion	+ + +			- -		17	+ / - + / -		7 7/10		6 13			14		14 14		
Ethanol	= -- --			-- --		=	-- --		-- --		- -- -- --			-- / =		= / -- -		
Glycerol	++ ++			= ++			++ ++		++ ++		= ++ ++ ++			++		++ ++		
Organic acids	TA		++ ++															
	VA																	
	LA																	
	AA							++ +++				++ = =			--			
Impacting Factors	NS / DEL			DEL / NS / SC		SPE	OX / NS / SC		DEL / GRA		DEL			SC		SPE / NS		
Interaction Mechanisms							COMP				TOX / COMP					Not TOX / Not COMP / CCC		
Reference	[75]			[12]		[36]	[67]		[60]		[49]			[69]		[68]		[14]

Species		<i>Saccharomyces cerevisiae</i> / <i>Torulaspora delbrueckii</i>													
Matrix		Synthetic Medium													
		SGJ	WYPD		SGJ	SGJ				SGJ				SGJ	SGJ
Sugar Initial Concentration (g/L)		210 (Glc)	200 (Glc)		210 (Glc)	220 (Glc 110 Fru 110)				200 (Glc 100 Fru 100)				200 (Glc 100 Fru 100)	200 (Glc 100 Fru 100)
YAN / PAN (mg/L)						YAN 324		YAN 176						YAN 410	200
Temperature (°C)		25	25		25	20				25				25	20
Oxygenation		+	++	+	+	+				-	+	++	+++	++	+
Inoculation delay between 2 strains (h)		0	0		0	0	0	48	48	0				0	0
Ratio Sc/NS		1:1	1:1		1:1	1:2	1:20	1:2	1:2	1:10				1:1	1:10
Sc population	Max	2	1	1	2	2	2	4	5	3	3	3	3	1/3	2
	Dominance	+	+	+	+										
	Decrease	-	-	-	-	-	-	-	-	-	+	+	+	-	-
NS population	Max	2	1	1	1	2	3	3	2	1	1	1	1	1	2
	Decrease	3	-	-	3	3	4	4	2	1	3	3	3	2/3	-
	Detectable End	Low 7			Low 6	Low 5								No 6 / Yes	
Fermentation completion		10			10	7	5	9							
Ethanol		= = - = = -- -- -- = --													
Glycerol		++ -- -- -- =													
Organic acids	TA														
	VA														
	LA														
	AA	= = --													
Impacting Factors		RAT / DEL OX / SPE SPE / NS SPE													
Interaction Mechanisms		Not TOX / Not COMP / CCC	Not TOX / Not COMP / Not QS / CCC		COMP	Not CCC / Not COMP / TOX				COMP				TOX	COMP / TOX
Reference		[25]	[26]		[27]	[51]				[29]				[65]	[95]

Species		<i>Saccharomyces cerevisiae</i> / <i>Torulaspora delbrueckii</i>							
Matrix		Grape Juice / Must (unspecified color)			White Grape Juice / Must			Red Grape Juice / Must	
		Grape must			Sauvignon blanc	Sauvignon blanc	Riesling	Shiraz	Tempranillo
Sugar Initial Concentration (g/L)		231			219 (Glc 110 Fru 109)	217	225	280 (Glc 140 Fru 140)	245
YAN / PAN (mg/L)		PAN 154 / NH <sub>3</sub> 22			YAN 588	YAN 170 (supp)	YAN 250 (supp)	YAN 170 (supp)	YAN 181
Temperature (°C)		25			20	15	20	25	
Oxygenation		+			+	+	+	++	+
Inoculation delay between 2 strains (h)		0			24	120	48	96	72
Ratio Sc/NS		1:1	1:100	1:10000	1:10	1:1	1:1	1:1	1:1
Sc population	Max	3	10	3	3		4		
	Dominance	2		15				+	
	Decrease	-	-	-	-		15		
NS population	Max	3	3	3	1		0		
	Decrease	3	10	15	-	+	4	+	+
	Detectable End	No 15	No 22	No 22		14	No 6	No 7	Yes 6
Fermentation completion		+	+	+	7	19	+	17	
Ethanol		--	--	--	-	=	=	=	--
Glycerol		--	--	--			++		++
Organic acids	TA	+	+	+	+	=	--		
	VA	--	--	--	--	=		=	--
	LA								
	AA					-	=		
Impacting Factors		RAT / SPE			SPE	SPE	DEL	SPE	SPE
Interaction Mechanisms									
Reference		[32]			[47]	[41]	[44]	[36]	[37]

Species		<i>Saccharomyces cerevisiae</i> / <i>Hanseniaspora guilliermondii</i>										
Matrix		Synthetic Medium							White Grape Juice / Must			
		SGJ							SGJ	SGJ	Malvasia fina - Arinto	Malvasia fina - Arinto
Sugar Initial Concentration (g/L)		200 (Glc 110 Fru 110)					100	220 (Glc 110 Fru 110)	220 (Glc 110 Fru 110)		234	234
YAN / PAN (mg/L)											YAN 175 / 387 (supp)	YAN 387
Temperature (°C)		18						20	25		20	20
Oxygenation		+						+	+		+	+
Inoculation delay between 2 strains (h)		0						0	0		0	0
Ratio Sc/NS		1:1	3:1	1:2	1:10	1:100	1:10	1:1	1:1	1:1	1:1	
Sc population	Max	2	3	7	7	7			2	1	1	
	Dominance											
	Decrease	-	-	-	-	-		-	-	-	-	
NS population	Max	1			3	3			2	1	1	
	Decrease	1	+	1	4	15	7	+	2	2	6	
	Detectable End	No 7	No 3	No 8	No 11	No 23	Yes 34	Low 4	No 4	No 12	Low 10	
Fermentation completion		20	20	25	25	25		+	9	+		
Ethanol												
Glycerol												
Organic acids	TA											
	VA											
	LA											
	AA											
Impacting Factors		RAT									MED	
Interaction Mechanisms		TOX / Not COMP							TOX		Not COMP	
Reference		[52]							[85]	[99]	[50]	[112]



Species	<i>Saccharomyces cerevisiae</i> / <i>Hanseniaspora uvarum</i>			
Matrix	Synthetic Medium			White Grape Juice / Must
	SGJ	SGJ	SGJ	Macabeo
Sugar Initial Concentration (g/L)	200 (Glc 110 Fru 110)	200 (Glc 100 Fru 100)	200 (Glc 100 Fru 100)	180
YAN / PAN (mg/L)		YAN 410	YAN 200	YAN 114.6
Temperature (°C)	18	25	20	20
Oxygenation	+	++	+	+
Inoculation delay between 2 strains (h)	0	0	0	0
Ratio Sc/NS	4:1	1:1	1:10	1:9
Sc population	Max	2		1
	Dominance			
	Decrease	-		8
NS population	Max		1	1
	Decrease	1	2	1
	Detectable End	No 3	No 4	Low 10
Fermentation completion	18			+
Ethanol		=	+	=
Glycerol			-	++
Organic acids	TA			
	VA			
	LA			
	AA			++
Impacting Factors	RAT	SPE / NS	SPE	SPE
Interaction Mechanisms	TOX / Not COMP	TOX	COMP / TOX	COMP
Reference	[52]	[65]	[95]	[48]

YAN = yeast assimilable nitrogen / PAN = primary amino nitrogen. Sc = *S. cerevisiae* / NS = Non-*Saccharomyces*. TA = total acidity / VA = volatile acidity / LA = lactic acid / AA = acetic acid. SGJ = Synthetic Grape Juice / Glc = Glucose / Fru = Fructose / WYPD = Yeast Peptone Dextrose medium modified for wine fermentation / Supp = with supplementation. Oxygen: - = anaerobia, +/- semi-anaerobia, low oxygenation, + = semi-anaerobia, ++ aerobia, +++ aerobia, with higher oxygenation. Population columns: Max = maximal population reached by day x / Dominance: + = dominance of *S. cerevisiae*, = similar populations, "x" = dominance obtained after x days / Decrease = decrease since day x / Low = low population since day x / No = population not detectable since day x / Yes = population still detectable at day x. Fermentation completion: x = reached at day x, +/- = reached/not reached during experimentation. Ethanol, glycerol and organic acids are compared to Sc pure culture: +++ very high increase ++ high increase, + increase, +/- slight decrease, = no change, - decrease, - - high decrease. Impacting factors: inoculation delay (DEL), inoculation ratio between *S. cerevisiae* and non-*Saccharomyces* yeast (RAT), yeast species (SPE), yeast strain *S. cerevisiae* (SC) or non-

*Saccharomyces* (**NS**), medium composition (**MED**), grape nature (**GRA**), temperature (**TEMP**), oxygenation (**OX**), type of reactor (lab, pilot, industrial) (**REAC**). **Interaction mechanisms:** involvement of quorum sensing mechanisms (**QS**), toxic compounds (including ethanol, antimicrobial peptides) (**TOX**), competition for nutrient (including oxygen) (**COMP**), cell-cell contact mechanisms (**CCC**) / **No** = mechanism involvement has been ruled out by the study.