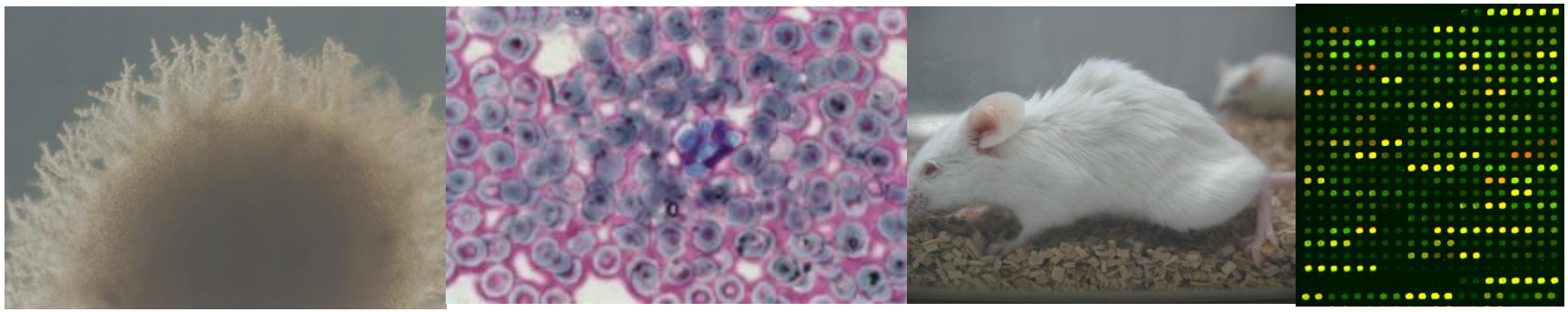
¿Las levaduras que se utilizan en la elaboración de alimentos son seguras?



Amparo Querol
Instituto de Agroquímica
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Valencia, Spain

More frequent yeast species	Origin (Food and beverage)	Major functions
Saccharomyces species	Wine, beer, sourdoughs, cider, sherry, cheese, indigenous fermented foods and beverages	- Sugars fermentation, - production of secondary metabolites, - pectinase and glycosidasic activities, - inhibitory effect on the growth of mycotoxin producing moulds, - degradation of some fractions of kasein - CO_2 evolution
Debaryomyces hansenii	Cheese, salami	- Lipolytic, proteolytic and urease activities, - increase of pH - production of growth factors of importance for bacteria
Hanseniaspora (*Kloeckera*) species	Wine, cider, indigenous fermented foods and beverages	- Proteolytic, glycosidasic and pectinolytic activities, - production of secondary metabolites
Candida fermenting species	Wine, sourdough, indigenous fermented foods and beverages	- Proteolytic, glycosidasic and pectinolytic activities, -production of secondary compounds - inhibitory effect on the growth of mycotoxin producing moulds
Yarrowia lipolytica	Cheese, salami	- Lypolytic, proteolytic and urease activities - Reduction of fat rancidity

YEASTS IN FOODS

Beneficial role

Fermentations

Negative aspects

Spoilage

Emergent pathogens

Yeast associated with foods and beverages

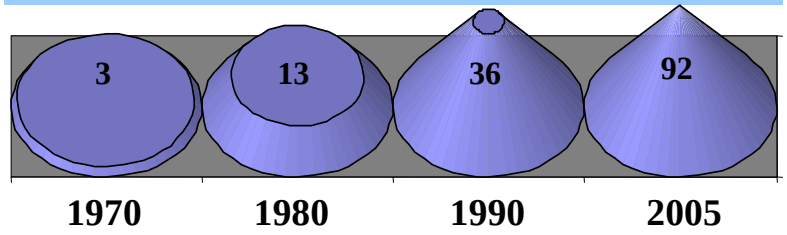
Candida kefyr
Candida krusei
Candida tropicalis
Rhodotorula mucilaginosa
Saccharomyces cerevisiae

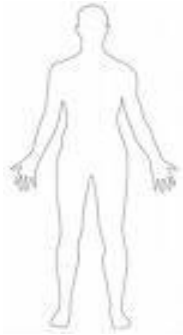
EMERGING PATHOGENS

Human infections

450 000 Candidiasis/year	×	0,5% *S. cerevisiae*	→	2250 *S. cerevisiae*/year

DOCUMENTED CLINICAL CASES RELATED WITH *S. cerevisiae*





- **Digestive tract**
- **Vagina**
- **Skin**
- **Oropharynx**
- **Sterile body sites: blood, organs (lungs, kidneys, heart..)**

Phenotypic traits associated to pathogenicity

Non-clinical isolates of *S. cerevisiae*

ISOLATES	ORIGIN	PLACE OF ORIGIN
S. boulardii (Ultralevure)	UPSA (Biocodex) *Lot R-08*	Madrid, Spain
References strains		
CECT 1895	Natural wine fermentation	Spain
CECT 1462	Fermentation	United Kingdom
CECT 1942	Brewery	The Netherlands
CECT 10.334	Sherry wines	Spain
CECT 10.551	Sherry wines	Spain
CECT 10.338	Sherry wines	Spain
CECT 1479	Sherry wines	Spain
Commercial wine strains		
ICV-3	Uvaferm CEG	California, USA
ICV-16	Cryoaromae	California, USA
ICV-17	Fermivin crio 7303	California, USA
ICV-30	Uvaferm 71B	California, USA
ICV-32	Uvaferm PM	California, USA
T 73	Lallemand	
Commercial baker's yeast		
Cinta Roja	Burns Philip Lesaffre	Australia
Plus Vital	International	Francia

Clinical isolates

Nº ISOLATES	ORIGIN
Vall D'Hebron Hospital (Barcelona, Spain)	
43	Faeces
31	Vagina
7	Pharynx
10	Sputum
2	Tracheal aspirated
1	Oral exuded
1	Bronchoalveolar lavage
1	Oral cavity
1	Pleural fluid
1	Bronchial aspirated
1	Low respiratory tract
1	Urine
1	Bile
La Fe Hospital (Valencia, Spain)	
4	Blood

Dietary supplements isolates of *S. cerevisiae*

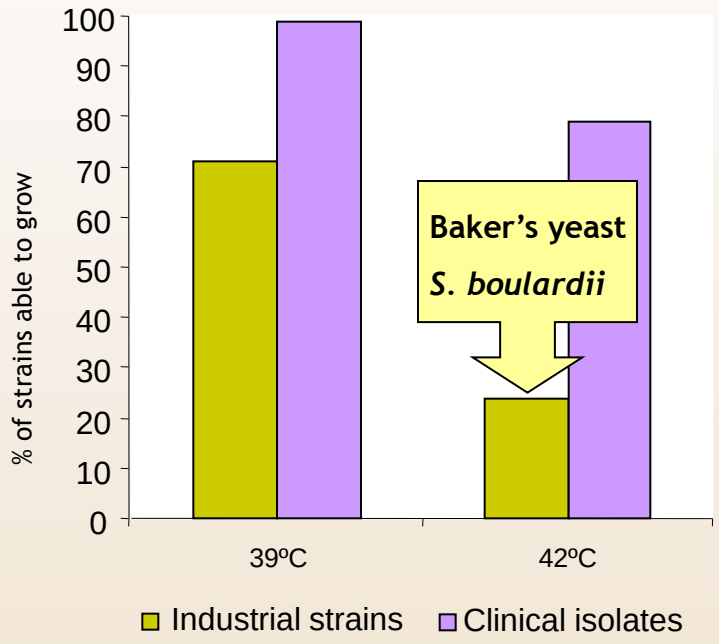
de Llanos R, Querol A, Pemán J, Gobernado M, Fernández-Espinar MT. 2006b. Int J Food Microbiol. 110(3):286-90.
de Llanos R, Querol A, Planes AM, Fernández-Espinar MT. 2004. Syst Appl Microbiol. 2004 Aug;27(4):427-35.

Phenotypic traits associated to pathogenicity

Ability to grow on GPYA plates at 28ºC, 37ºC, 39ºC and 42ºC.

- 28ºC is the optimal growth temperature for *S. cerevisiae*
- 37ºC and 39ºC are temperatures observed in febrile patients
- 42ºC was tested because it is reported as an important characteristic of virulent isolates (McCusker et al., 1994)

CLINICAL AND FOOD STRAINS



DIETARY SUPPLEMENTS ISOLATES

ISOLATES	Growth at 28ºC	Growth at 37ºC	Growth at 39ºC	Growth at 42ºC
2	+	+	+	+
4	+	+	+	+
5	+	+	+	+
6	+	-	-	-
14	+	+	+	+
23	+	-	-	-

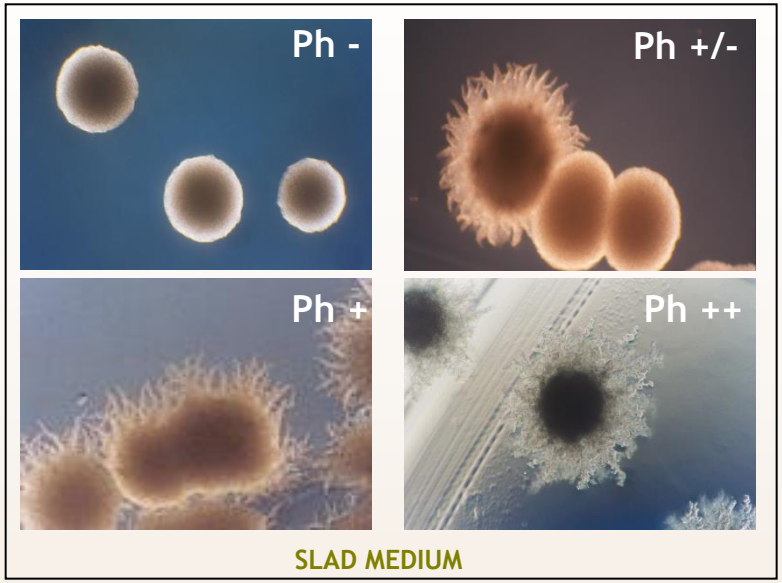
de Llanos, R., M. T. Fernandez-Espinar, and A. Querol. 2006. Antonie Van Leeuwenhoek 90:221-231

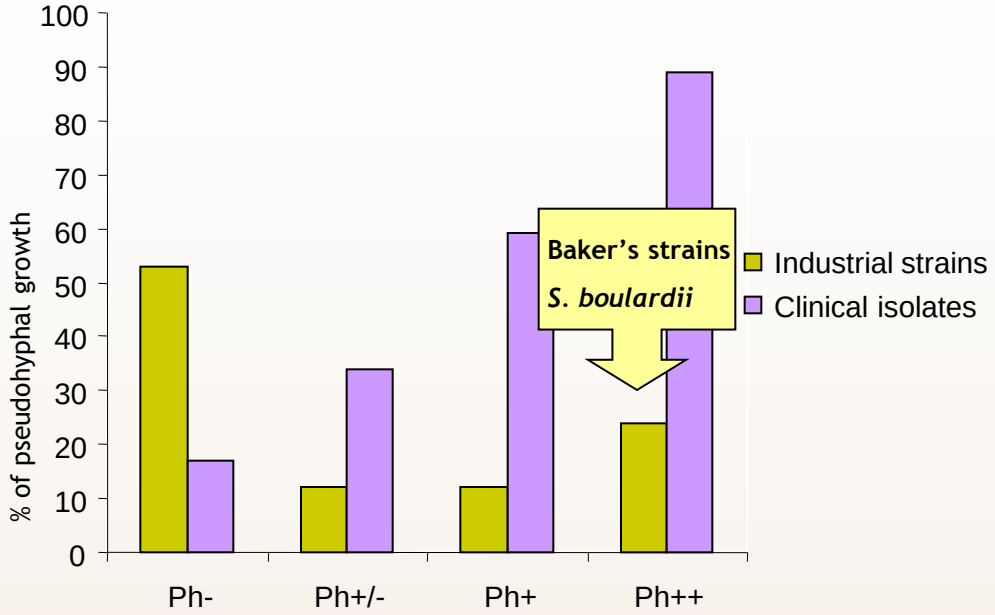
Llopis et al., Plost One (2015)

Phenotypic traits associated to pathogenicity

Pseudohyphal growth

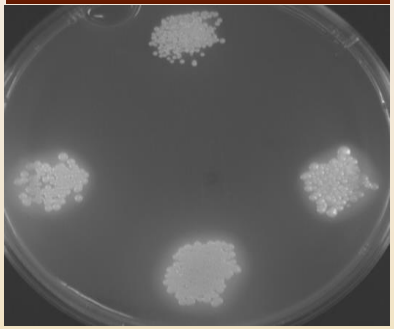
Degrees of pseudohyphal growth

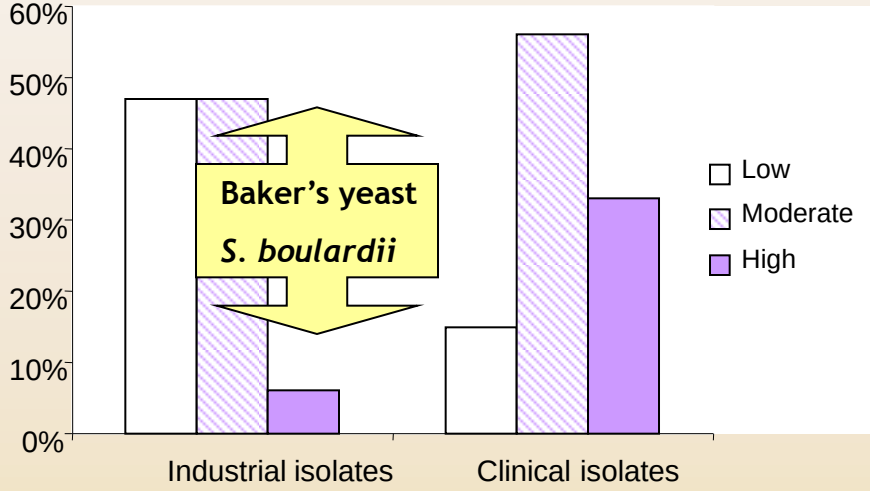




Extracellular secretion of degradative enzymes: proteases and phospholipases.

PHOSPHOLIPASES





To study the pathogenicity in murine models

Phenotypical traits associated with virulence

Strains	Growth to 37°C	39°C	42°C	Protease activity	Phospholip. activity	Ph growth	Invasive growth
Industrial strains							
S.boulardii	++	++	+	++	+	+	-
ICV-17	++	++	-	++	-	+	+/-
T73	++	++	-	++	++	-	-
CECT10.431	+	-	-	-	-	-	+/-
Baker yeast	++	++	+	+	++	++	-
Clinical isolates							
F27 (Blood)	++	++	+	++	++	++	-
# 75 (vagina)	++	++	-	++	++	++	-
# 20 (faeces)	++	++	++	+	+	++	+
# 60 (vagina)	++	++	++	++	+	++	+
# 102 (Respirat.)	++	++	++	+	+	++	+

S. cerevisiae STRAINS

Three murine models: mice with different immunity states

IMMUNOCOMPETENT MICE: BALB/C

IMMUNODEFICIENT MICE: DBA/2N. (deficient in complement factor C5)

IMMUNODEPRESSED MICE ICR/Swiss (neutropenic mice by cyclophosphamyde)

TO MIMIC THE WHOLE HUMAN POPULATION WITH DIFFERENT SUSCEPTIBILITIES TO *S. cerevisiae* INFECTION

de Llanos, R., S. Llopis, G. Molero, A. Querol, C. Gil, and M. T. Fernandez-Espinar. 2011. Int. J. Food Microbiol. 144:393-399

To study the pathogenicity in murine models

Intravenous inoculation in the lateral tail vein of mice.

S. cerevisiae dose: 2x 10^7 UFCs at a volume of 0,5ml of PBS buffer.

5 ICR/Swiss mice

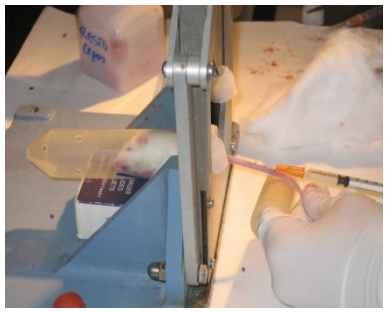
IMMUNODEPRESSED

Neutropenia was induced by intraperitoneal injection of 200 mg/Kg of cyclophosmamide,1 day before infection and 5 days after the first.

MICE WERE HANDLED IN STERILE CONDITIONS!!

10 DBA/2N mice

IMMUNODEFICIENT C5-



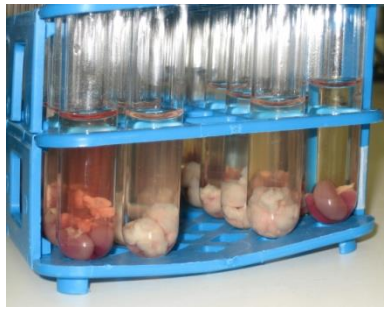
10 BALB/c mice

IMMUNOCOMPETENT

Survival experiment continue until day 30

2 mice were euthanized at 7, 15 day.

Pooled sera was obtained by cardiac puncture



Brain and kidneys were aseptically removed

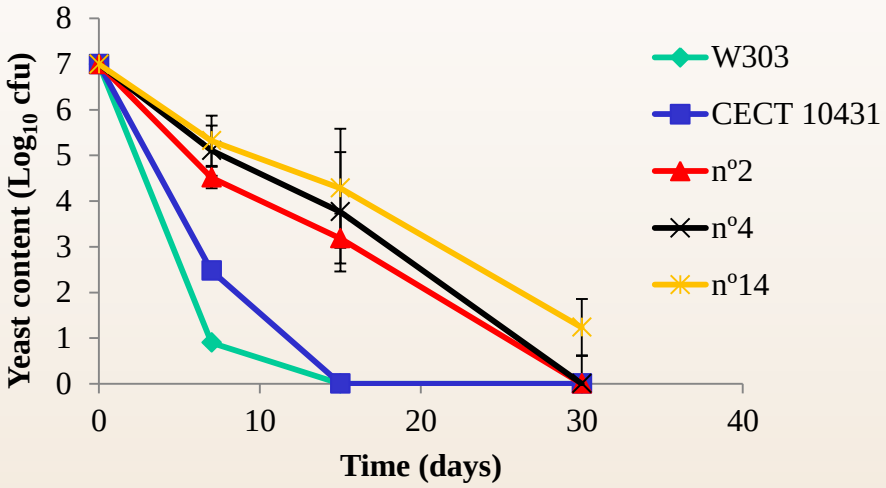
Both organs were homogenized diluted serially in sterile saline and plated onto GPYA plates with chloramphenicol

The plate were incubated for 48h at ambient temperature, and colonies were counted

Pathogenicity in murine models

YEASTS INFECTIONS IN MURINE MODEL

KIDNEY'S BURDENS



MICE DEATHS

Immunocompromised

Strains	# Dead	Day dead
Genital # 60	4/5	Day 4 (1) 5 (2) 6 (1)
Respiratory # 102	2/5	Day 4
Baker yeast	2/5	Day 4
S.boulardii	1/5	Day 4
Blood F27	1/5	Day 4

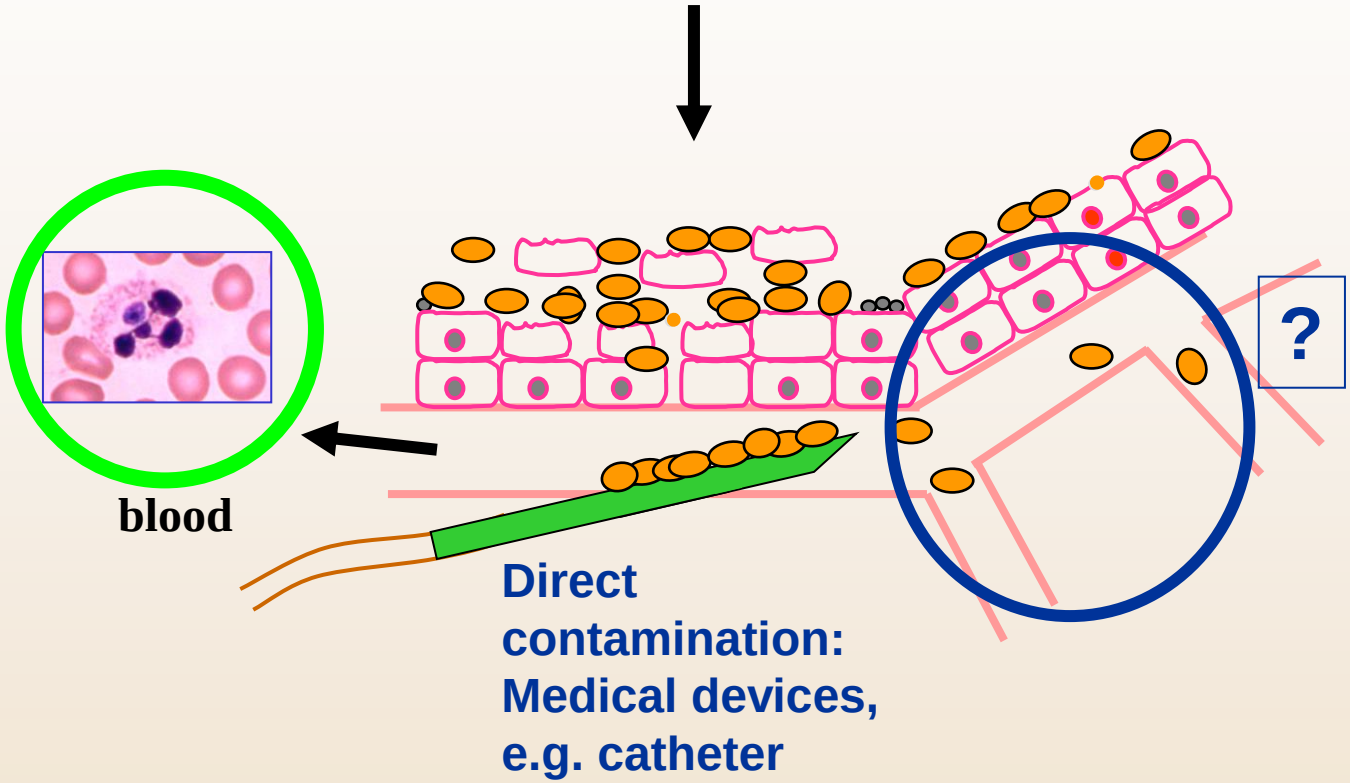
Immunocompetent

Strains	# Dead	Day dead
Genital # 60	2/10	Day 4
Respiratory # 102	2/10	Day 3
Baker yeast	3/10	Day 3
S. boulardii	2/10	Day 4
Strain D14	3/10	Day 2

(de llanos et al. IJFM, 2010)
(Llopis et al., IJFM 2011)

Portal of entry of *S. cerevisiae*

Penetration of epithelial cells from mucosal surfaces



Llopis et al. BMC Genomics 2012

Protocol

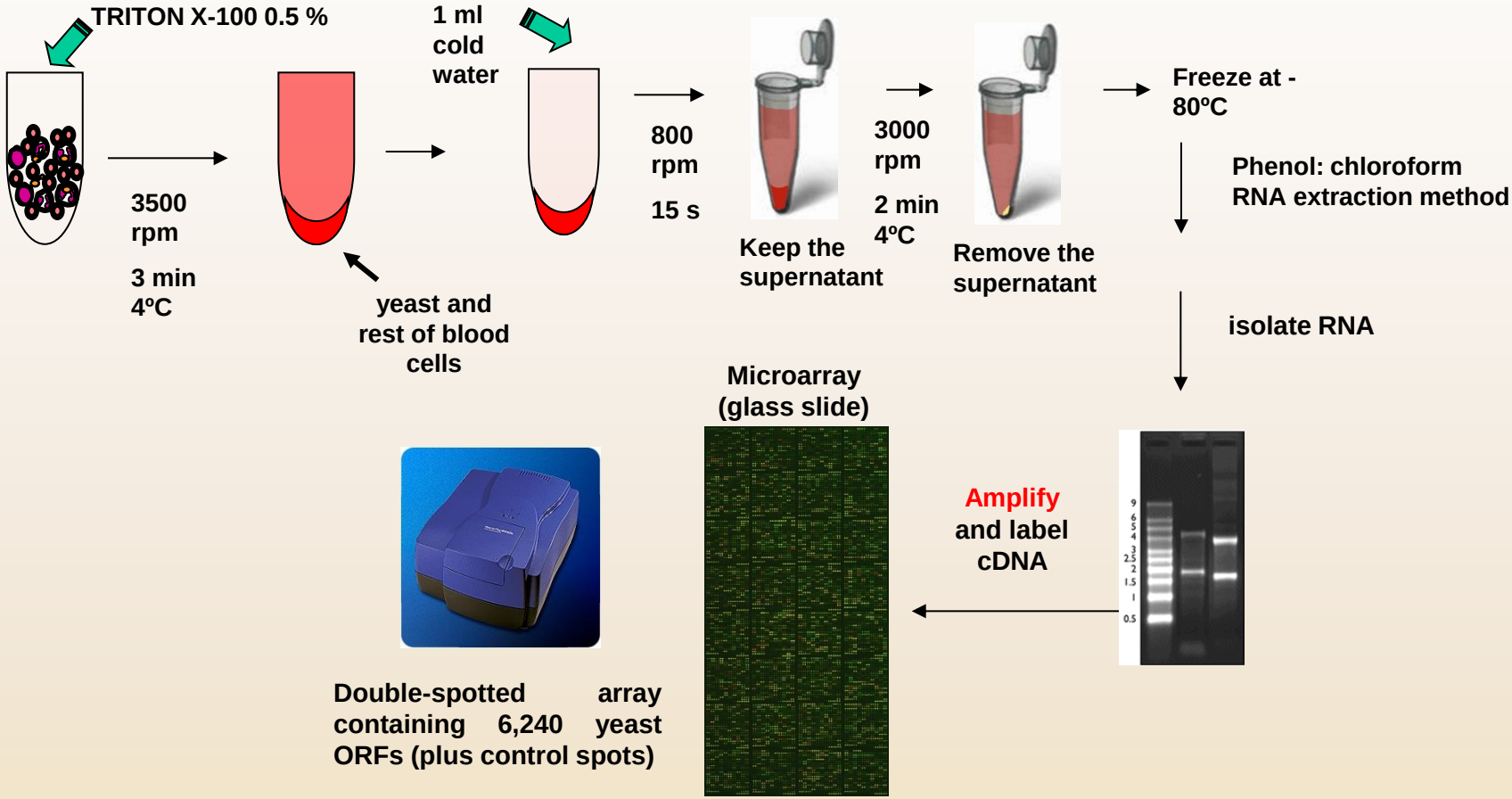
We take human peripheral venous blood from healthy volunteers

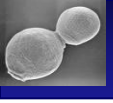
Preincubation yeasts (1x10^7 cells/ml) in PBS at 37ºC at 120 rpm.

***S. cerevisiae* strains virulent (60, D14) and non–virulent (W303, CECT 10431)**

Incubation yeasts with blood with a ratio of 2:1 (yeast:leukocyte)

Sample at 0, 15, 30 and 60 minutes





Survival in blood

Yeasts survival in whole blood and fractions

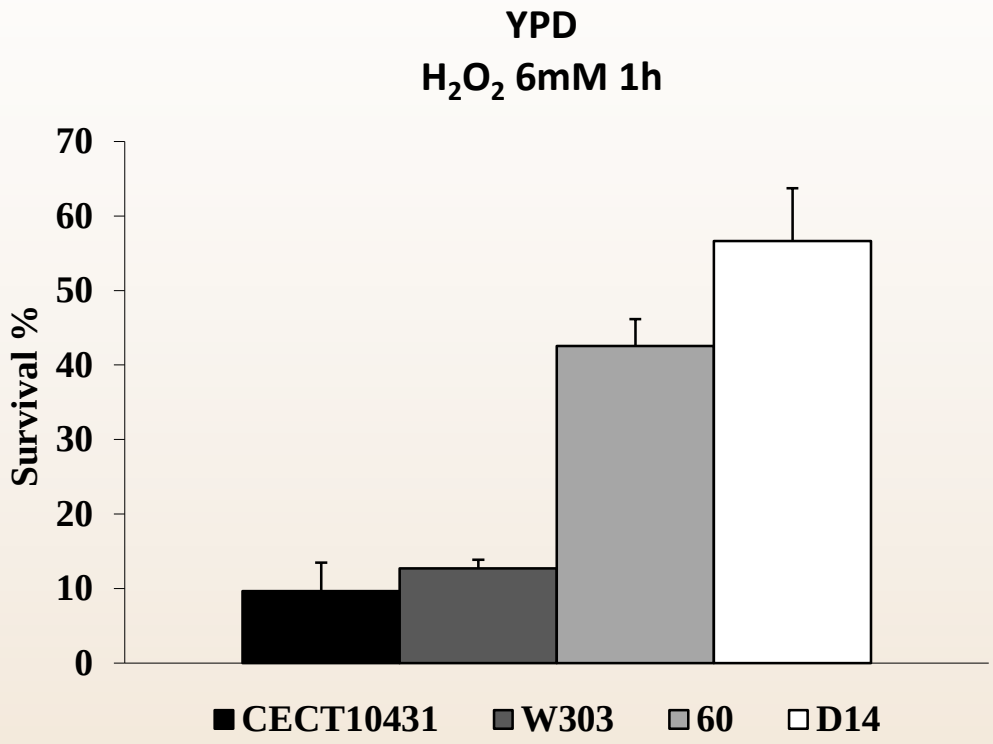
	Species	*C. albicans*	*S. cerevisiae*	*S. cerevisiae*	*S. boulardii*	*S. cerevisiae*	*S. cerevisiae*
	Strain	SC5314	60	D14	Ultralevure	W303	10431
10 min							
	Blood	87.4 ± 8.7	98.8 ± 8.7*	76.5 ± 13.8	76.0 ± 13.8	66.0 ± 11.2	-
	PMNs	81.0 ± 5.6#	84.0 ± 6.3#	37.3 ± 7.3	35.3 ± 6.3	34.8 ± 6.3	-
	MNCs	96.4 ± 5.8#	83.9 ± 4.9	95.5 ± 6.5#	98.0 ± 6.5#	72.7 ± 7.5	-
60min							
	Blood	56.4 ± 3.7*	56.4 ± 3.7*	60.5 ± 5.8*	60.0 ± 5.8*	35.7 ± 4.8	50,8 ± 9.8
	PMNs	52.7 ± 3.8#	48.3 ± 5.0*	17.5 ± 5.0	14.0 ± 5.0	22.1 ± 3.8	-
	MNCs	85.2 ± 3.9**	67.3 ± 3.6*	63.3 ± 5.1#	57.3 ± 4.4	49.0 ± 4.4	-

Strains individually compared to W303 strain presented the following p-values: # p < 0.05, * p < 0.005 and **p < 0.0001.

Functional groups comparing virulent with avirulent

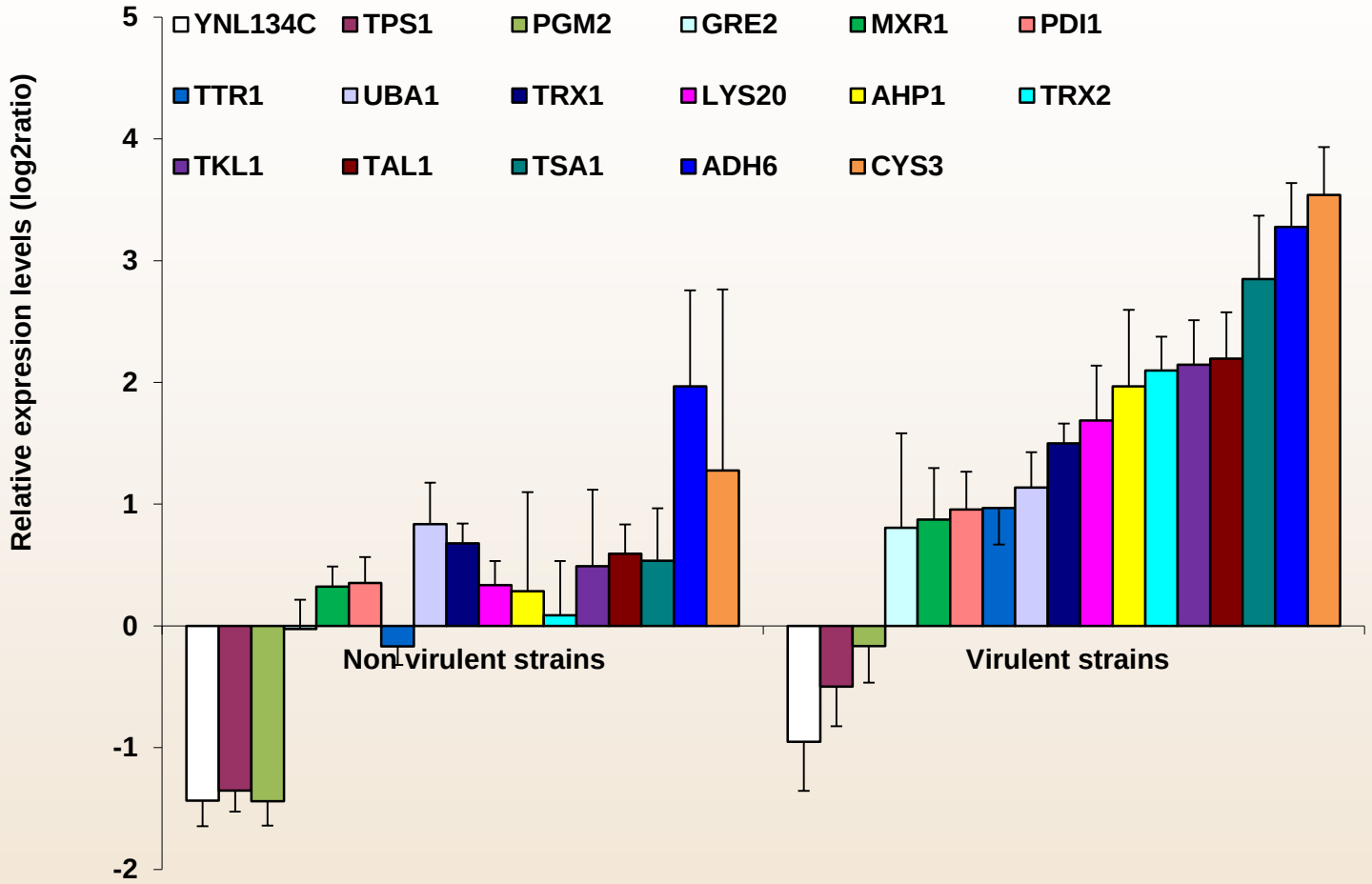
Functional groups	Time (min)			Genes
	15	30	60	
Base-excision repair	+	-	-	POL30, POL31, RAD27, APN1, OGG1
Vacuole organization	+	+	-	CMD1, VPS3, CUP5, VTC1, SFK1, VID24/ MD1/ VPS45/ TRX2/ TRX1/ VAC7/ TPM1/ VTC3/
Xenobiotic transporter	+	-	-	PDR5, SNQ2, PDR12
Amino acid biosynthetic process/ Amine biosynthetic process/ Nitrogen compound biosynthetic process	-	+	+	ARO4/ ARO3/ LYS4/ HIS1/ ARG5,6/ SER3/ILV1/ SER33/ HIS5/ LYS1/ TRP3/ ARG1/ ARG8/ LEU9/ ORT1/ SAM4
Amino acid metabolic process	-	+	+	ADH5/ ARO4/ ARO3/ LYS4/ HIS1/ ARG5,6/ SER3/ ILV1/ADH4/ SER33/ HIS5/ LYS1/ URA2/ TRP3/ MSE1/ ARG1/ ARG8/ LEU9/ ORT1/ SAM4/
Phosphatase activity	-	+	+	PTC3/ PHO3/ PHO5/ DPP1/ LPP1/ SDT1/ PHO12/ INP51/ SAP185/ SAC1/ PPZ1/ TSL1/ YMR087WTPS3/ FCP1/ YNL217W/ PHO12
Transmembrane transporter activity	-	+	-	CTP1/ GGC1/ ATP17/ GNP1/ PIC2/ HXT10/ VPS73/ ZRT1/ TPO2/ TNA1/ AVT1/ VMA5/ ZRT3/ YBT1/ AQY2/ SMF3/ ZRT2/ ATR1/ ITR2/ ORT1/ ODC2/ PDR12/ YMC1/ ANT1/ TPO3/
Cell redox homeostasis	-	-	+	TTR1/ TRX2/ TRX1/ TSA1/ GLR1/ YNL134C/ TPS1/ PGM2/ GRE2/ MXR1/ PDI1/ UBA1/ LYS20/ AHP1/ TKL1/TAL1/ ADH6/ CYS3

Resistance to oxidative stress

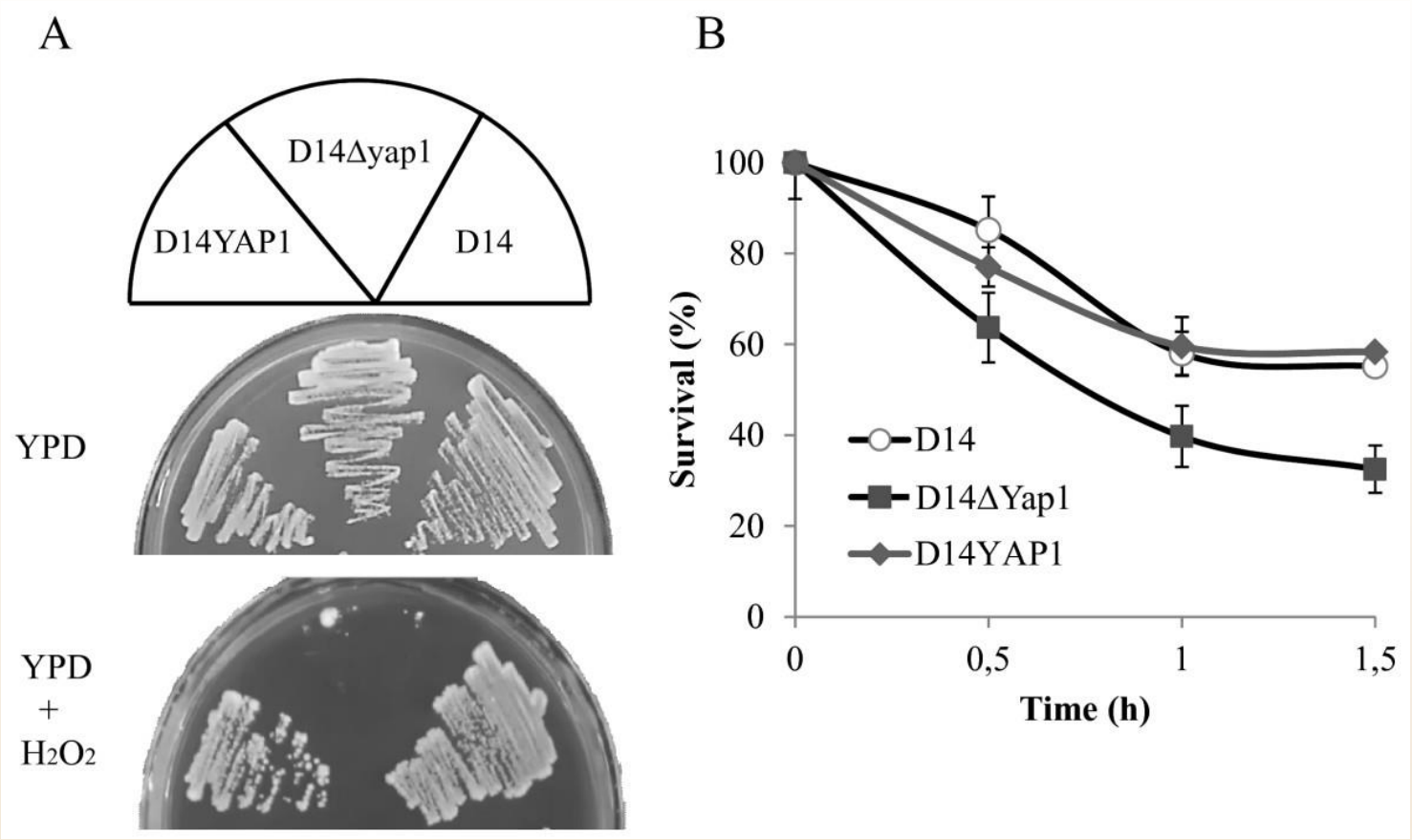


Expression of oxidative stress genes is increased in virulent strains

Yap1p regulated genes



Resistance to oxidative stress: survival in blood

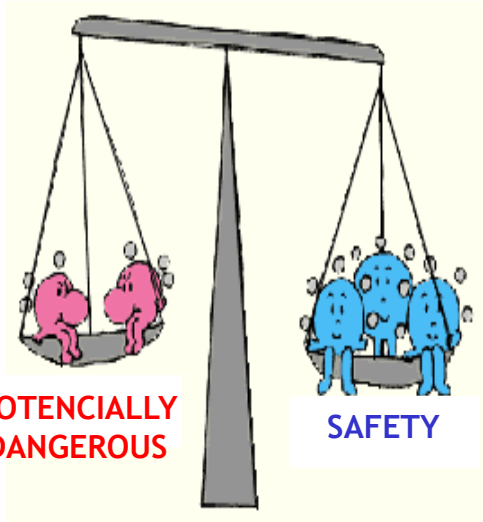


Conclusions

S. *cerevisisae* could be considered an opportunistic pathogen with low virulence, but the agrochemical industry and hospital would be wise to exercise caution.

- To warn consumers and the medical profession about the dangers of ingesting enriched food with yeast (brewer's yeast tablets) as part of healthy diets mainly in people with weakened health.

- The ability to distinguish pathogenic from non-pathogenic strains would be of great benefit to Agrochemical industry, using as selection criteria: the ability to grow at 42°C, pseudohyphal growth and high levels of phospholipase activity.



- Caution needs to be exercised by people that work in the industrial and biotechnological sector, from bakery industry and from associating with individuals who work in close contact with yeast . Also to hospital staff that handle the capsules and packets of *S. boulardii*.

- Caution in handling of catheters, may be a nosocomial infection.

- Consider an alternative treatment to *S. boulardii* to treat patients with diarrhea, mainly if they are inmunodepressed.

The* transcriptional analysis indicate that the amino acids synthesis pathway and oxidative stress resistance are two molecular mechanisms involved in the virulence in *S. cerevisisae

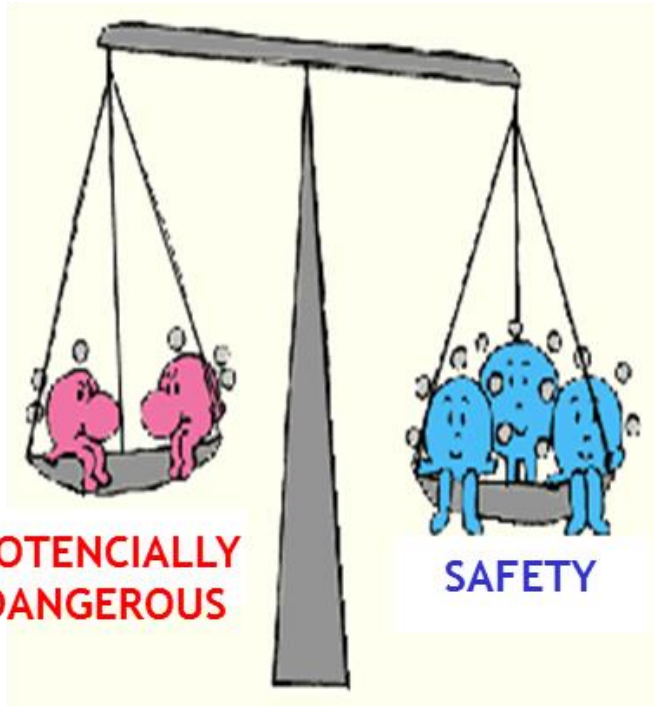
Yeast other than *Candida* and *Cryptococcus* species causing opportunistic infections in humans (obtained from (Fleet and Roostita, 2006).)

Species	References
Saccharomyces cerevisiae	Eschete *et al*. (1980); Aucott *et al*. (1990); Bassetti *et al*. (1998); McCullough *et al*. (1998a); Murphy and Kavanagh (1999); Wheeler *et al*. (2003); Llanos *et al*. (2004)
Saccharomyces cerevisiae var *boulardii*	McCullough *et al*. (1998b); Piarroux *et al*. (1999); Lherm *et al*. (2002); Cassone *et al*. (2003)
Rhodotorula spp	Papadogeorgakis *et al*. (1999); Petrocheilou-Paschou *et al*. (2001); Braun and Kaufmann (1999); Diekema *et al*. (2005)
Pichia anomala	Murphy *et al*. (1986); Haron *et al*. (1988); Klein *et al*. (1988); Yamada *et al*. (1995); Garcia-Martos *et al*. (1996); Cermeno-Vivas *et al*. (1999); Georgiev (2003)
Pichia farinosa	Garcia-Martos *et al*. (1996)
Pichia membranifaciens	Garcia-Martos *et al*. (1996)
Issatchenkia orientalis	Merz *et al*. (1986); Goldman *et al*. (1993); Abbas *et al*. (2000); Georgiev (2003)
Kluyveromyces marxianus	Lutwick *et al*. (1980); Nielsen *et al*. (1990); Garcia-Martos *et al*. (1996)
Hanseniaspora uvarum	Garcia-Martos *et al*. (1999)
Yarrowia lipolytica	Shin *et al*. (2000); Georgiev (2003)

Host factors contributing to human infections with opportunistic yeast pathogens (from: Hazen, 1995; Annaissie *et al*., 1989; Hobson, 2003)

• Weak health; hospitalisation
• Cancer, AIDS
• Immunodeficiency; treatment with immunosuppressive drugs; chemotherapy
• Treatment with broad spectrum antibacterial agents
• Intravenous or central venous catheters
• Recent surgery (especially gastrointestinal tract)
• Total parenteral nutrition

¿Las levaduras que se utilizan en la elaboración de alimentos son seguras?



Introduction

EFSA is requested to assess the safety of a broad range of microorganisms in the context of notifications for market authorisation as sources of food and feed additives, enzymes and plant protection products.

http://www.efsa.europa.eu/

QPS scope

- The QPS assessment is intended for **EFSA's own use**
- To provide a generic safety assessment approach applicable across EFSA's scientific Panels
- Unambiguously defined taxonomic units are assessed for potential safety concerns
- Sufficient body of knowledge covering field of application for which an authorisation is sought

QPS recommended list

List of notifications for QPS assessment

Taxonomic level & body of knowledge

Safety concerns

List of QPS recommended microorganisms and viruses

QPS recommended list

List of notifications for QPS assessment

Taxonomic level & body of knowledge

Safety concerns

List of QPS recommended microorganisms and viruses

Yeasts			
Species			**Qualifications ******
Debaryomyces hansenii			
Hanseniaspora uvarum			
Kluyveromyces lactis	*Kluyveromyces marxianus*		
Pichia angusta		*Pichia jadinii*	QPS applies only when the species is used for enzyme production
Komagataella pastoris			
Saccharomyces bayanus	*Saccharomyces cerevisiae*†	*Saccharomyces pastorianus*	
Schizosaccharomyces pombe			
Wickerhamomyces			QPS applies only when

******Absence of resistance to antimycotics used for medical treatment of yeast infections. In the case of *Saccharomyces cerevisiae* this qualification applies for yeast strains able to grow above 37 ° C.**

Amparo Querol

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