PECULIARITA' DEI LIEVITI FLOR

Marilena Budroni, Ilaria Mannazzu, Severino Zara, Giacomo Zara, Giovanni Antonio Farris
DISAABA
Università di Sassari

"La Vernaccia di Oristano"
Oristano, 15 maggio 2009

1. Chi sono e cosa fanno i lieviti flor
2. Gli stress
3. Le peculiarità genetiche e metaboliche
4. Il comportamento sociale
5. Applicazioni

•*Specie*

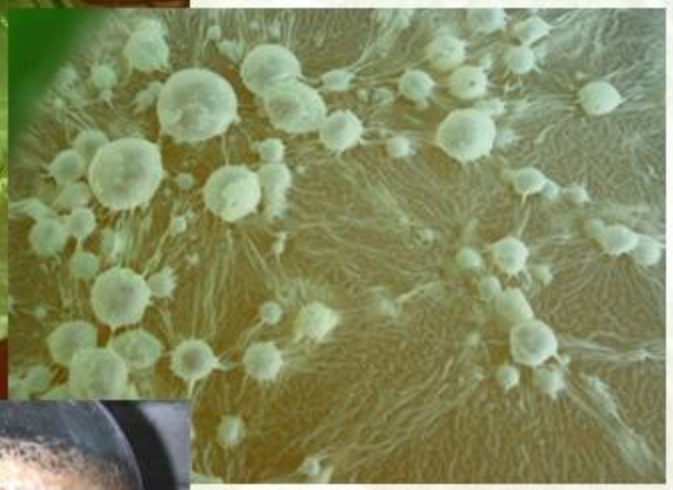
Saccharomyces cerevisiae

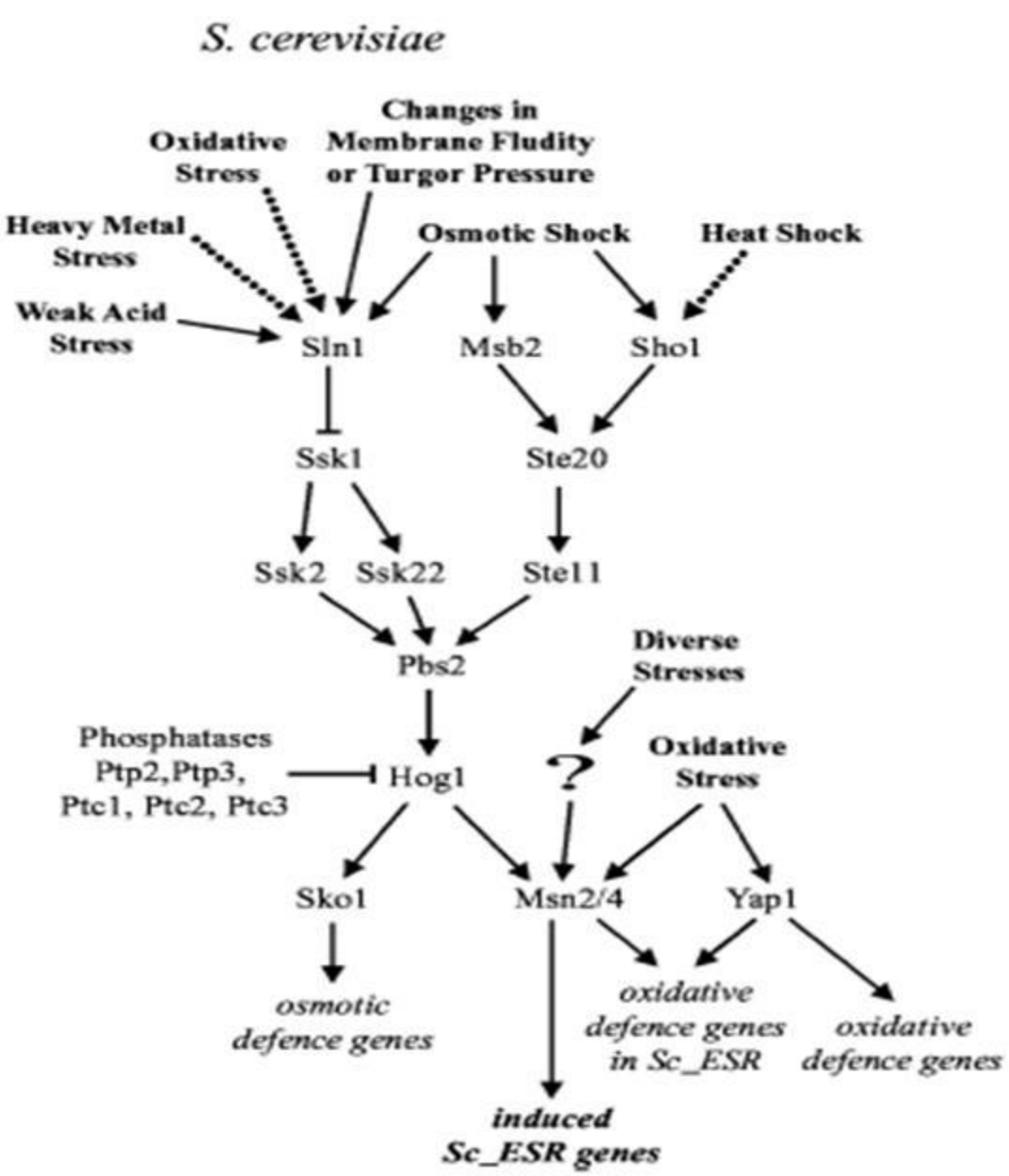
• *Modalità di azione*

Verso la fine della fermentazione alcolica risalgono sulla superficie del vino formando un biofilm o flor

• *Peculiarità*

Sono fondamentali per l'affinamento di alcuni vini speciali come gli Sherry spagnoli ed i vini sardi Malvasia di Bosa e Vernaccia di Oristano




S. cerevisiae
Changes in
Membrane Fludity
or Turgor Pressure
Oxidative
Stress
Heavy Metal
Stress
Osmotic Shock
Heat Shock
Weak Acid
Stress
Sln1
Msb2
Sho1
Ssk1
Ste20
Ssk2
Ssk22
Ste11
Pbs2
Diverse
Stresses
Phosphatases
Ptp2,Ptp3,
Ptc1, Ptc2, Ptc3
Hog1
?
Oxidative
Stress
Sko1
Msn2/4
Yap1
osmotic
defence genes
oxidative
defence genes
in Sc_ESR
oxidative
defence genes
induced
Sc_ESR genes

PECULIARITA' GENETICHE

ANALISI dei cariotipi e del profilo di restrizione dei FLOR

- 52 ceppi isolati da Vernaccia, Malvasia ed Arvisionadu
- 16 diversi cariotipi

Vernaccia

1 cariotipo più frequente

(analisi Dna cromosomale e mitocondriale)

Budroni, M., Nobile, C., Roggio, T., Pinna, G., Bardi, L., Farris, G.A. 1996.
J. Wine Res. 7: 201-205
Pinna G., Budroni M., Giordano G., Usai S., Farris G.A. 2001.
Annals of Microbiology, 50, 177-182

Journal of Applied Microbiology 2000, 89, 657–662

A genetic study of natural *flor* strains of *Saccharomyces cerevisiae* isolated during biological ageing from Sardinian wines

M. Budroni, G. Giordano, G. Pinna and G.A. Farris

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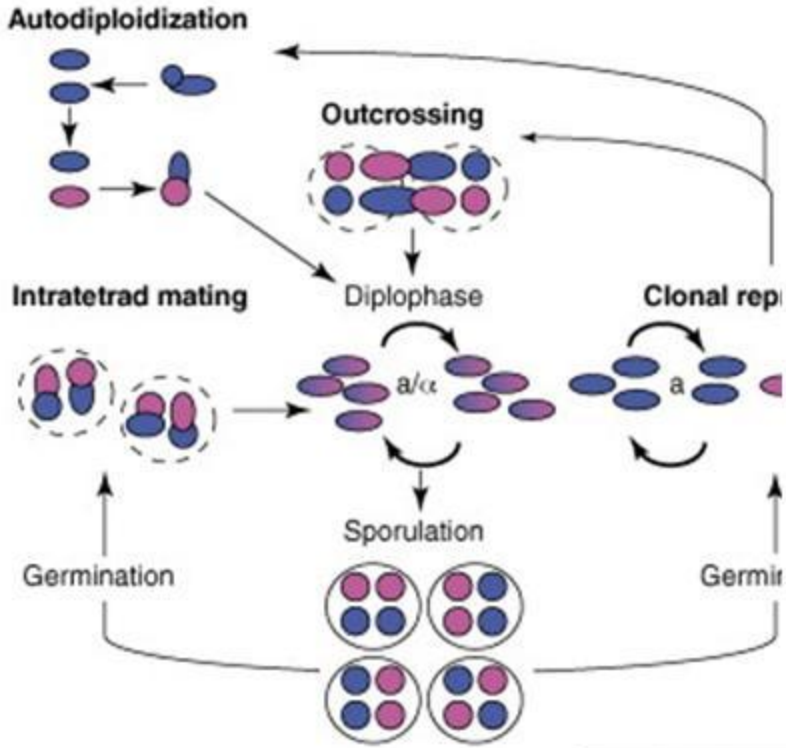
173/2/2000: received 11 February 2000, revised 30 May 2000 and accepted 7 June 2000

M. BUDRONI, G. GIORDANO, G. PINNA AND G.A. FARRIS. 2000. In this study, three *flor* strains of *Saccharomyces cerevisiae* were genetically characterized. They were isolated from biofilms on Sardinian sherry-like wines produced at family-run wineries where pure cultures of yeasts were not used. The study aimed to investigate the life cycle of these naturally-occurring *flor* strains, using a genetic procedure supplemented by analysis of subsequent meiotic generations. A semi-homothallic life cycle was found in three strains that could be helpful in a genetic improvement programme.



TRENDS in Ecology & Evolution

Antonie van Leeuwenhoek 85: 29–36, 2004.

Diversity of Y region at *HML* locus in a *Saccharomyces cerevisiae* strain isolated from a Sardinian wine

Giorgia Pirino, Severino Zara, Giovanni Pinna, Giovanni Antonio Farris and Marilena Budroni.*

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Received 15 October 2002; accepted in revised form 24 April 2003

Key words: Flor strain, Mating type switching, *Saccharomyces cerevisiae*, Semi-homothallic life cycle

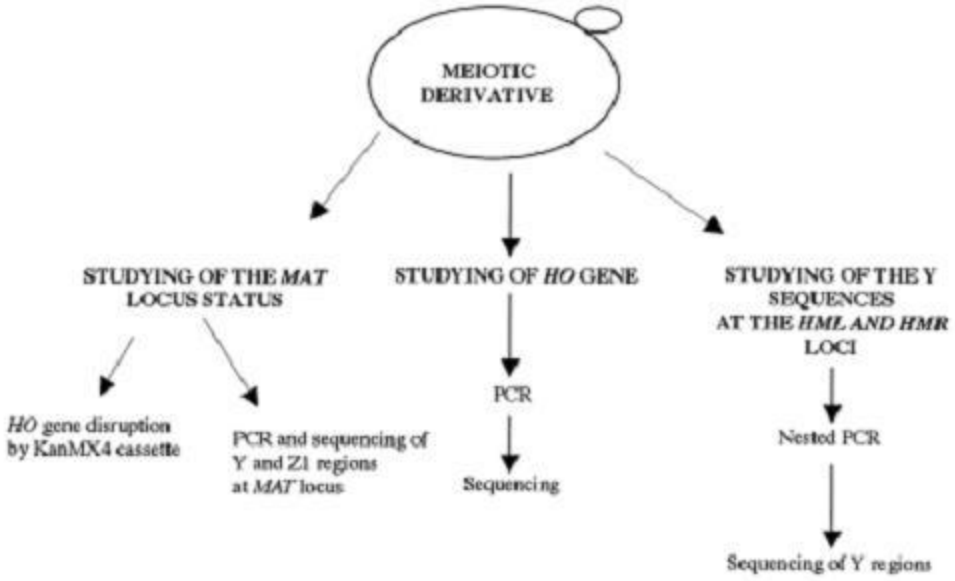

Figure 1. Scheme of strategy.

Exploitation of the semi-homothallic life cycle of *Saccharomyces cerevisiae* for the development of breeding strategies

Giacomo Zara, Ilaria Mannazzu, Maria Lina Sanna, Davide Orro, Giovanni Antonio Farris & Marilena Budroni

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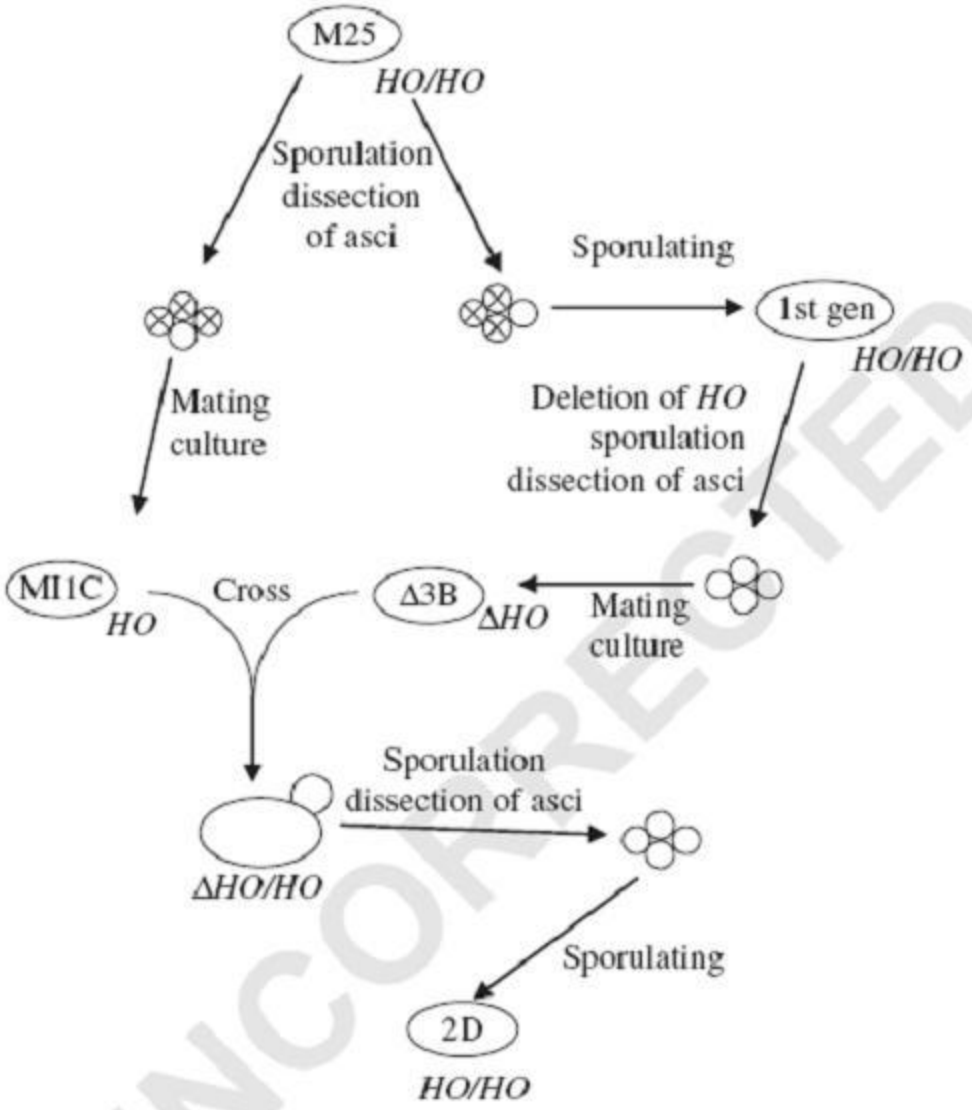
DOI:10.1111/j.1567-1364.2008.00393.x

Editor: Patrizia Romano

Keywords
Saccharomyces cerevisiae; flor; biofilm; semi-homothallic life cycle; genetic stability; wine strains.



PECULIARITA' METABOLICHE

ELSEVIER

FEMS Yeast Research 5 (2005) 951–958

FEMS
Yeast Research
www.fems-microbiology.org

Peculiarities of *flor* strains adapted to Sardinian sherry-like wine ageing conditions

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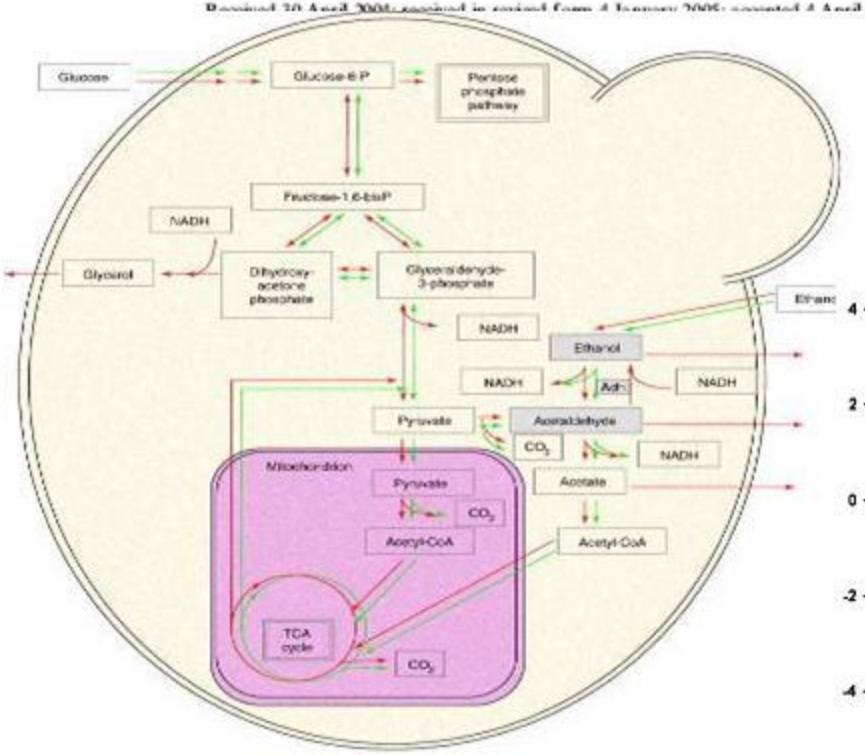
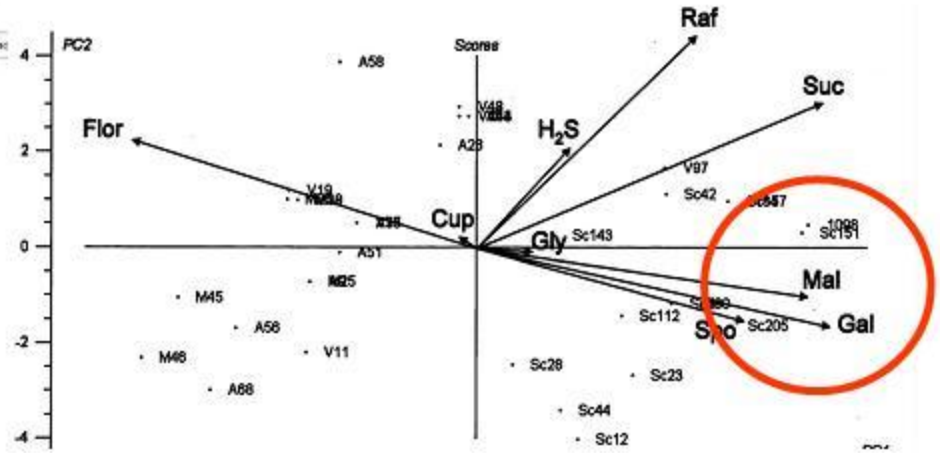
Received 30 April 2004; received in revised form 4 January 2005; accepted 4 April 2005





IN FERMENTAZIONE

Journal of Applied Microbiology ISSN 1364-5072

ORIGINAL ARTICLE

Correlation between cell lipid content, gene expression and fermentative behaviour of two *Saccharomyces cerevisiae* wine strains

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Keywords
fermentative behaviour, gene ex
lipids, quantitative RT-PCR, *Sacch*
cerevisiae

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2007

doi:10.1111/j.1365-2672.2007.0

RESEARCH ARTICLE

Oxygen is required to restore flor strain viability and lipid biosynthesis under fermentative conditions

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Editor: Guenther Daum

Keywords
Saccharomyces cerevisiae; flor yeast; lipid biosynthesis; fermentation; real-time PCR.

Abstract

To further elucidate the biosynthesis of lipids in flor strains under fermentative conditions, the transcription levels of the lipid biosynthetic genes *ACS1*, *ACS2*, *ACC1*, *OLE1*, *ERG1*, *ERG11*, *ARE1* and *ARE2*, as well as the lipid composition and cell viability of a flor strain were compared with that of a non-flor strain during hypoxic and aerobic fermentations in the absence of lipid nutrients. While no significant differences in transcription levels or lipid compositions were observed between the two strains when oxygen was not limiting, significant differences were seen during hypoxic fermentation. In this last condition, the flor strain, in spite of higher levels of transcription of hypoxic genes, lost the abilities to desaturate fatty acids and complete ergosterol biosynthesis, and showed a dramatic loss of viability. In contrast, the non-flor strain, which showed lower transcription levels, was able to reach a balanced lipid composition and maintained a higher cell viability. One possible explanation is that the flor strain requires a higher amount of oxygen than the non-flor strain in order to carry out the oxygen-dependent steps of lipid biosynthesis under fermentative conditions.

EFFETTO DELL'OSSIGENO

L'aggiunta di ossigeno favorisce lo sviluppo dei lieviti

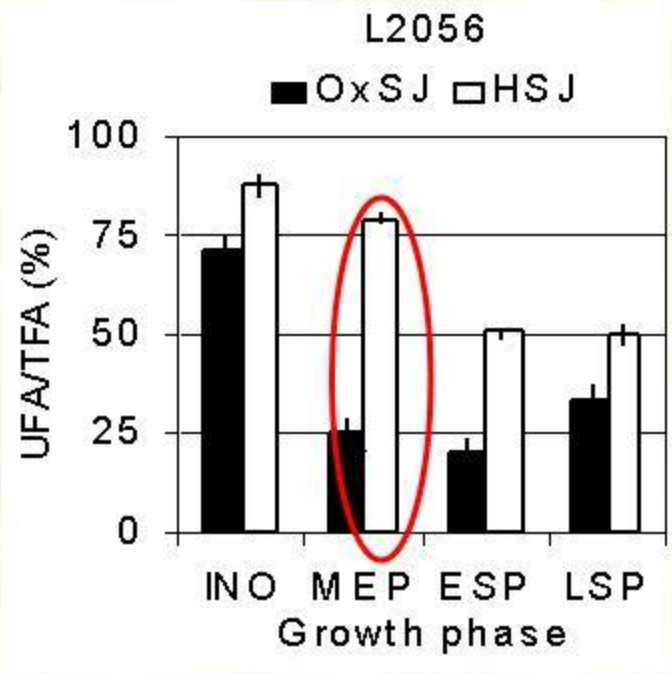
Table 2. Number of generations produced by the two strains during growth under aeration and progressive oxygen depletion.

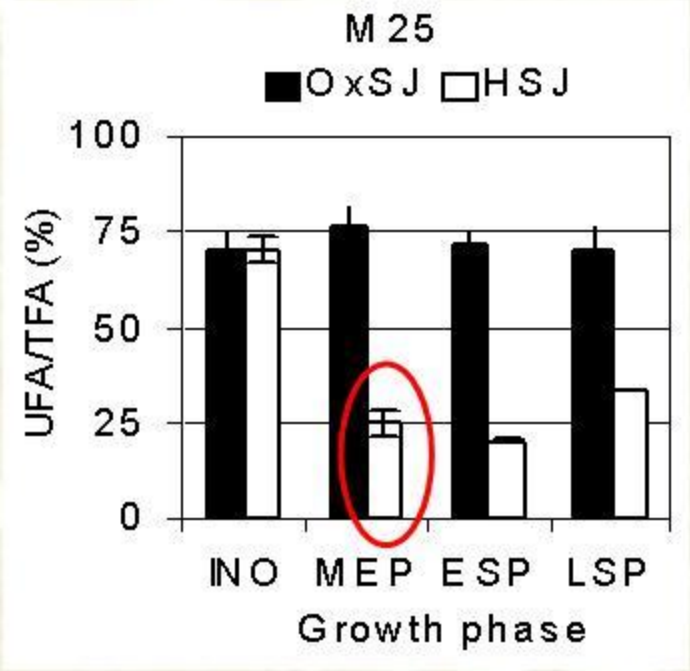
Strain	Number of generations	
	OxSJ	HSJ
M25	7.2 ± 0.0^{a}	5.1 ± 0.2^{b}
L2056	7.1 ± 0.1^{a}	4.9 ± 0.1^{b}

Zara et al., 2009

EFFETTO DELL'OSSIGENO

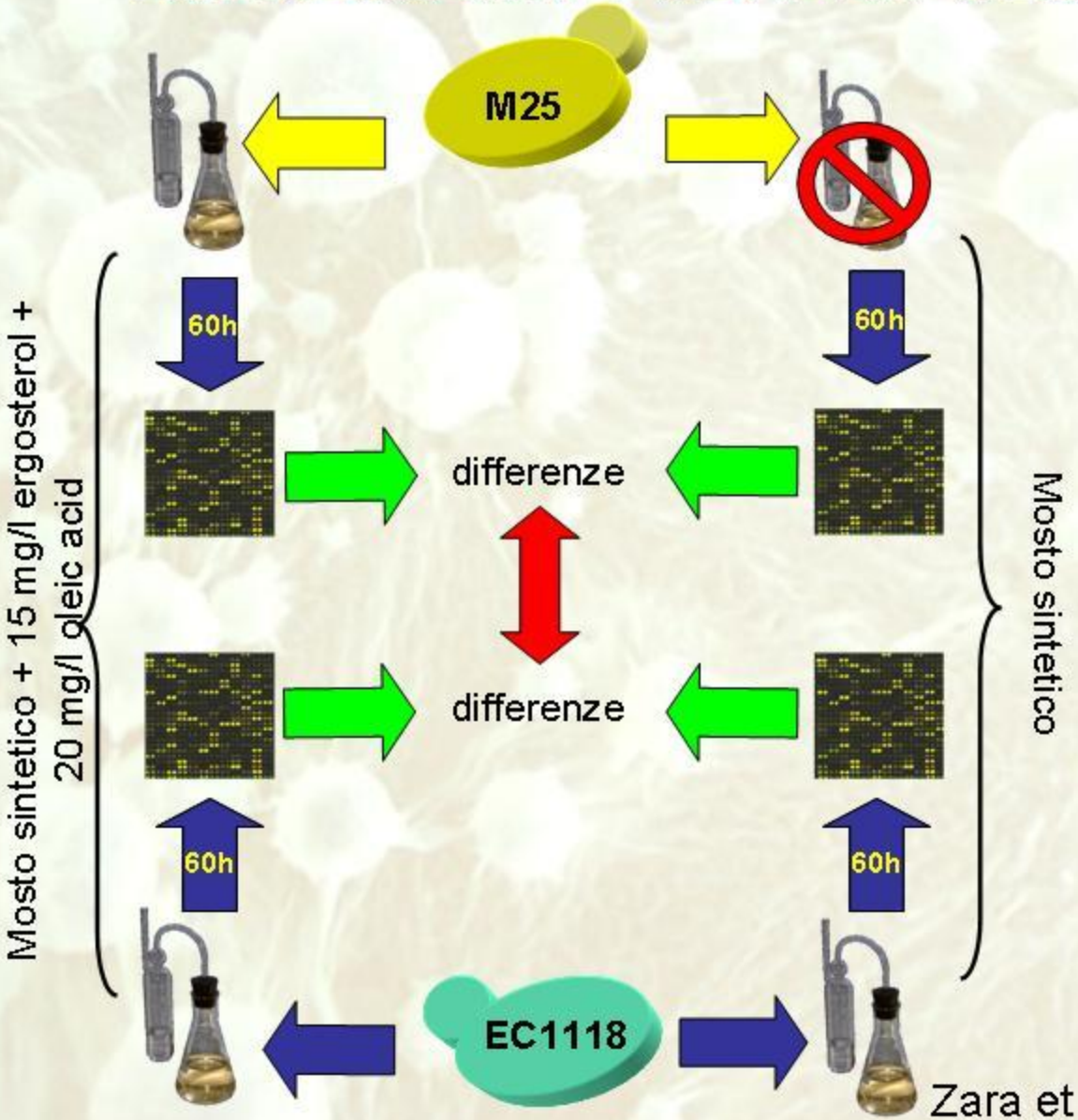
Tuttavia M25, in anaerobiosi non riesce a modificare il rapporto fra UFA e SFA.





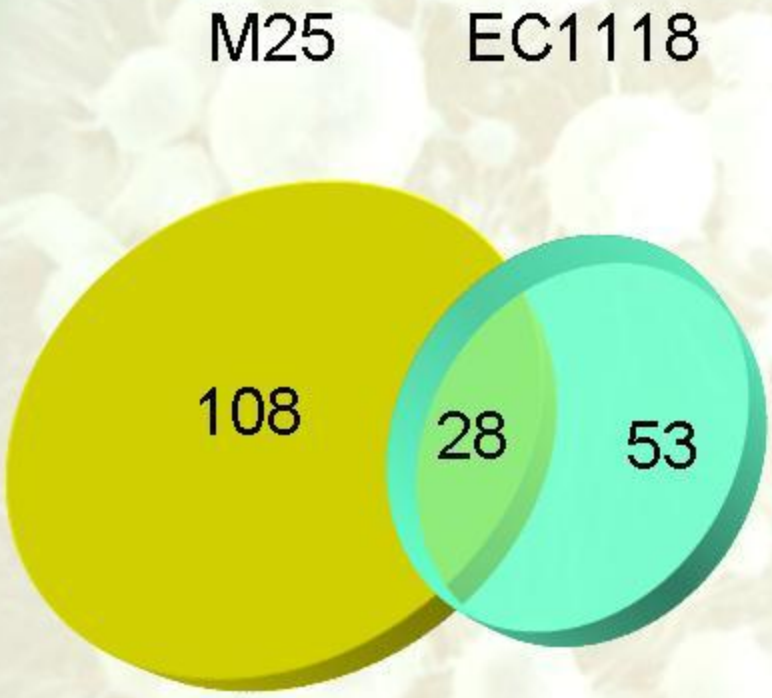
Zara et al., 2009

ESPRESSIONE GENICA GLOBALE 1

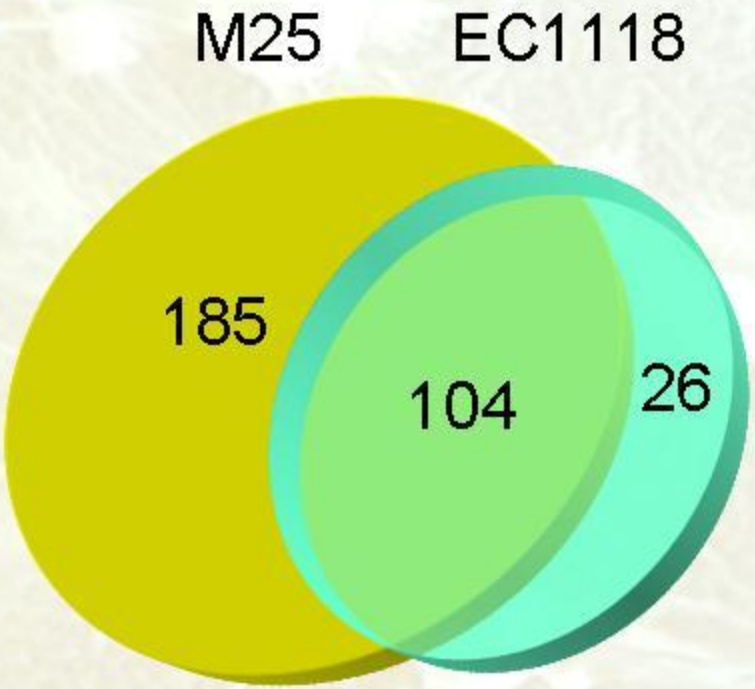


Zara et al., (in preparation)

ESPRESSIONE GENICA GLOBALE 1



Geni sottoespressi in mosto sintetico



Geni sovraespressi in mosto sintetico

Zara et al., (in preparation)

PATHWAY COMUNI AI DUE CEPPI

- **Induzione dei geni HSP**
- **Sintesi di ergosterolo**
- **Fosforilazione ossidativa**

Zara et al., (in preparation)

DIFFERENZE DI ESPRESSIONE IN EC1118 E M25

- **Metabolismo del glucosio**
- **Produzione di trealosio**
- **Sintesi di mannoproteine**

Zara et al., 2008 (in preparation)


IL COMPORTAMENTO
SOCIALE:
IL BIOFILM

Che cosa è il comportamento sociale

Comportamento sociale dei lieviti

- Capacità di aggregrarsi a formare strutture cell più o meno stabili
- Conseguenza della plasticità adattiva dei lieviti alle variazioni ambientali di tipo nutrizionale (carenza di N, G), che modula l'espressione genica
- Che si esprime fenotipicamente con la rimodellazione della composizione della parete cellulare e del suo contenuto in adesine o flocculine.

Saccharomyces cerevisiae

può aggregarsi:

• In terreno liquido: vino, t. sintetico, acque reflue

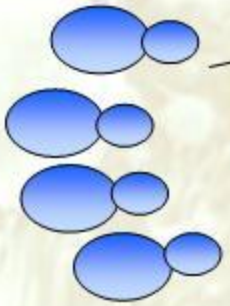
• In t. solido: soft-agar, superfici plastiche, vetro

• Stimoli: nutrizionali (carenza di nutrienti, presenza di alcooli).

• La *scelta* del tipo di aggregazione dipende da fattori genetici, epigenetici, ploidia, subtrato, *quorum sensing*

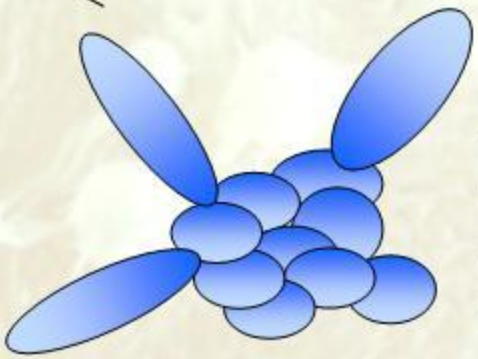
Le modalità di aggregazione cellulare

colonie

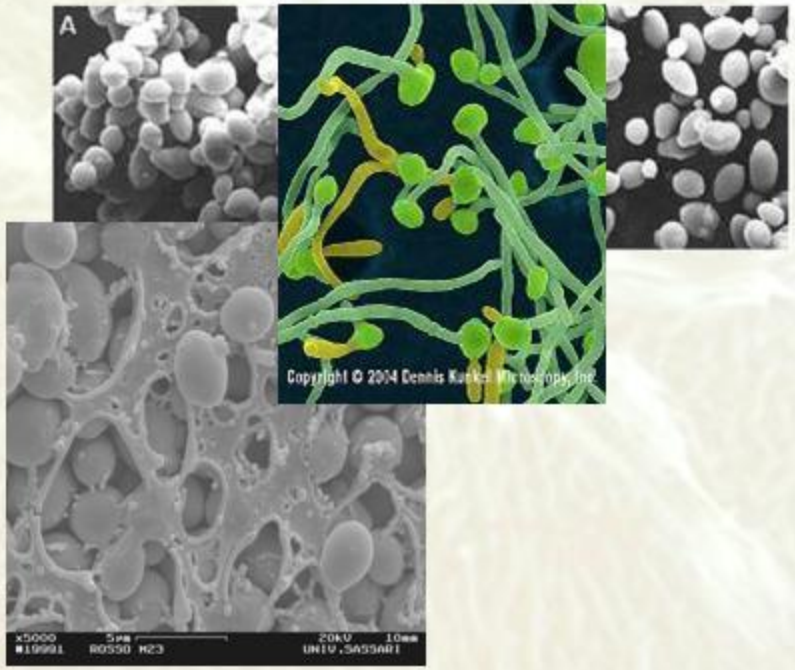


Mancanza N
generazione segnale

Accesso ai nutrienti
Repressione sintesi segnale e risposta al segnale



Triptofolo,
feniletanolo



APPLIED AND ENVIRONMENTAL MICROBIOLOGY, June 2005, p. 2934–2939 Vol. 71, No. 6
0099-2240/05/$08.00+0 doi:10.1128/AEM.71.6.2934–2939.2005

FLO11-Based Model for Air-Liquid Interfacial Biofilm Formation by *Saccharomyces cerevisiae*

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Maria Antonietta Demontis,[1] and Marilena Budroni[1]*

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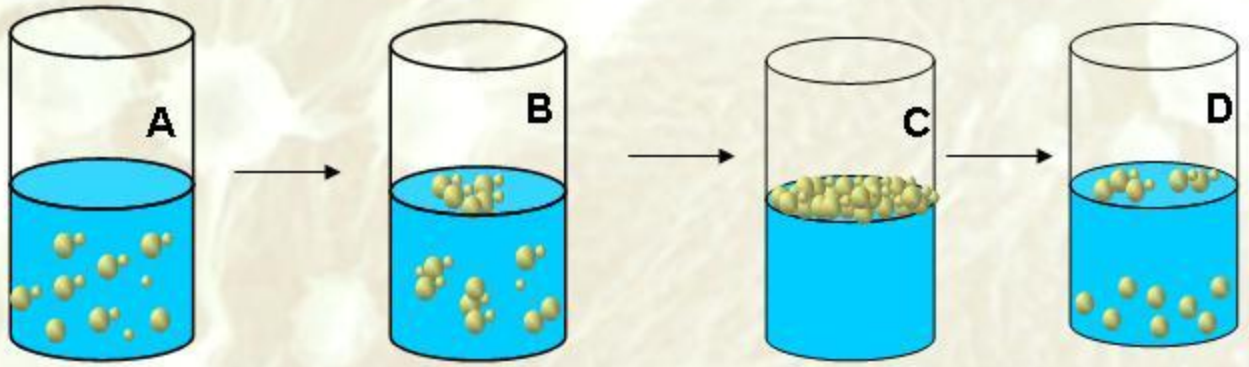
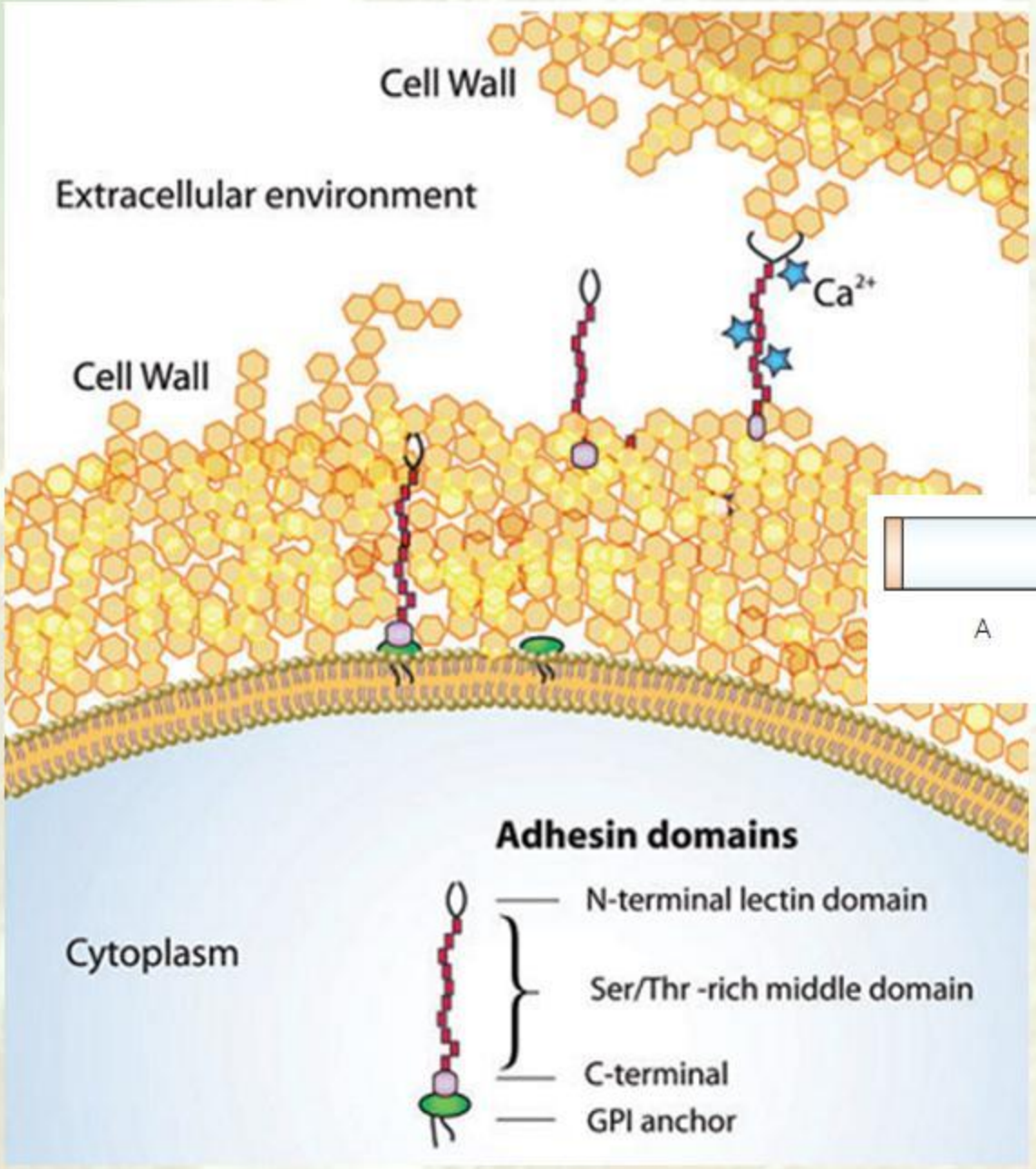
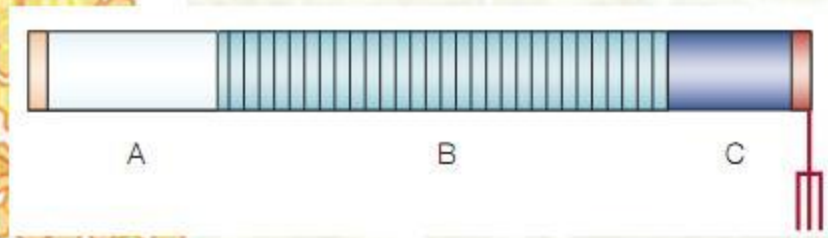
Sardinian wine strains of *Saccharomyces cerevisiae* used to make sherry-like wines form a biofilm at the air-liquid interface at the end of ethanolic fermentation, when grape sugar is depleted and further growth becomes dependent on access to oxygen. Here, we show that *FLO11*, which encodes a hydrophobic cell wall glycoprotein, is required for the air-liquid interfacial biofilm and that biofilm cells have a buoyant density greater than the suspending medium. We propose a model for biofilm formation based on an increase in cell surface hydrophobicity occurring at the diauxic shift. This increase leads to formation of multicellular aggregates that effectively entrap carbon dioxide, providing buoyancy. A visible biofilm appears when a sufficient number of hydrophobic cell aggregates are carried to and grow on the liquid surface.







Verstrepen et al., 2006

FLO11

Flo11 è una flocculina che appartiene alla famiglia delle adesine, proteine della parete cellulare coinvolte nei fenomeni di adesione cellula-cellula e cellula-substrato

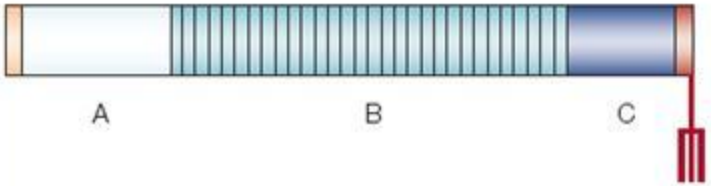
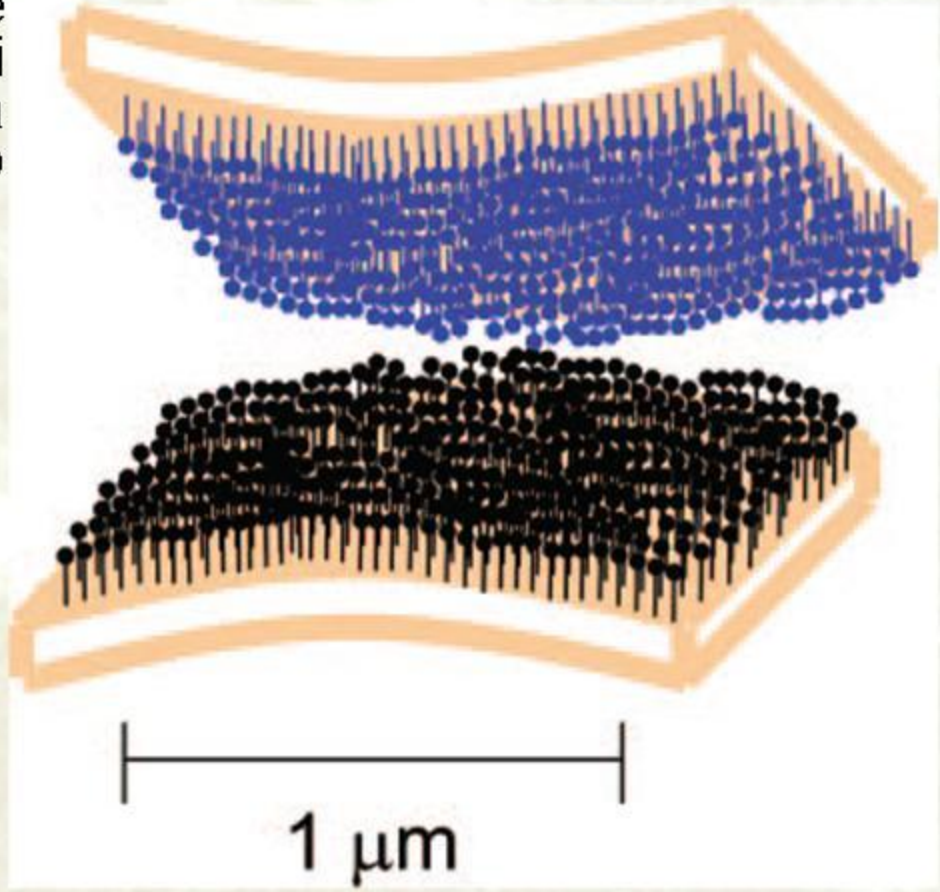


Figure 2 | **Domain structure of adhesins.** Adhesins comprise three domains — A, B and C — which are preceded by an amino-terminal signal sequence. The N-terminal domain (A) is thought to confer adhesion. The central domain (B) contains a serine/threonine-rich region that is encoded by many repeated nucleotide sequences. The carboxy-terminal domain (C) contains a site for the covalent attachment of a glycosyl phosphatidylinositol anchor (shown in red).

(Verstrepen et al., 2006);



(Dranginis *et al.*, 2007)

A: logarithmic phase; B: stationary phase; C: flor medium

CWPs		M23			3238-32			ΔFLO11			EC1118	
		A	B	C	A	B	C	A	B	C	B	C
GPI	Cwp1	×	×	×	×	×	×	×	×	×	×	×
GPI	Ssr1	×		×	×	×	×	×	×	×	×	×
GPI	Crh1	×	×	×	×	×	×			×		×
GPI	Crh2							×		×		
GPI	Gas1	×	×	×	×	×	×	×		×		
GPI	Gas3	×		×	×	×	×	×	×	×		×
GPI	Gas5		×	×						×		×
ASL	Pir1	×	×	×	×	×	×			×	×	×
ASL	Pir2	×	×	×	×		×	×	×	×	×	×
ASL	Pir3		×	×	×	×	×		×	×	×	×
ASL	Pir4	×	×	×						×	×	×
ASL	Scw4	×	×	×	×	×	×	×		×	×	×
ASL	Scw10			×	×	×	×		×	×		
GPI	Ecm33	×	×	×	×	×	×	×	×	×	×	×
GPI	Yps1		×									
GPI	**Flo11**		×		×		×					
GPI	Tip1	×	×	×	×		×	×		×		
GPI	Hpf1'									×		
GPI	Tip1					×						
GPI	Sag1				×	×	×		×	×		

(Fancellu, F., in preparazione)

Microarray

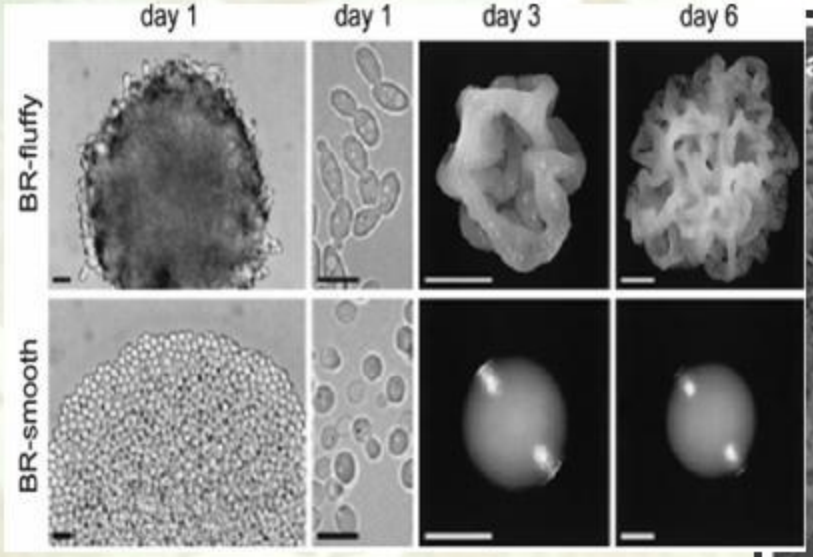
Cellule del biofilm (flor) VS cellule del fondo

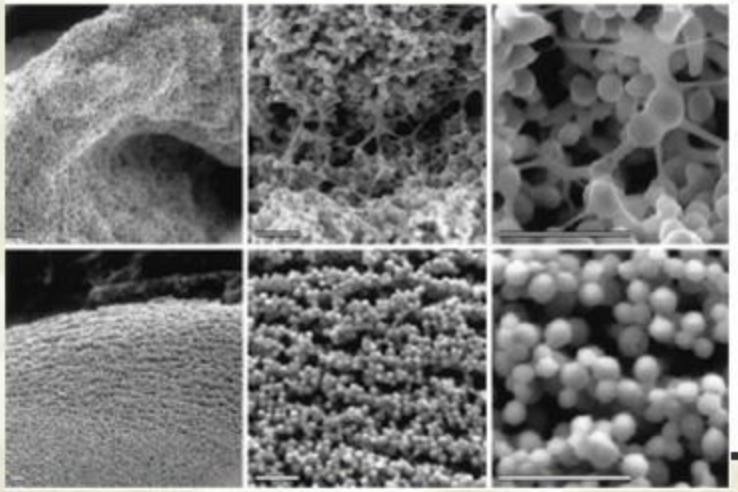
- ribosome biogenesis
- nuclear organization and biogenesis
- amino acid and derivative metabolic process
- translation
- cytokinesis
- heterocycle metabolic process
- cell budding
- DNA and RNA metabolic processes
- anatomical structure morphogenesis
- protein folding

I rapporti delle frequenze delle categorie GO per processi associati con la crescita suggeriscono che le cellule del biofilm crescono più velocemente rispetto a quelle del fondo

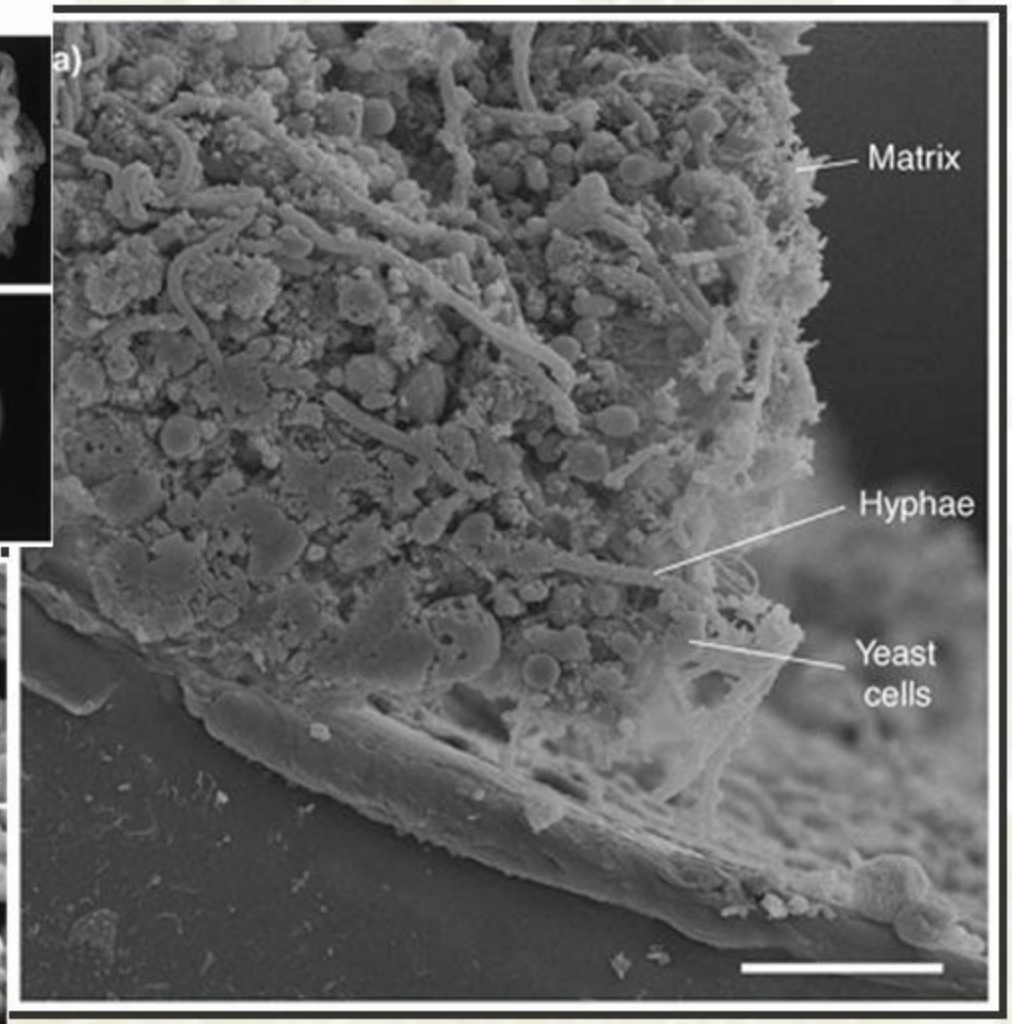
(Zara et al., in preparazione)

La matrice esocellulare

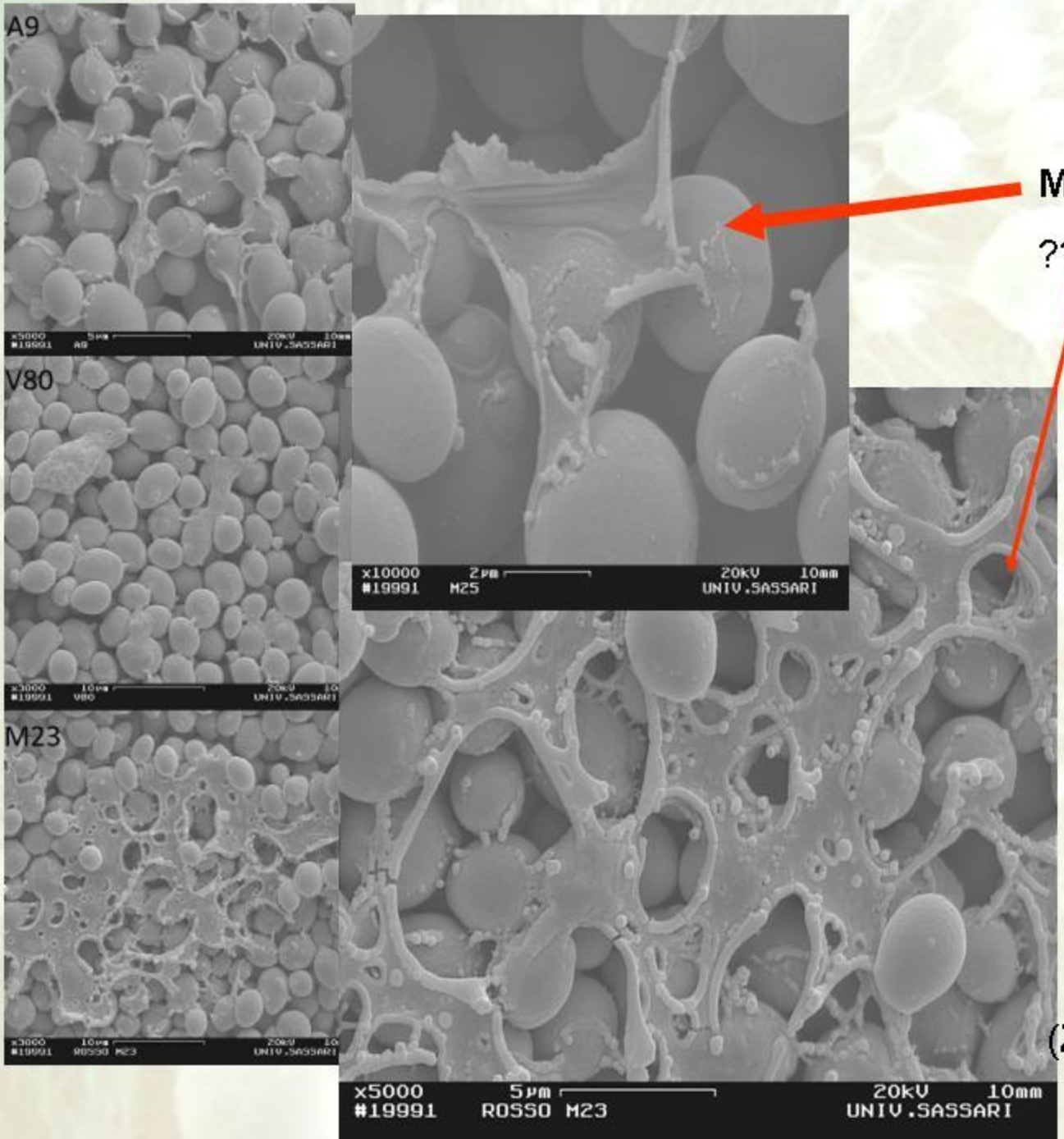




(Kuthan *et al.*, 2003).



(Nett and Andes, 2006).



(Zara et al.,2009; revision)

Conclusioni

- **Grande conoscenza di questi ceppi**
- **Importanza del modello**

- Miglioramento genetico
- OGM?
- Altre applicazioni:

  Biocontrollo,

  Smaltimento reflui di cantina


grazie
x5000 5µm 20kV 10mm
#19991 ROSSO M23 UNIV.SASSARI


Et là ! Tu comprends pourquoi je milite chez les anti OGM de GreenPeace !?