The Chinese University Of Hong Kong
Joint Graduate Student Seminar
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Quorum Sensing in Yeast

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Contents

Introduction to quorum sensing

Discovery of quorum sensing:

- Marine bioluminescent bacteria
- High cell concentration: luminesce
  Below threshold: do not luminesce
- Accumulation of secreted autoinducer signaling molecules
  → Transcription of luciferase coding gene
- Similar phenomena and molecules in other bacteria

Introduction to quorum sensing

Quorum sensing:

- Cell-to-cell communication
- Cell-density-dependent gene expression
  - Accumulation of quorum sensing molecules(QSMs)
    →Reaching a critical threshold in concentration
    →Activation of specific signaling pathway

Introduction to quorum sensing

Criteria of quorum sensing molecules

- By Winzer and colleagues(2002)

1. Be produced
   - During specific stages of growth
   - Under certain physiological conditions
   - In response to environmental changes

Introduction to quorum sensing

Criteria of quorum sensing molecules

- By Winzer and colleagues(2002)

2. Accumulate extracellularly & recognized by specific receptor

3. Trigger concerted response when concentration reaches threshold

4. Responses beyond metabolization or detoxification

Introduction to quorum sensing

- Physiological reactions:

  Biofilm formation
  Bioluminescence
  Virulence
  Antibiotic secretion
  Phenotype switch
  etc.

Efficient and effective usage of energy!

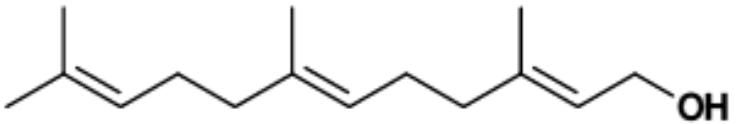
Quorum sensing phenomenon in yeast?

Quorum sensing phenomenon in yeast?

Inoculum size effect

- Yeast-mycelium dimorphism
- Initial cell concentration
  → Predomination of one morphology
- E.g. *Candida albicans*
  $\geq 10^6$ cells/ml: budding yeast
  $< 10^6$ cells/ml: germ tubes and mycelia

Discovery of farnesol



(E,E)-Farnesol

http://wildflowerfinder.org.uk/Flowers/M/MockOrange/(E_E)Farnesol.png

- Isolated by Hornby and colleagues(2001)
- First QSM identified in fungi
- Isolated from supernatant of *C. albicans* culture
- Inhibits filamentation

Control:
% mycelia >>
% budding
yeast

TABLE 1. QSM cross-reactivity of A72 with five other strains of *C. albicans*

ell type	Morphology results (mean % ± SD)[a] with: 37°C control	Supernatant from A-72[b]	A-72 cells[c]
72	16 ± 1 (Y) 83 ± 1 (M)	71 ± 4 (Y) 26 ± 4 (M)	73 ± 4 (Y) 24 ± 3 (M)
MEN	49 ± 6 (Y) 47 ± 7 (M)	99 ± 1 (Y) 1 ± 1 (M)	61 ± 3 (Y) 37 ± 2 (M)
SG 5314	8 ± 1 (Y) 92 ± 1 (M)	44 ± 6 (Y) 53 ± 2 (M)	61 ± 3 (Y) 39 ± 3 (M)
LGH 1095	19 ± 2 (Y) 78 ± 3 (M)	88 ± 3 (Y) 10 ± 2 (M)	73 ± 1 (Y) 27 ± 1 (M)
SG 3314	20 ± 9 (Y) 80 ± 9 (M)	45 ± 9 (Y) 50 ± 10 (M)	74 ± 4 (Y) 21 ± 5 (M)
SG 10261	25 ± 7 (Y) 72 ± 8 (M)	57 ± 12 (Y) 34 ± 15 (M)	44 ± 9 (Y) 55 ± 10 (M)

+ Supernatant:
% budding yeast ↑, % mycelia ↓

Hornby, J. M., Jensen, E. C., Lisec, A. D., Tasto, J. J., Jahnke, B., Shoemal
Dimorphic Fungus Candida albicans Is Mediated by Farnesol.*Applied and Environmental Microbiology*, *67*(7), 2982–2992.

Discovery of farnesol

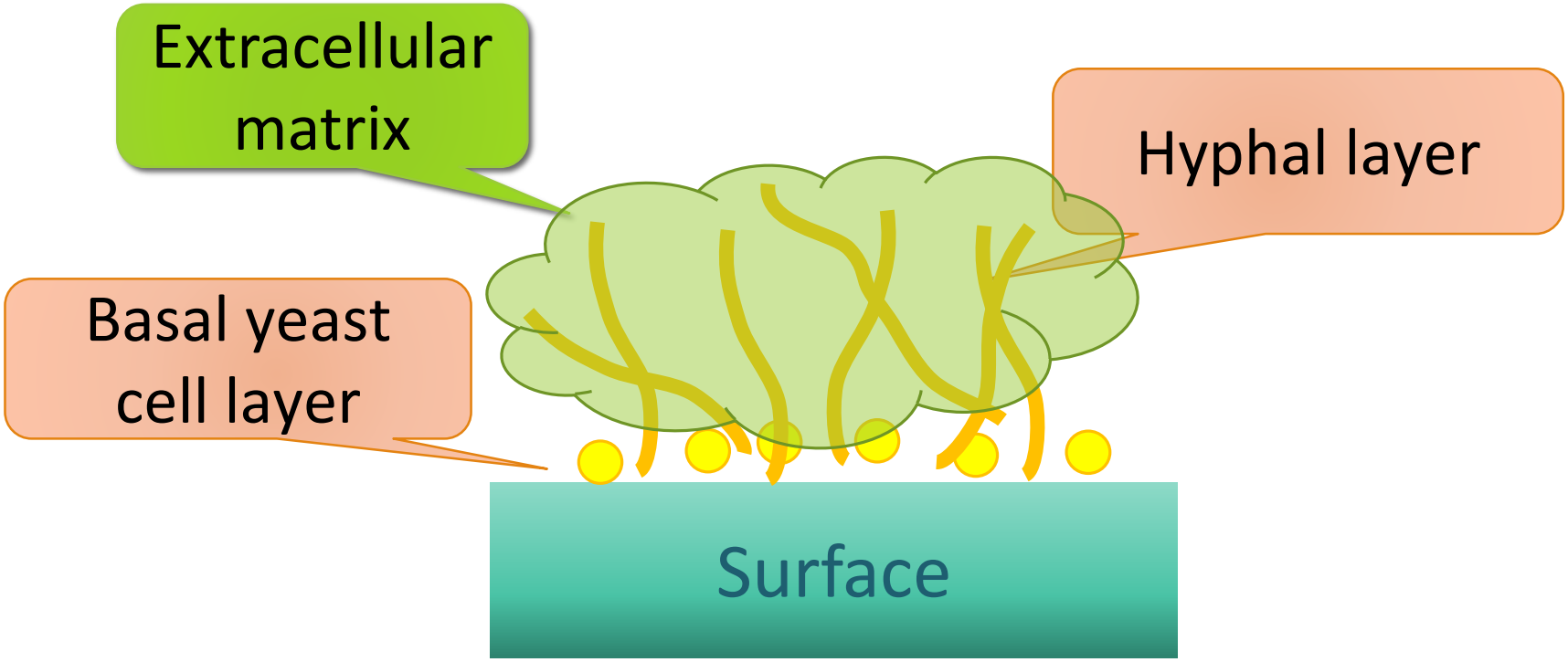
Other properties found by Hornby:

- Commercial farnesol has same effect
- Production:
  - Roughly proportional to cell density
  - Independent of growth media, carbon or nitrogen source
- No effects on growth rate
- Inhibit germ tube formation inducers: L-proline, N-acetylglucosamine and serum

Other physiological effects of farnesol on *C. albicans*

Farnesol disturb formation of biofilm structure

Mature *C. albicans* biofilm structure:

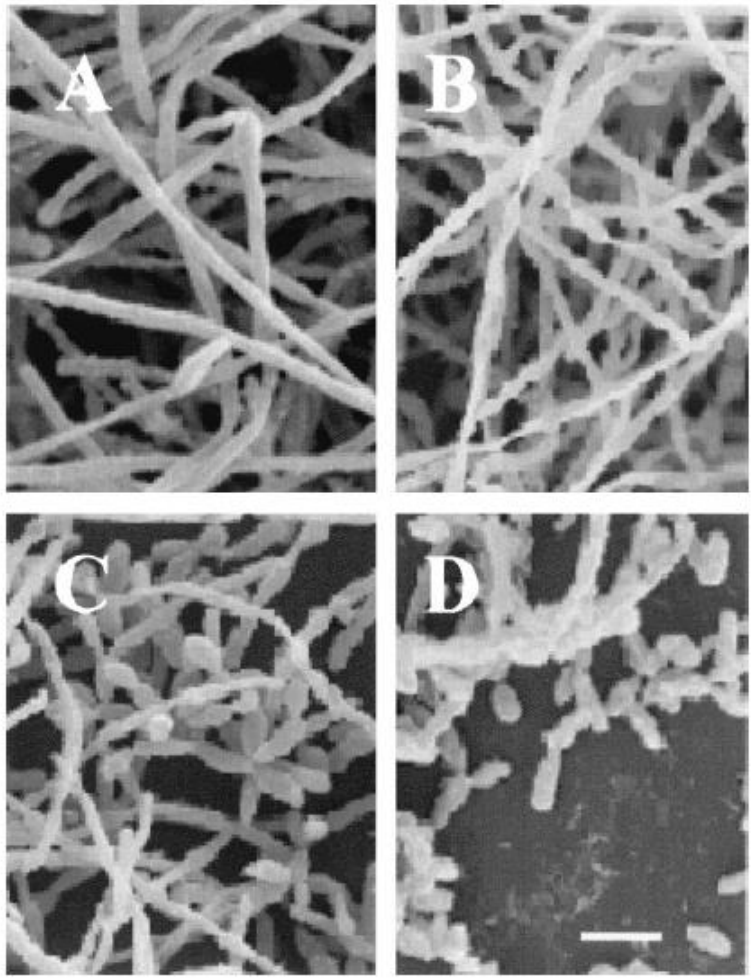


Ramage, G., Saville, S. P., Wickes, B. L., & López-Ribot, J. L. (2002). Inhibition of Candida albicans Biofilm Formation by Farnesol, a Quorum-Sensing Molecule. *Applied and Environmental Microbiology*,*68*(11), 5459–5463.

Other physiological effects of farnesol on *C. albicans*

Farnesol disturb formation of biofilm structure

Control (w/o fanesol)



3μM Farnesol

30μM

300μM

Ramage, G., Saville, S. P., Wickes, B. L., & López-Ribot, J. L. (2002). Inhibition of Candida albicans Biofilm Formation by Farnesol, a Quorum-Sensing Molecule. *Applied and Environmental Microbiology*,*68*(11), 5459–5463.

Ramage, G., Saville, S. P., Wickes, B. L., & López-Ribot, J. L. (2002). Inhibition of Candida albicans Biofilm Formation ... *pplied and Environmental Microbiology*,*68*(11), 5459–5463.

Before filamentation, inhibition

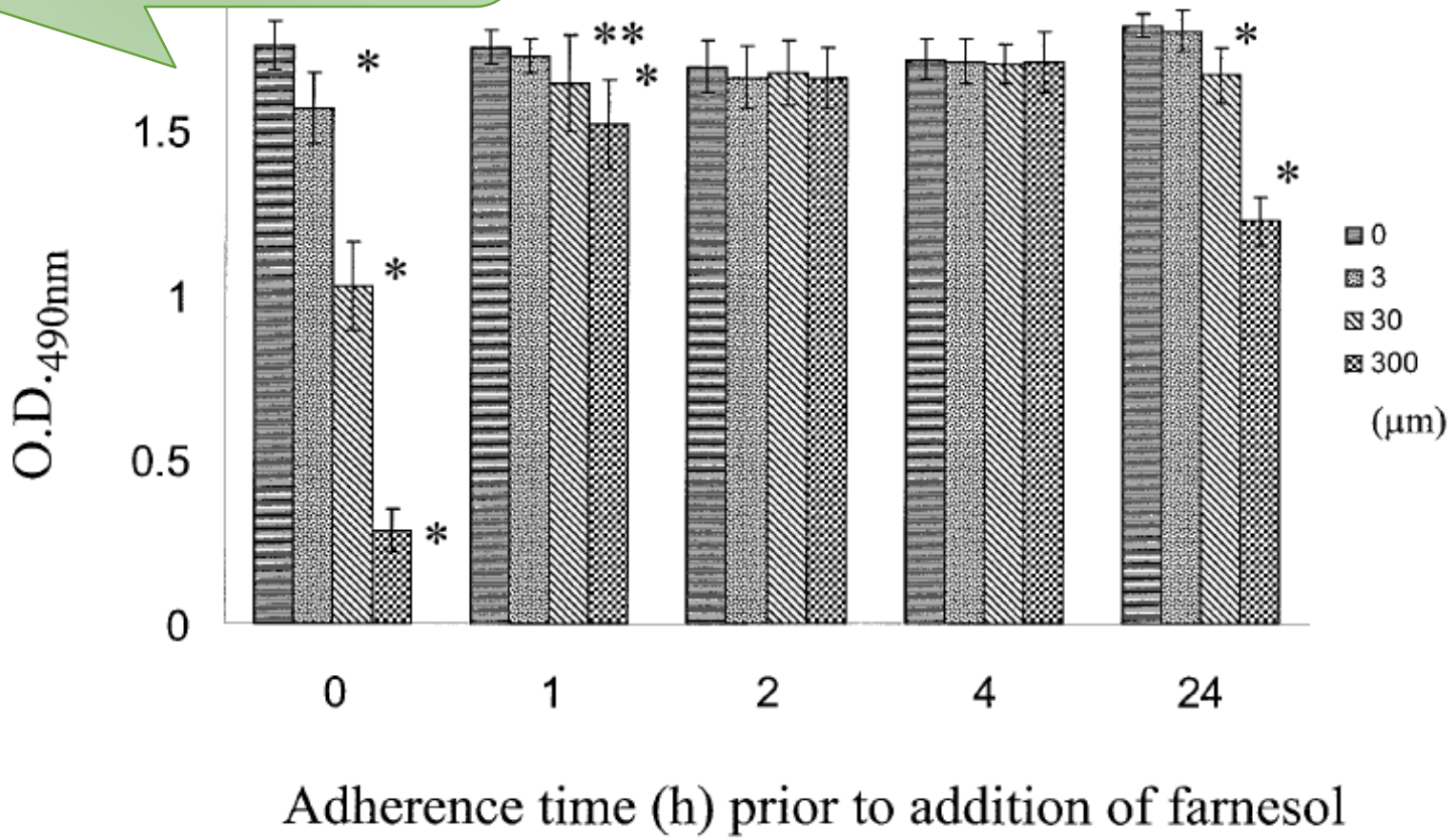
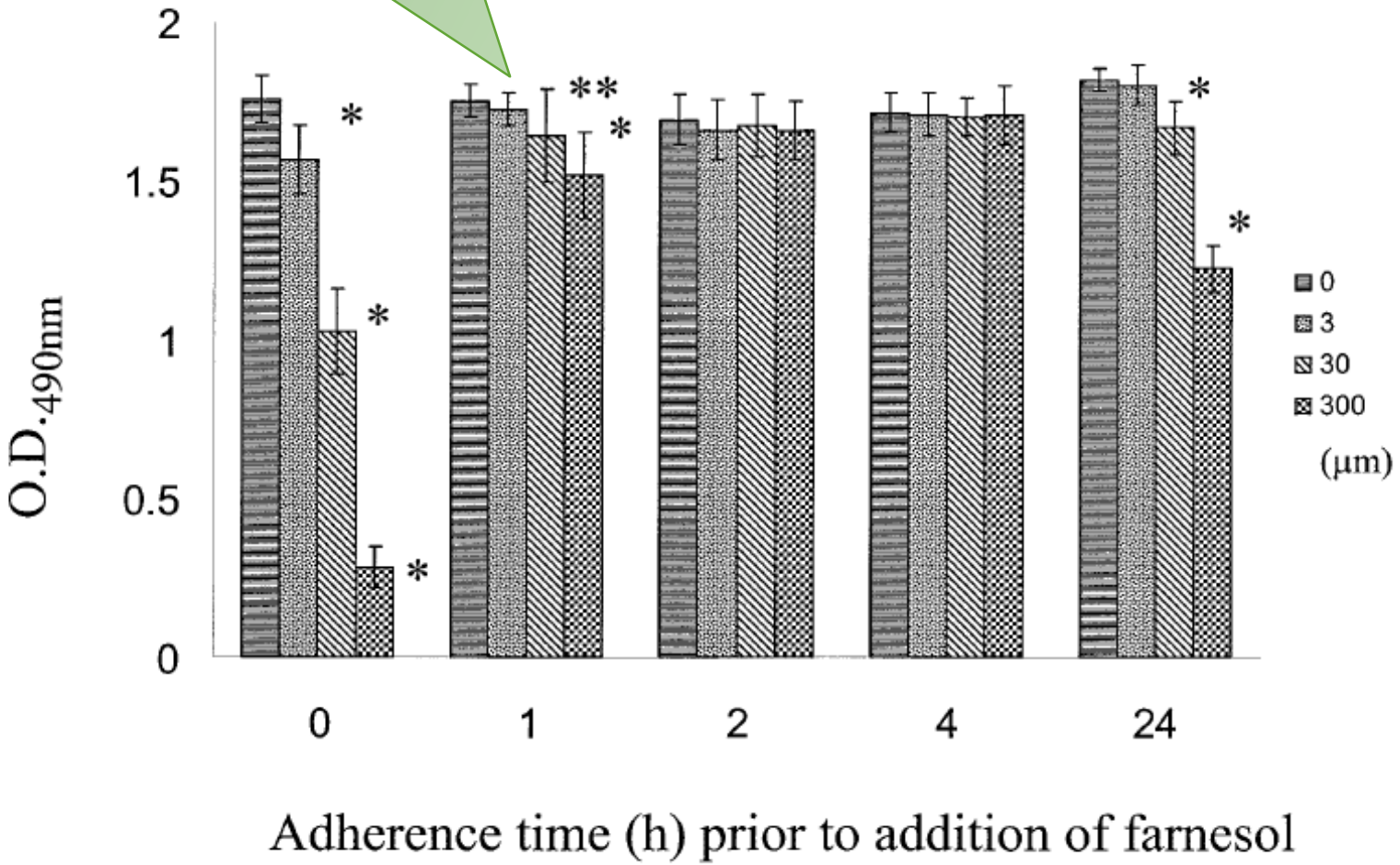


FIG. 1. Effect of farnesol on *C. albicans* biofilm formation. Different farnesol concentrations (0, 3, 30, and 300 μm) were added to *C. albicans* cells at different times after attachment (0, 1, 2, and 4 h), and the cells were incubated under biofilm-growing conditions; farnesol was also added to preformed (24-h) biofilms, which were incubated for an additional 24 h. The extent of biofilm formation was estimated by the XTT reduction assay. The values are mean absorbance values and standard deviations for 10 independent biofilms. Statistically significant differences (as determined by Student's *t* test, compared to biofilms formed in the absence of farnesol) are indicated as follows: one asterisk, $P < 0.01$; two asterisks, $P < 0.05$. $O.D._{490nm}$, optical density at 490 nm.

Rama bot, J. L. (2002). Inhibition of Candida albicans Biofilm Formation by Fa *and Environmental Microbiology*,*68*(11), 5459–5463.

After filamentation initiates, no effect



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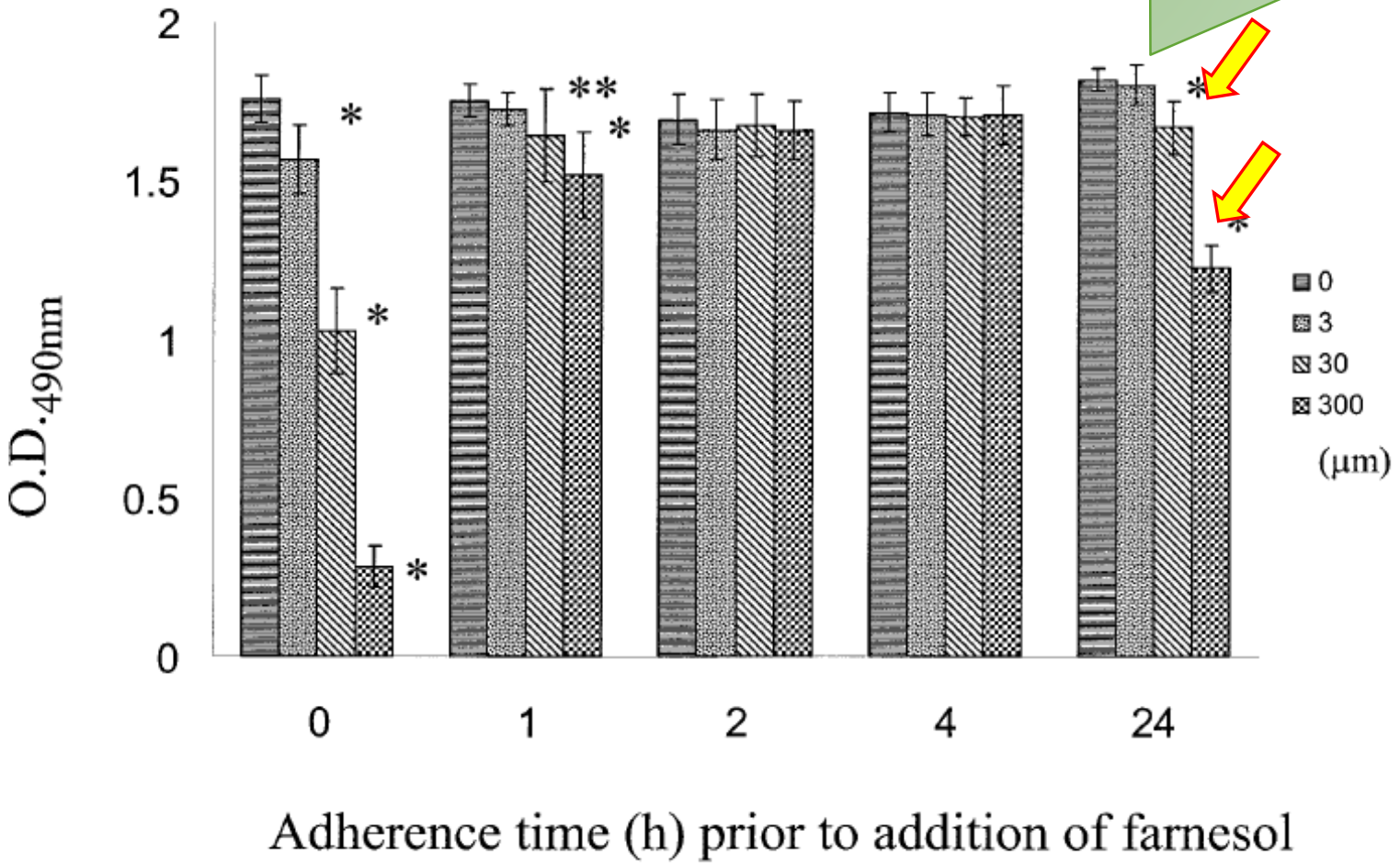
Ramage, G., Saville, S. P., Wickes, B. L., & López-Ri
by Farnesol, a Quorum-Sensing Molecule. *Applied*

New yeast cells: response to farnesol possibly related to biofilm dispersal



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Other physiological effects of farnesol on *C. albicans*

Oxidative stress

- Promote protection against oxidative stress

Induction of apoptosis

Modulation on drug efflux pump

- Specifically on ABC multidrug transporters
- Synergistic effect with azole & amphotericin B

Physiological effects of farnesol on other microbes

Biofilm inhibition	Growth inhibition	Apoptosis	Cell death	Filamentation inhibition
Candida dubliniensis	*Candida parapsilosis*	*Aspergillus fumigatus*	*Saccharomyces cerevisiae*	*Candida dubliniensis*
Candida parapsilosis	*Acinetobacter baumannii*	*Aspergillus nidulans*	*Staphylococcus aureus*	*Aspergillus nidulans*
Staphylococcus aureus	*Aspergillus fumigatus*	*Fusarium graminearum*	*Staphylococcus epidermidis*	*Paracoccidiodes brasiliensis*
Streptococcus mutans	*Paracoccidiodes brasiliensis*			
	Saccharomyces cerevisiae			
	Streptococcus mutans			

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Another QSM from *C. albicans* -- Tyrosol

Tyrosol is a quorum-sensing molecule in *Candida albicans*

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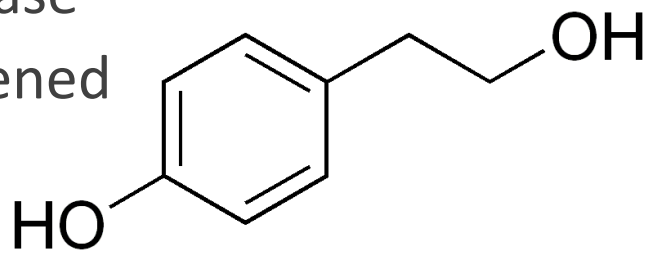
Contributed by Gerald R. Fink, February 27, 2004

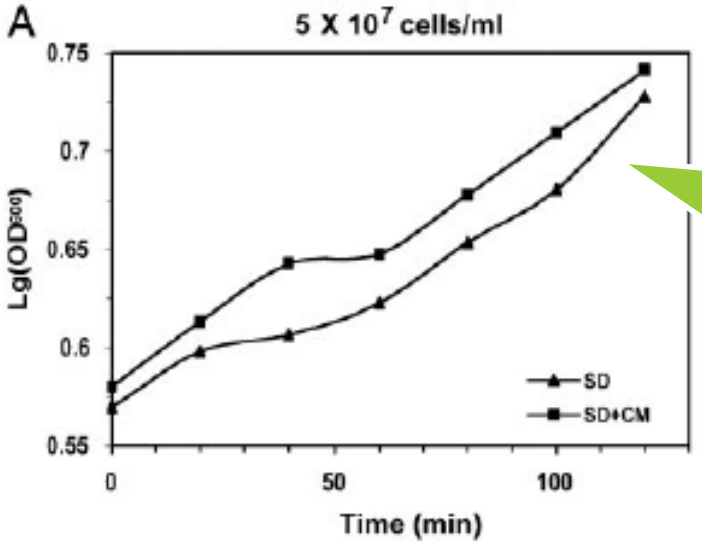
By Chen and colleagues(2004)

Long lag phase for diluted *C. albicans* inoculum

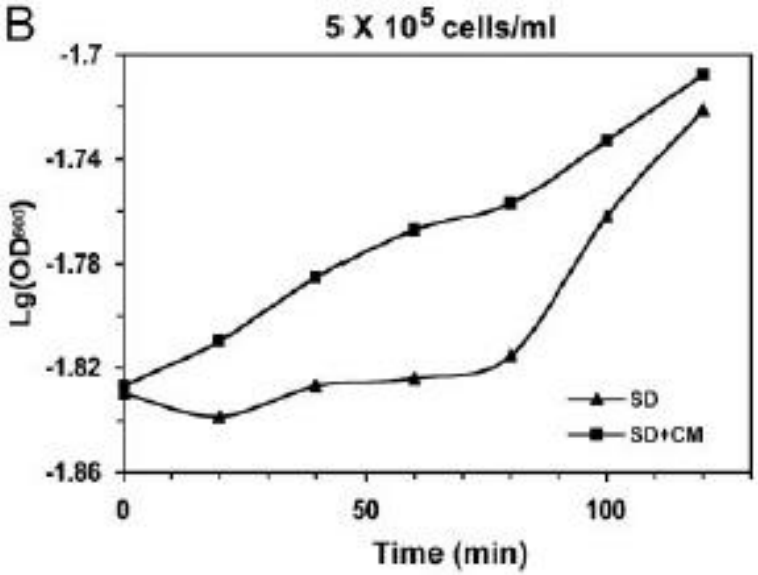
- More diluted, longer the lag phase
- + Spent media, lag phase shortened
- Active molecule: Tyrosol

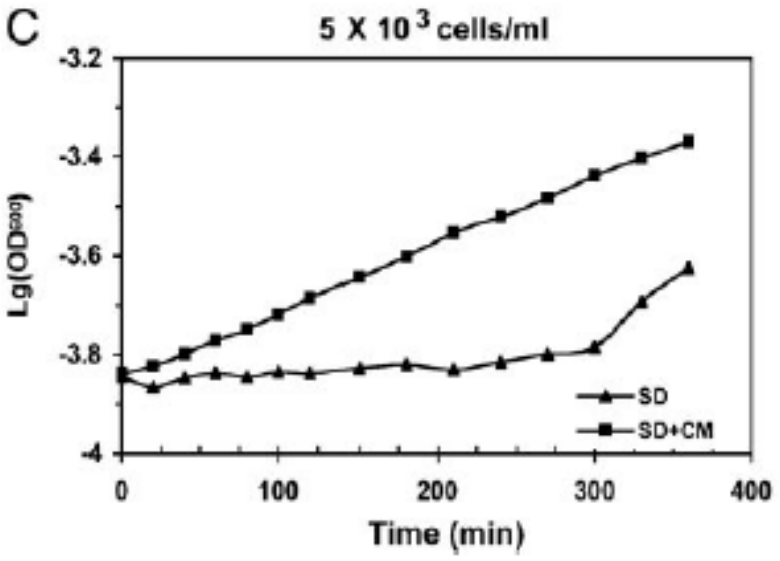


https://upload.wikimedia.org/wikipedia/commons/a/a6/Tyrosol.png

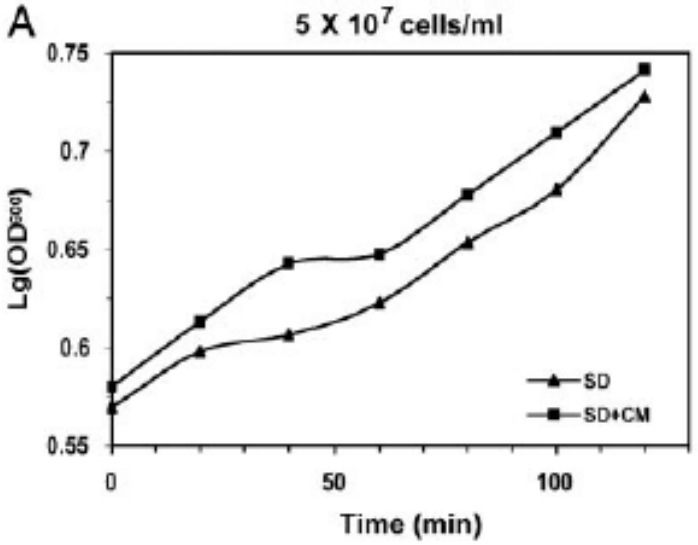


$5x10^7$ cells/ml
High inoculum concentration:
No lag phase

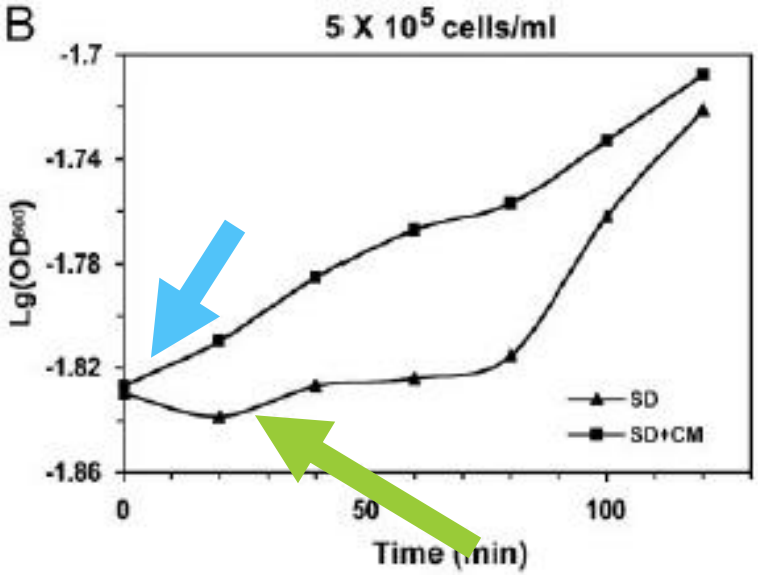


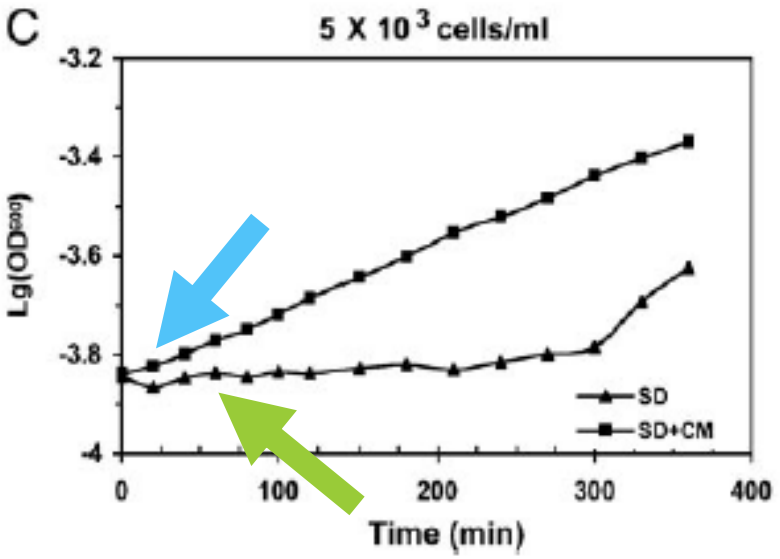


Chen, H., Fujita, M., Feng, Q., Clardy, J., & Fink, G. R. (2004). Tyrosol is a quorum-sensing molecule in Candida albicans. *Proceedings of the National Academy of Sciences of the United States of America*,*101*(14), 5048–5052.



$5x10^5$ & $5x10^3$ cells/ml
Low inoculum concentration:
Lag phase presences





+ Conditional media: lag phase shortens

Chen, H., Fujita, M., [illegible] albicans. *Proceedings of the National Academy of Sciences of the United States of America*,*101*(14), 5048–5052.

Other properties of tyrosol

- Commercial tyrosol: same effect
- Production roughly proportional to cell density
- Transcriptional regulation of DNA-replication machinery and cell-cycle-control proteins
- Promote germ tube formation

Other properties of tyrosol

- Production in biofilms > in planktonic cells
  - Early and intermediate stages:
    Tyrosol's effect > Farnesol's effect
  - Mature biofilm:
    Farnsol's effect & concentration > Tyrosol's
    →May be linked to biofilm dispersal

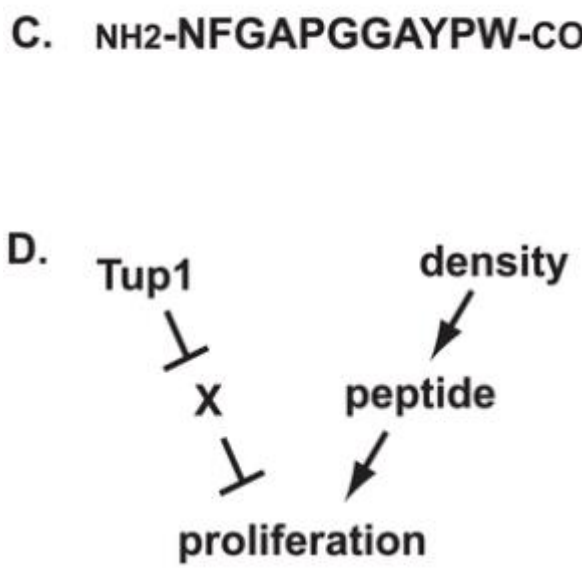
Examples of other possible QS system found in yeast

Saccharomyces cerevisiae

- QSMs: Typtophol, Phenylethanol
  - Control filamentation in low nitrogen conditions

Cryptococcus neoformans

- QS-like behavior in mutants of TUP1 of *C. neoformans*: No growth if [inoculum] $<10^3$cells/ml
- + Conditional medium from high density culture → growth rescued
- Active molecule: peptide



Madhani, H. D. (2011). Quorum Sensing in Fungi: Q&A. *PLoS Pathogens*, *7*(10).

Possible applications of yeast QSMs

Clinical purposes:

- Farnesol from *C. albicans*
  - Preventing biofilm on catheter and implant
  - New type of antifungals or antibiotics
- Developing quorum sensing inhibitors

Industrial purposes:

- Monitoring and controlling fermentation process
- Studying interactions between microbes

Conclusion

- Quorum sensing
  - Modulation of gene expression
  - Cell-density-dependent manner
- Quorum sensing molecules in yeast:
  - Farnesol: Inhibit filamentation
  - Tyrosol: Shorten lag phase, promote germ tube formation
  - New ways to prevent and cure microbial infection

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