Joint Graduate Seminar
Department of Microbiology
The Chinese University of Hong Kong

Bacterial Growth Conditions: That is the question.

Angela Leung

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Supervisor: Professor Margaret Ip

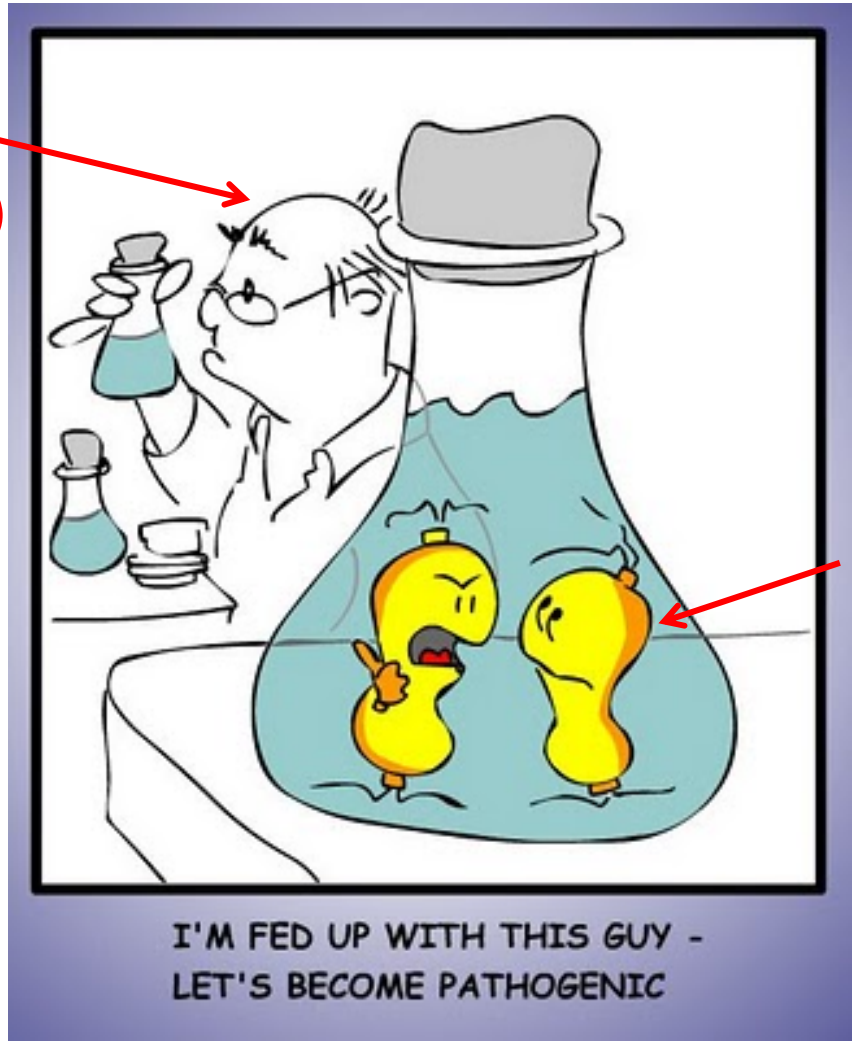
Objective

- To illustrate the importance of simulating real host conditions in experiments that involve growing bacterial cultures

Introduction

- Virulence depends on:

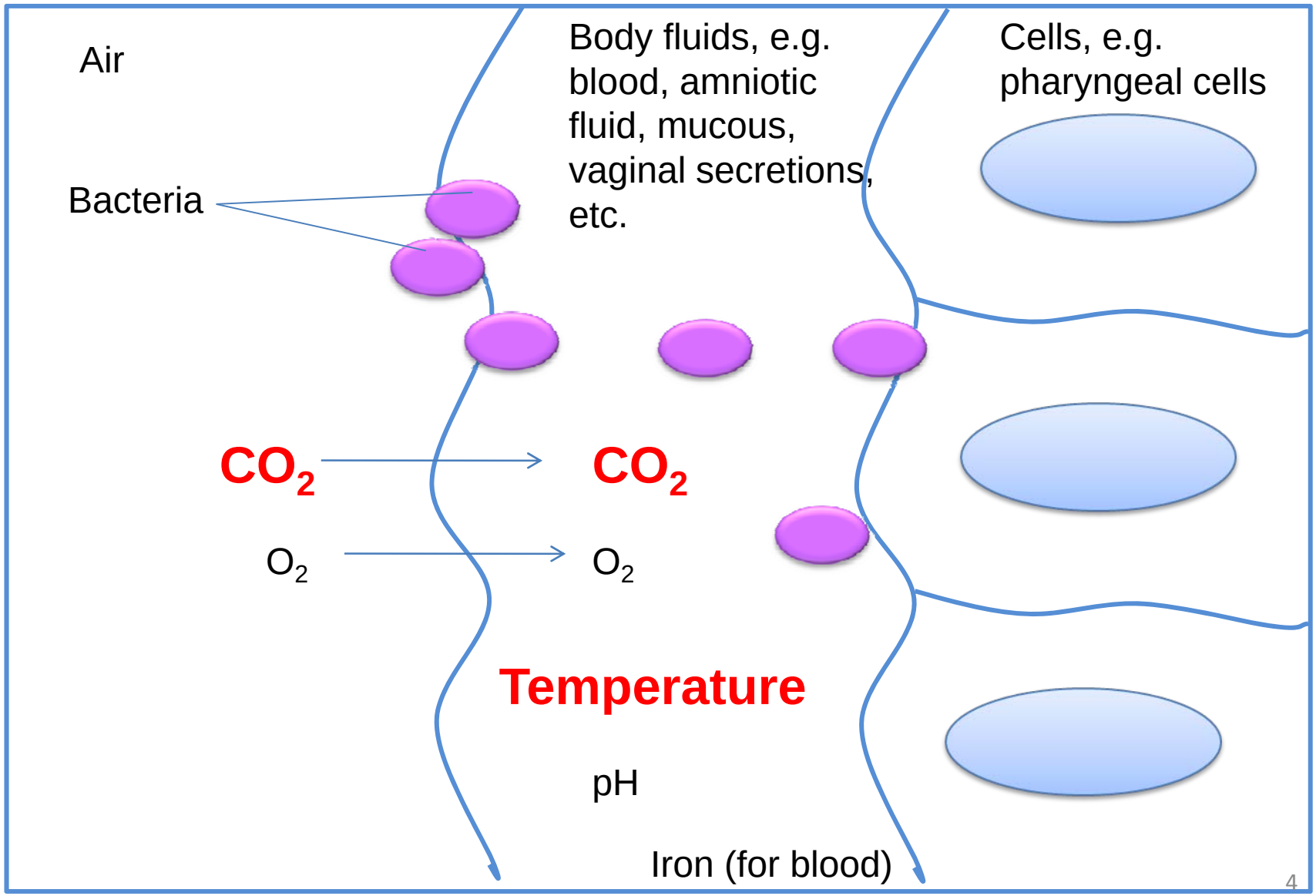
Host factors (predisposition)



Environmental conditions

The bacteria

Bacterial interactions with humans



Host micro-environment

Group B Streptococcus

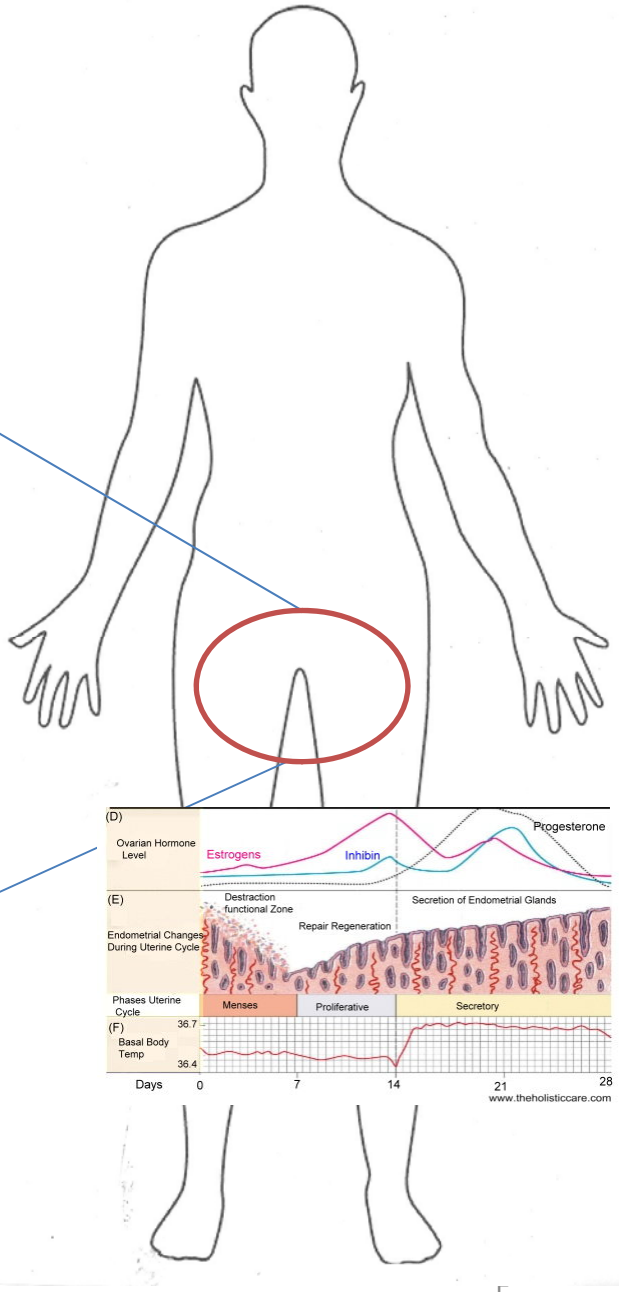
Transcriptomic changes

RNA

Proteins

Proteomic changes

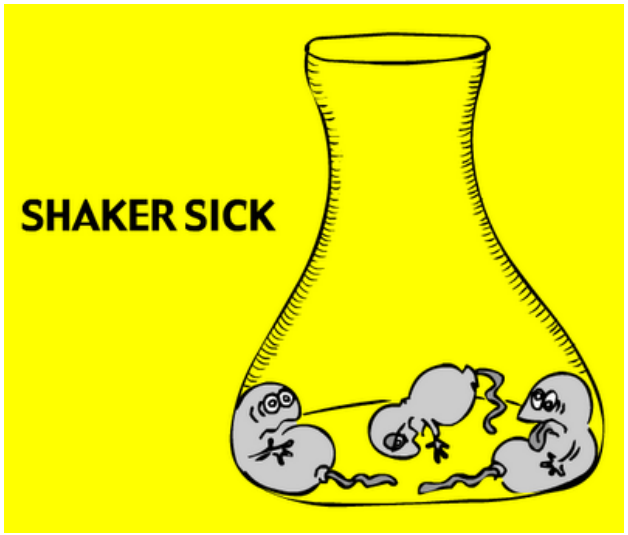
Secretory virulence factors



Quality of *in vitro* studies

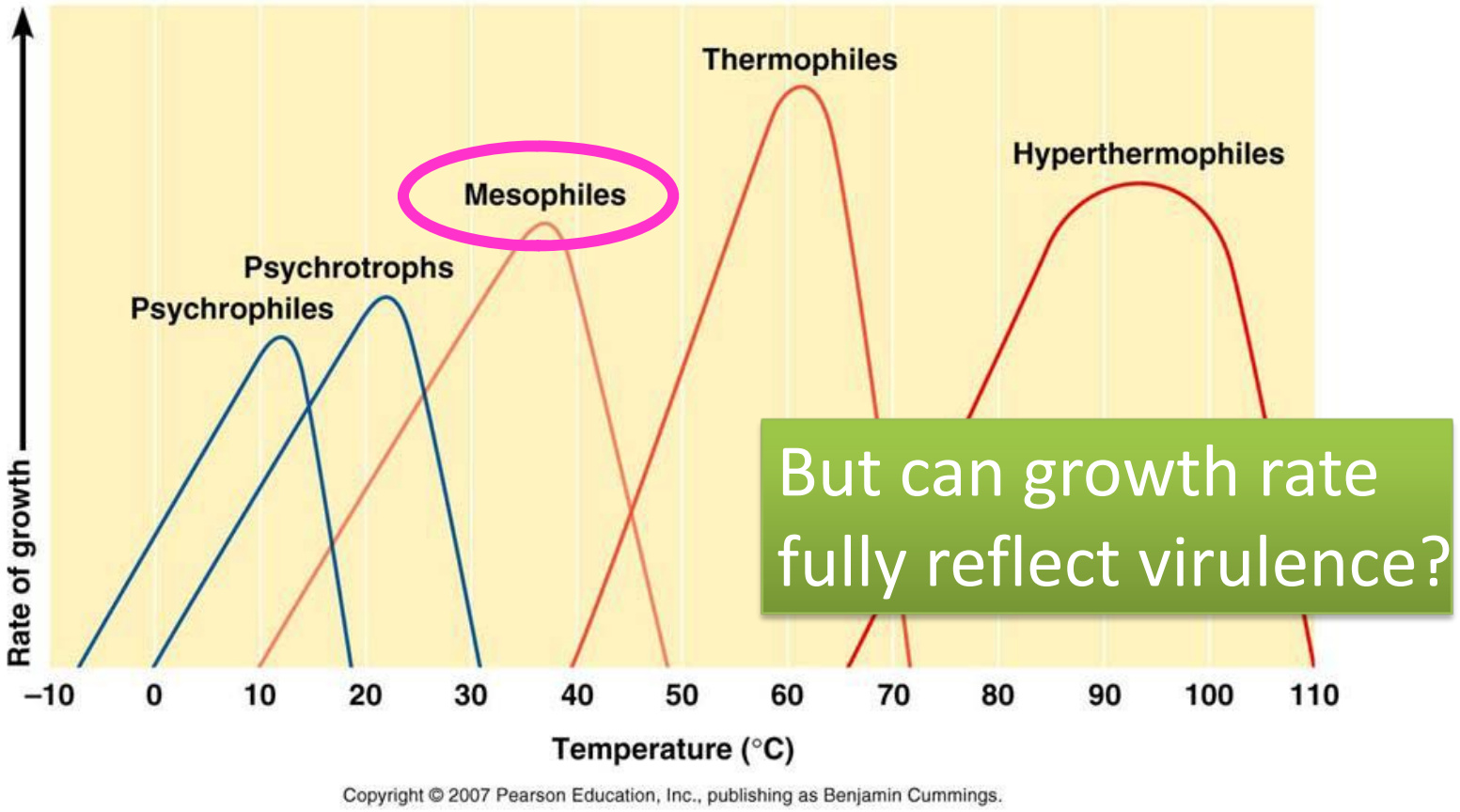
More similar, better

In vitro ⟺ *In vivo*



Animal models

Bacterial growth rate depends on temperature



But can growth rate fully reflect virulence?

http://classes.midlandstech.edu/carterp/Courses/bio225/chap06/Slide1.JPG

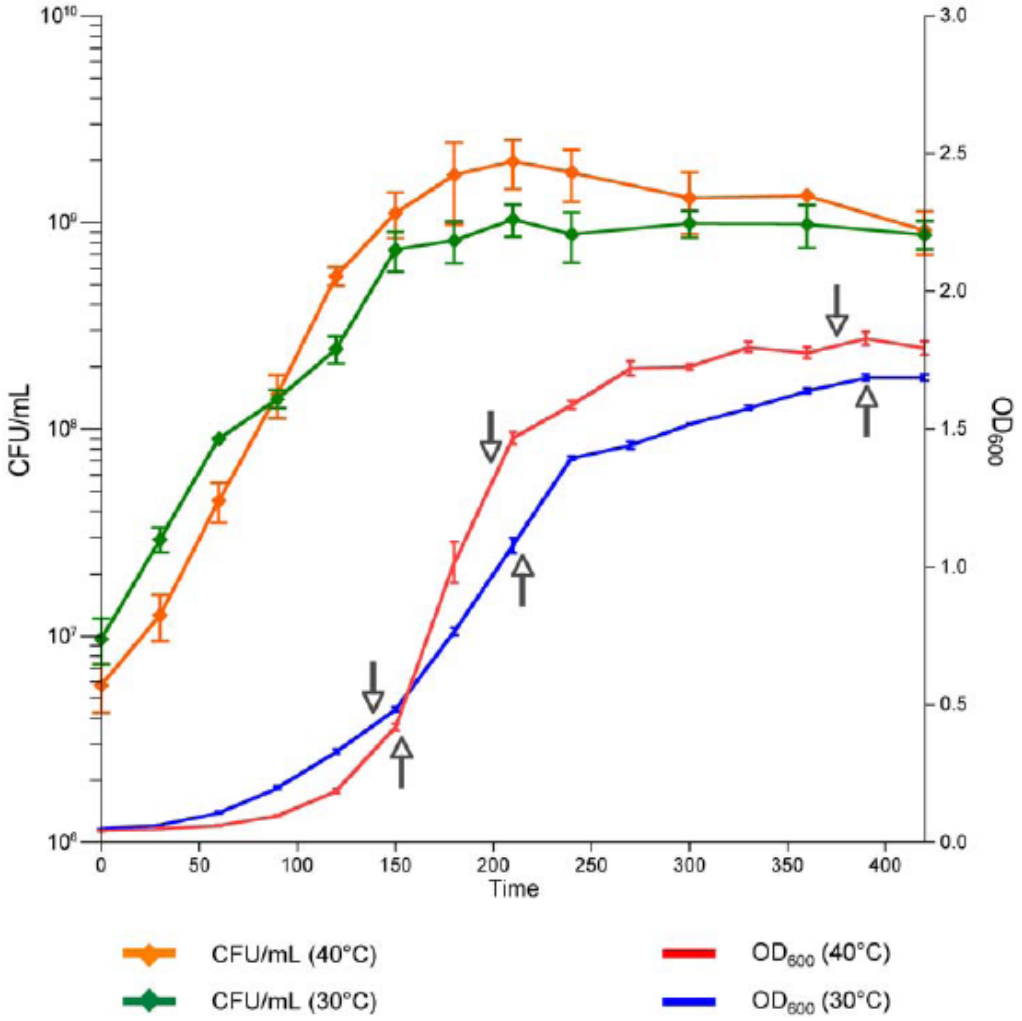
Different behaviours of GBS at 30°C and 40°C

Temp	Natural environment
30°C	Cow mammary gland surface (where the bacteria do not cause disease)
40°C	Patients with severe infections and high fever

Question:
Does the population size of GBS increase much at 40°C?

Mereghetti *et al.* (2008) *PLoS One* 3: e2785

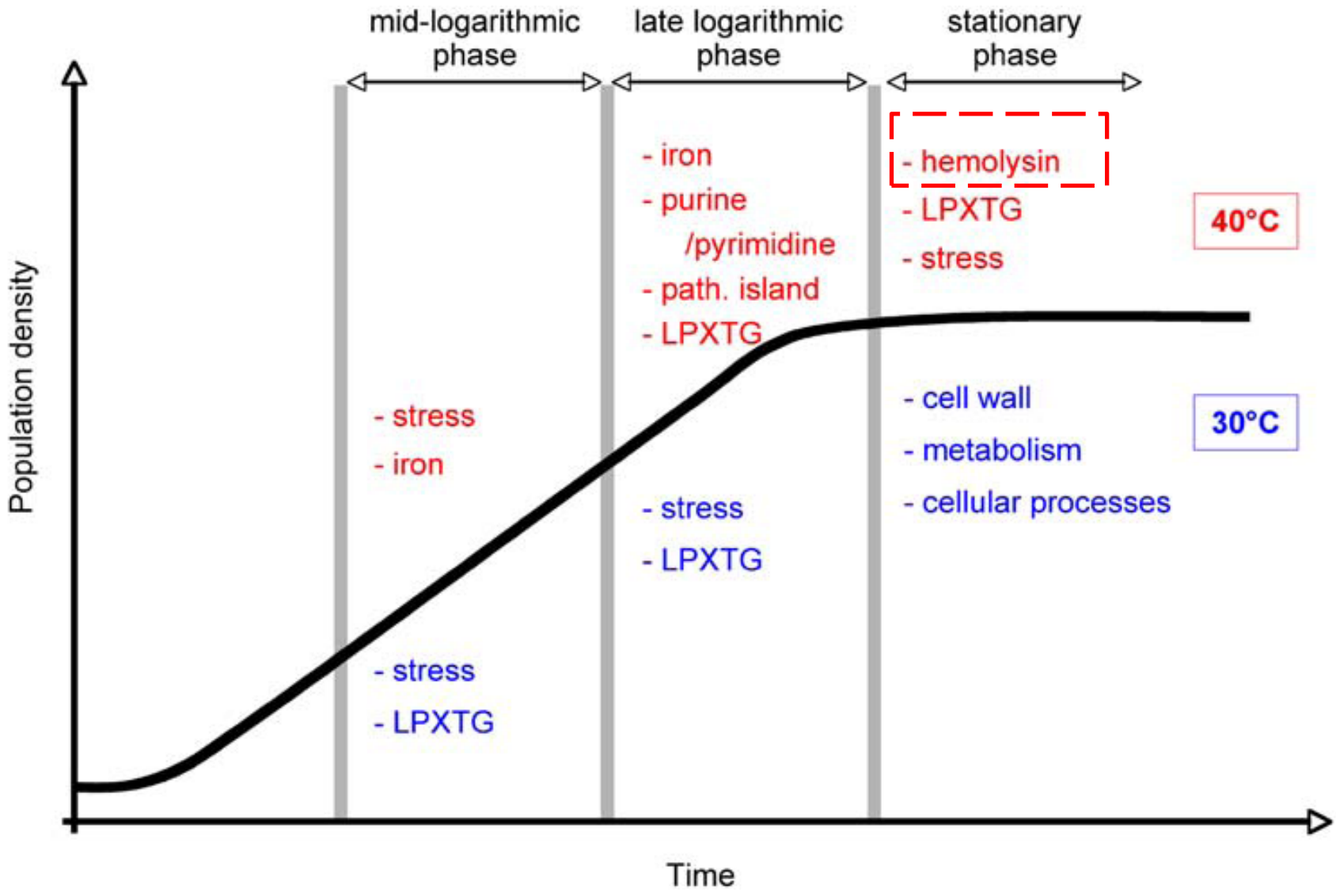
Growth temperatures do not cause great changes to GBS growth curves



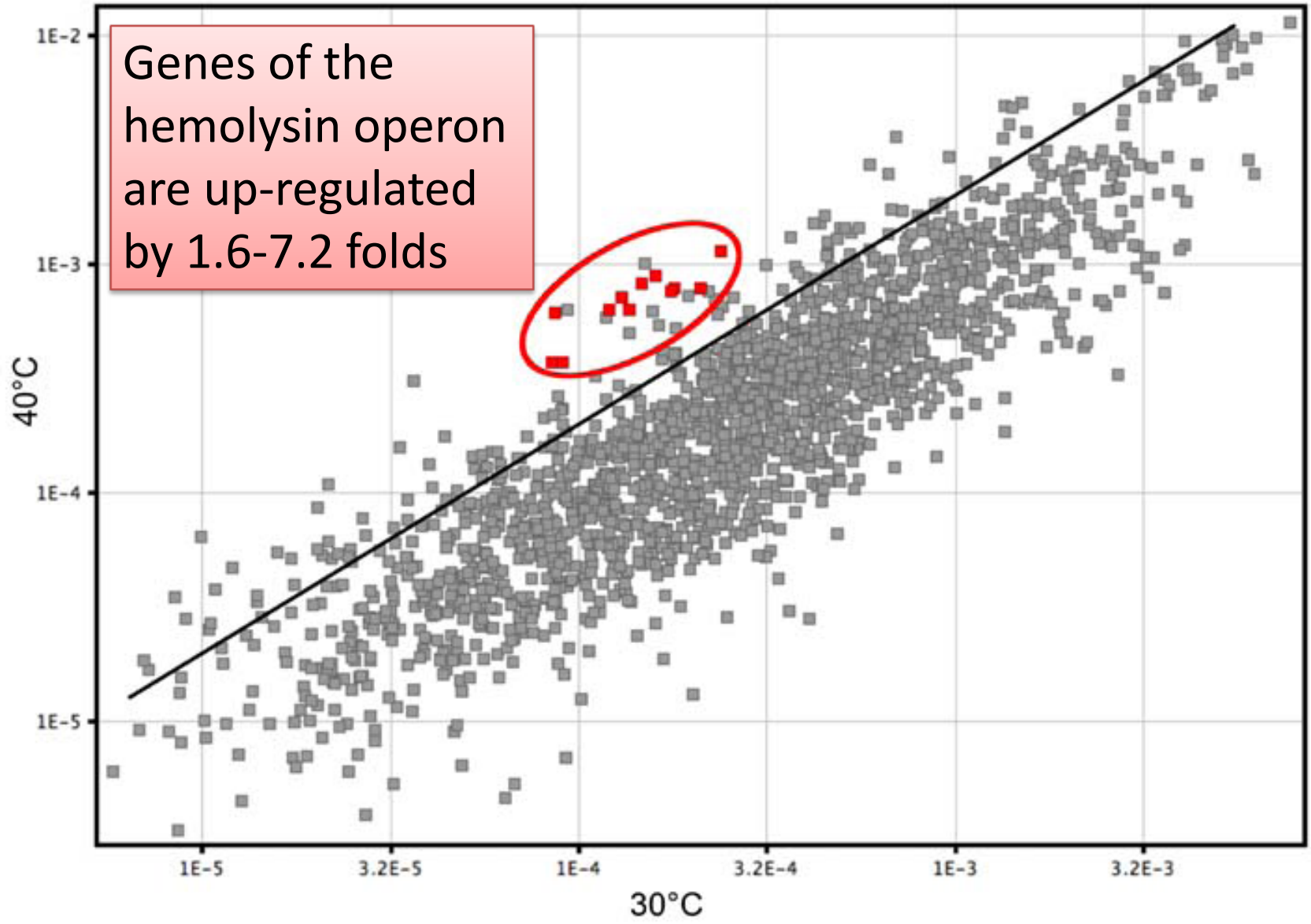
However…

Mereghetti *et al.* (2008) *PLoS One* 3: e2785

Transcriptonal response of GBS to change in growth temperature



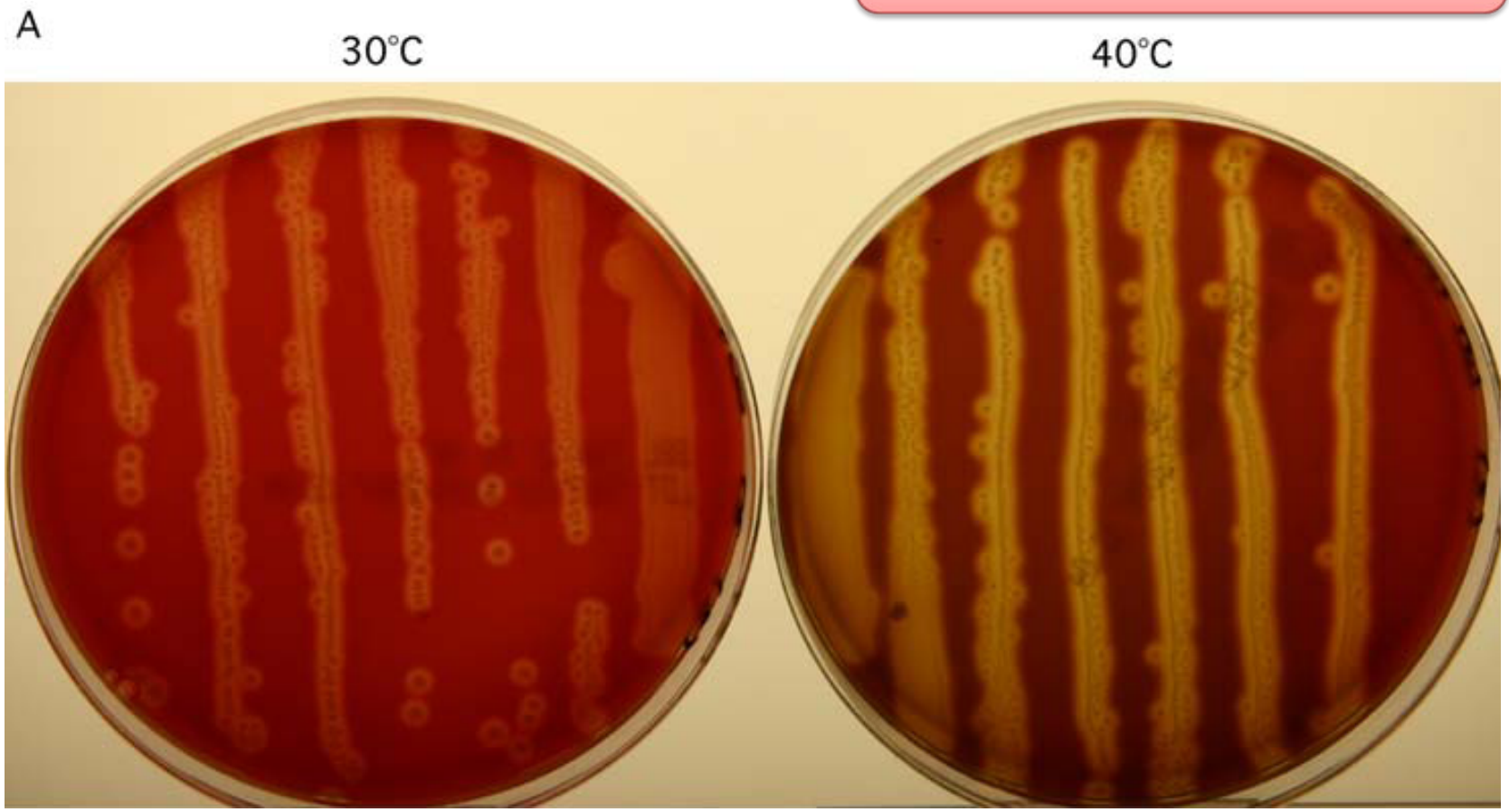
Mereghetti *et al.* (2008) *PLoS One* 3: e2785



Mereghetti *et al.* (2008) *PLoS One* 3: e2785

Growth temperature affects hemolytic activity

Hemolysin is up-regulated at 40°C



Mereghetti *et al.* (2008) *PLoS One* 3: e2785

Effect of CO_2 on bacterial growth and virulence factor production

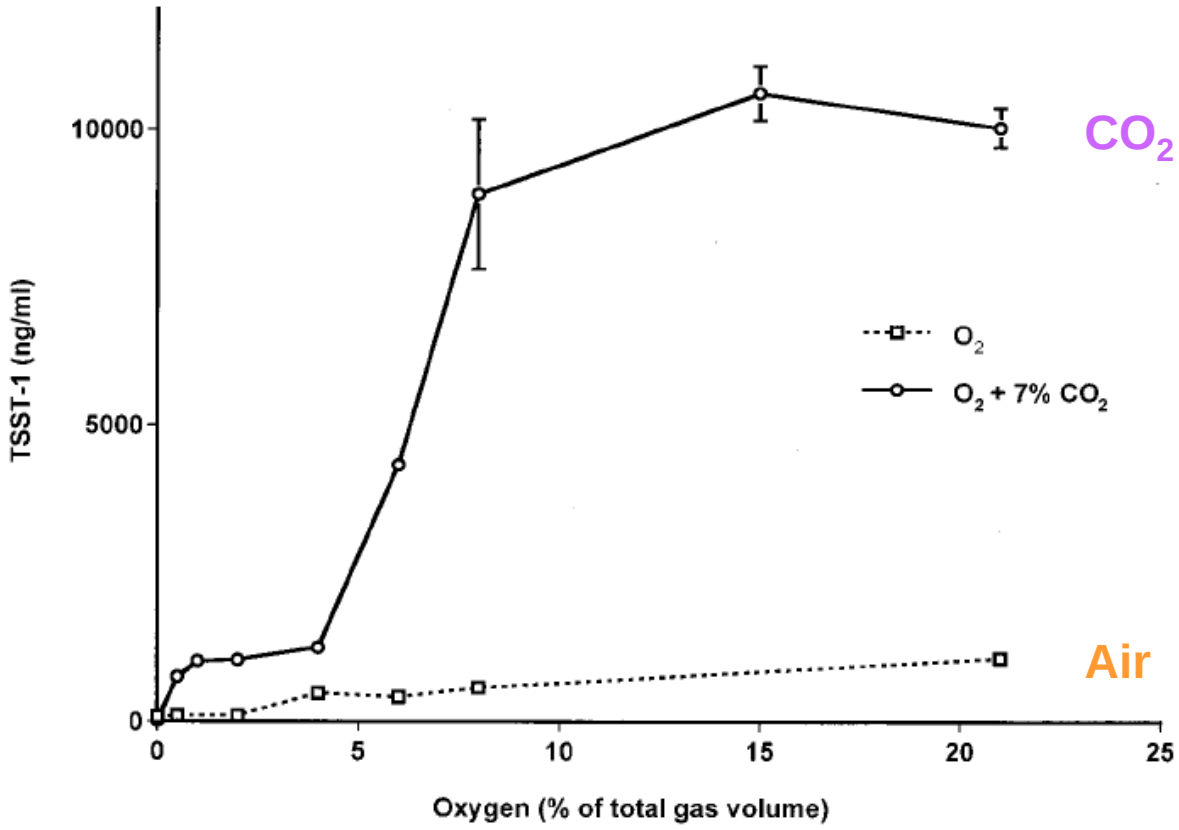
- Toxic shock syndrome (TSS)
- Menstrual TSS is associated with tampon use
- Tampon insertion changes vaginal microenvironment (e.g. gas composition)

- Toxin responsible for the syndrome:

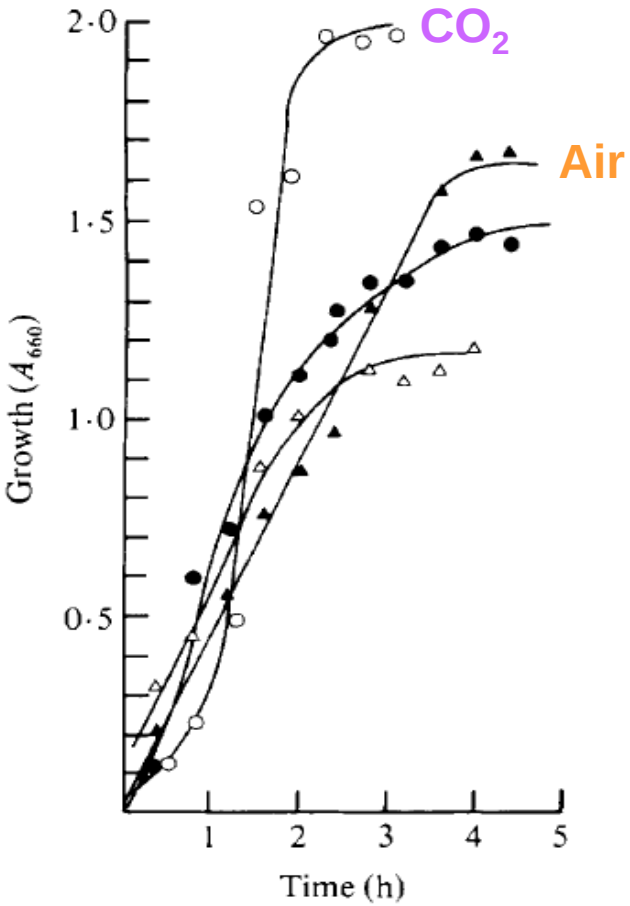
  Toxic shock syndrome toxin 1 (TSST-1)

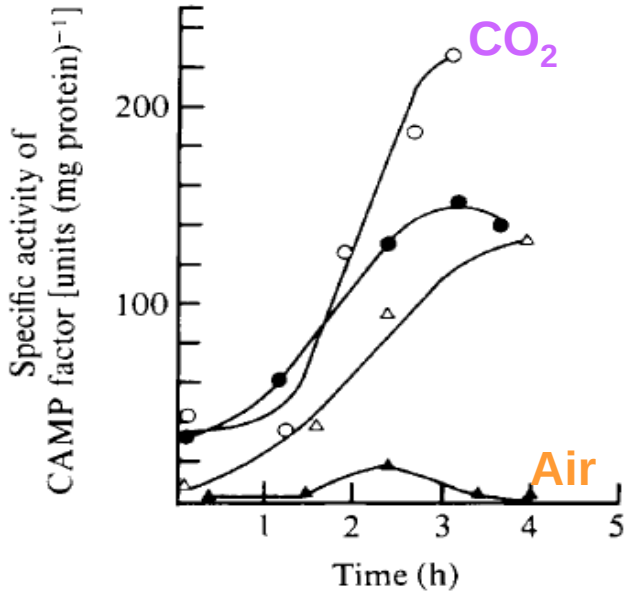
Wong & Bergdoll (1990) *Infect Immun* 58: 1026

TSST-1 production is greatly increased by CO2 supply



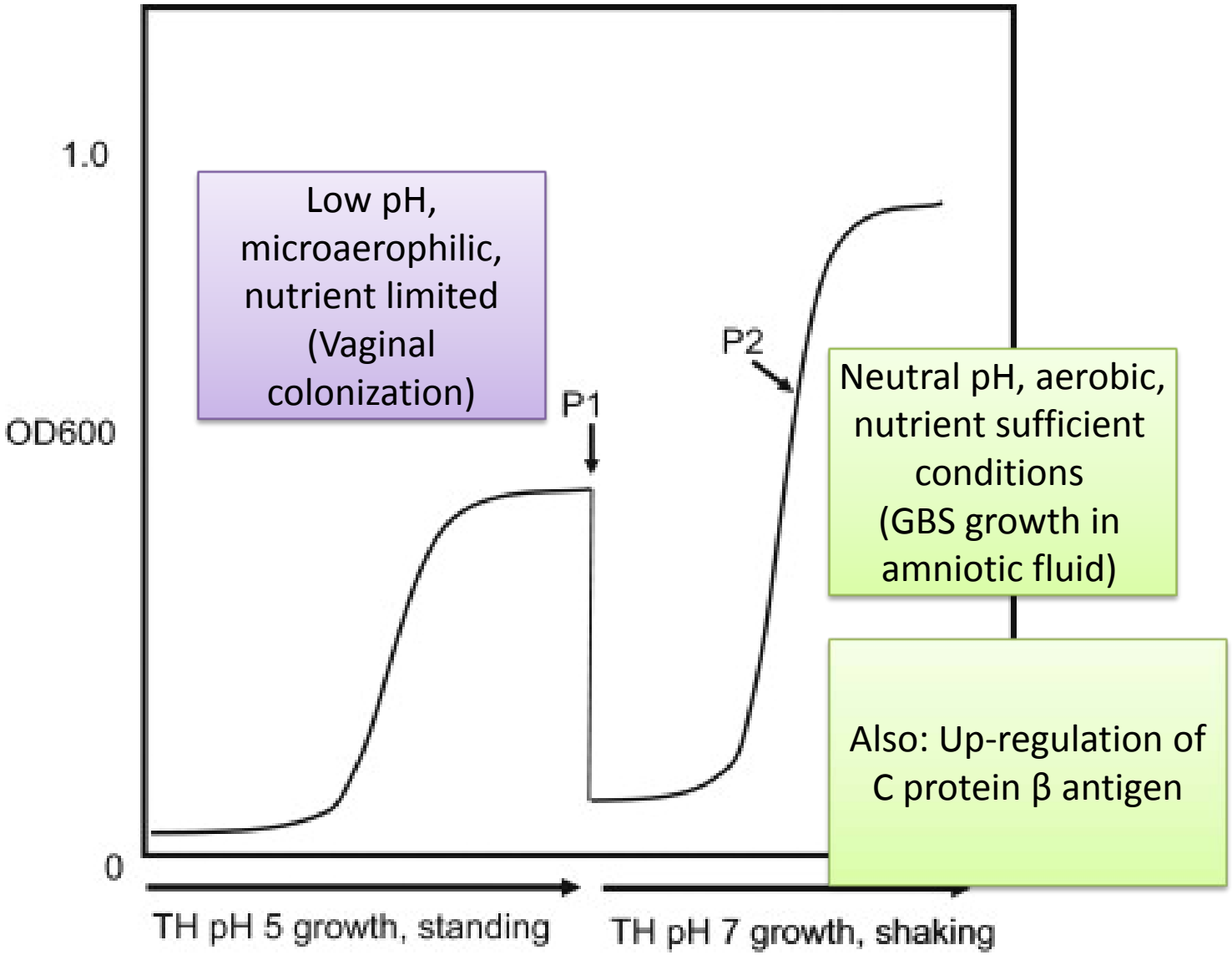
CO_2 shifts GBS growth curve to the left and increases production of CAMP (an extracellular protein)





Huser *et al.* (1983) *J Gen Microbiol* 129: 1295

Using growth conditions associated with real infection sites



Yang *et al.* (2010) *Int J Med Microbiol* 300: 331

Co-culture of human samples to study changes in bacterial cells

Bacteria species	Co-culture with…	Effects studied	Reference
GAS and GBS	Amniotic fluid 	Transcriptome adaptations	Sitkiewicz (2010) *PLoS One* 5: e9785 Sitkiewicz (2009) *PLoS One* 4: e6114
GBS	Blood 	Transcriptome	Mereghetti *et al.* (2008) *PLoS One* 3: e3143
GAS	Plasma	Protein expression	Johansson *et al*. (2005) *J Proteome Res* 4: 2302
GAS	Pharyngeal cells	Phage toxin	Broudy *et al*. (2001) *Infect Immun* 69: 1440

Summary

- Bacteria encounter different conditions in different micro-environments in hosts

  → Different transcriptomic / proteomic responses (whole remodeling / up-regulation of certain virulence factors)

- Mimicing host conditions when growing bacterial cultures allows us to know how the bacteria behave in the hosts

Growth condition(s)	To mimic…	Phenomenon observed
40°C	High fever	Higher hemolytic activities in GBS
CO_2 supply	Vagina environment when tampon is used	Higher TSST-1 toxin production in *S. aureus*
Neutral pH, aerobic, nutrient sufficient	Amniotic fluid	Up-regulation of C protein β antigen
Co-culture with human samples e.g. blood	Different micro-environments in hosts	Transcriptomic / Proteomic changes

Thank You!