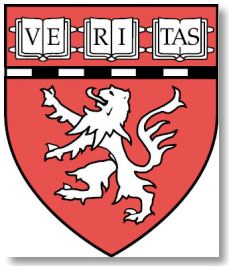
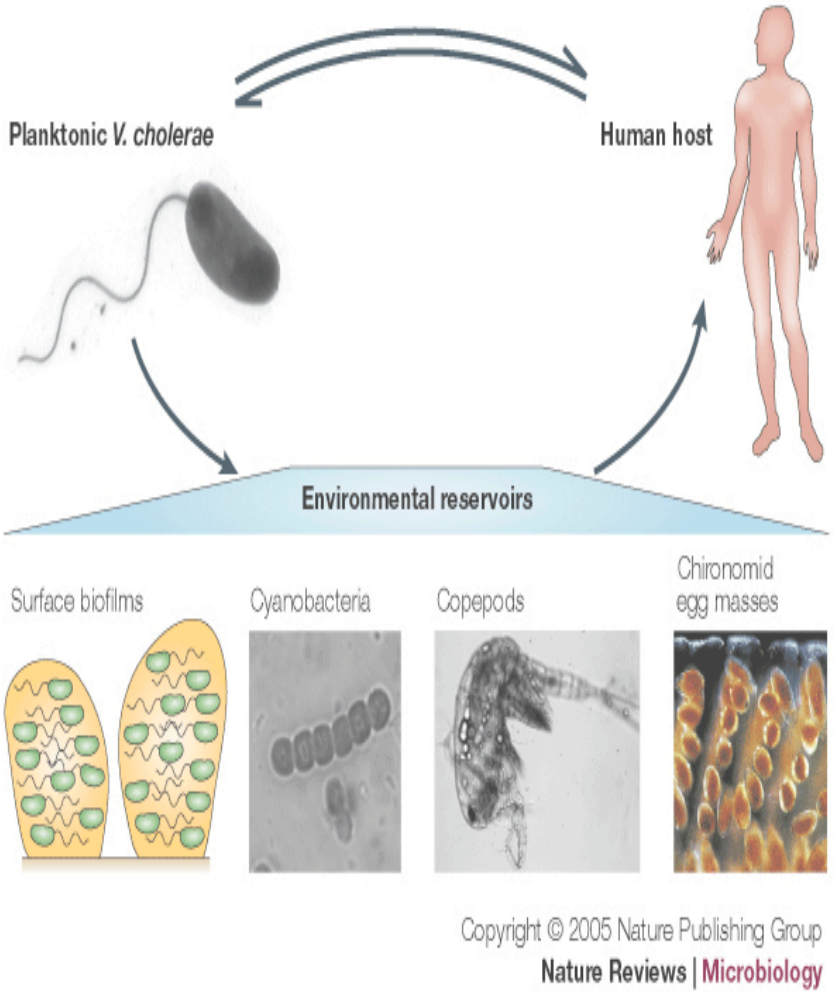
Regulation of Vibrio cholerae Virulence Gene Expression and Pathogenesis in Response to Microaerophilic Growth Conditions

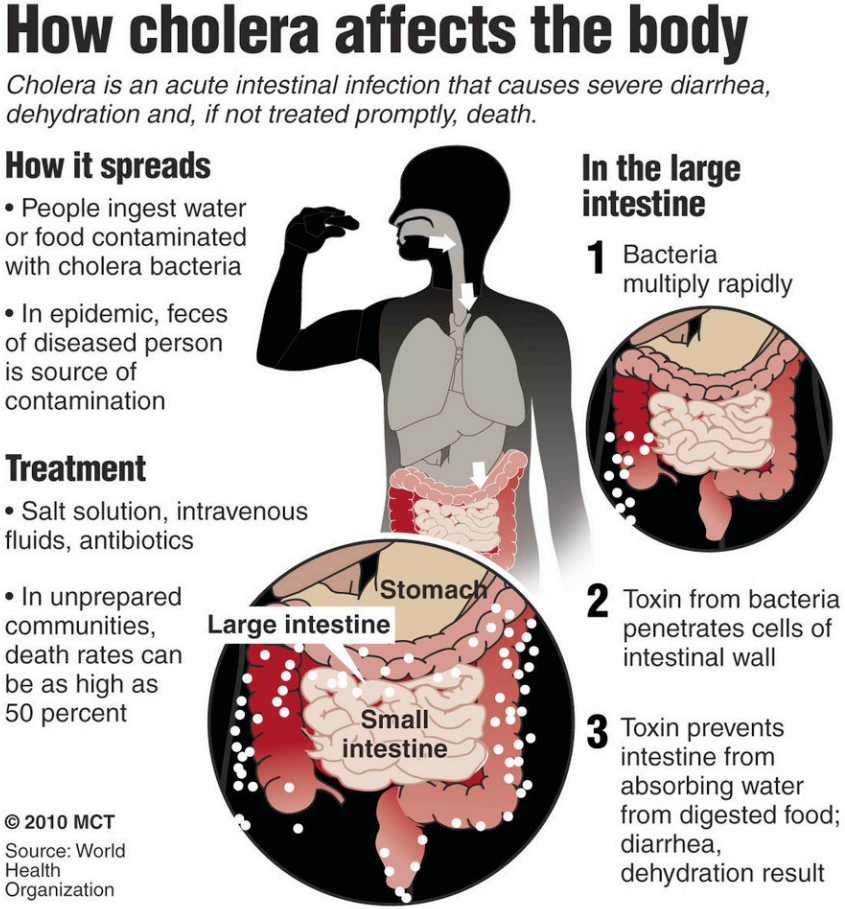
Pratik Shah. Ph.D.

Postdoctoral Research Fellow

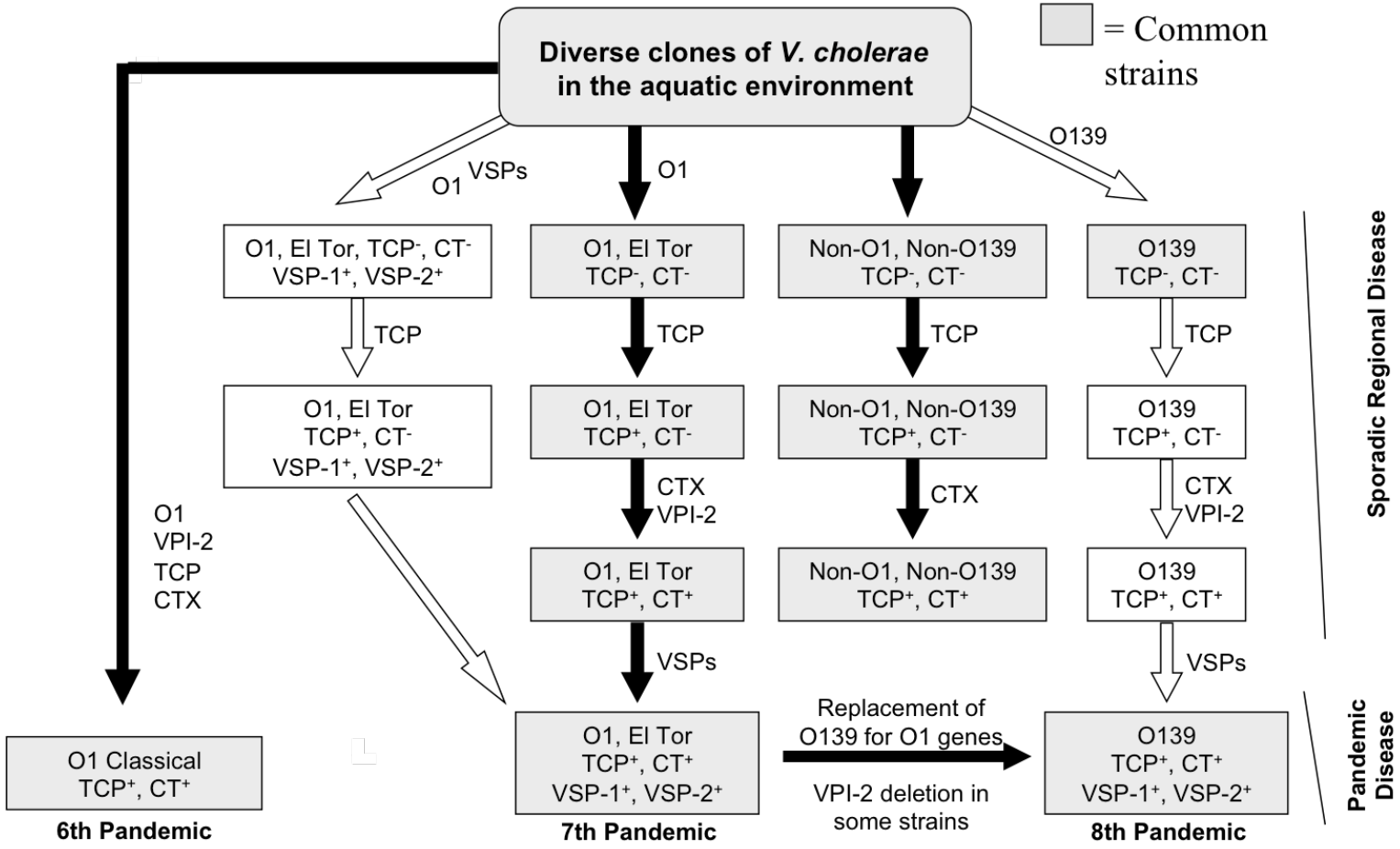


Vibrio cholerae life-cycle

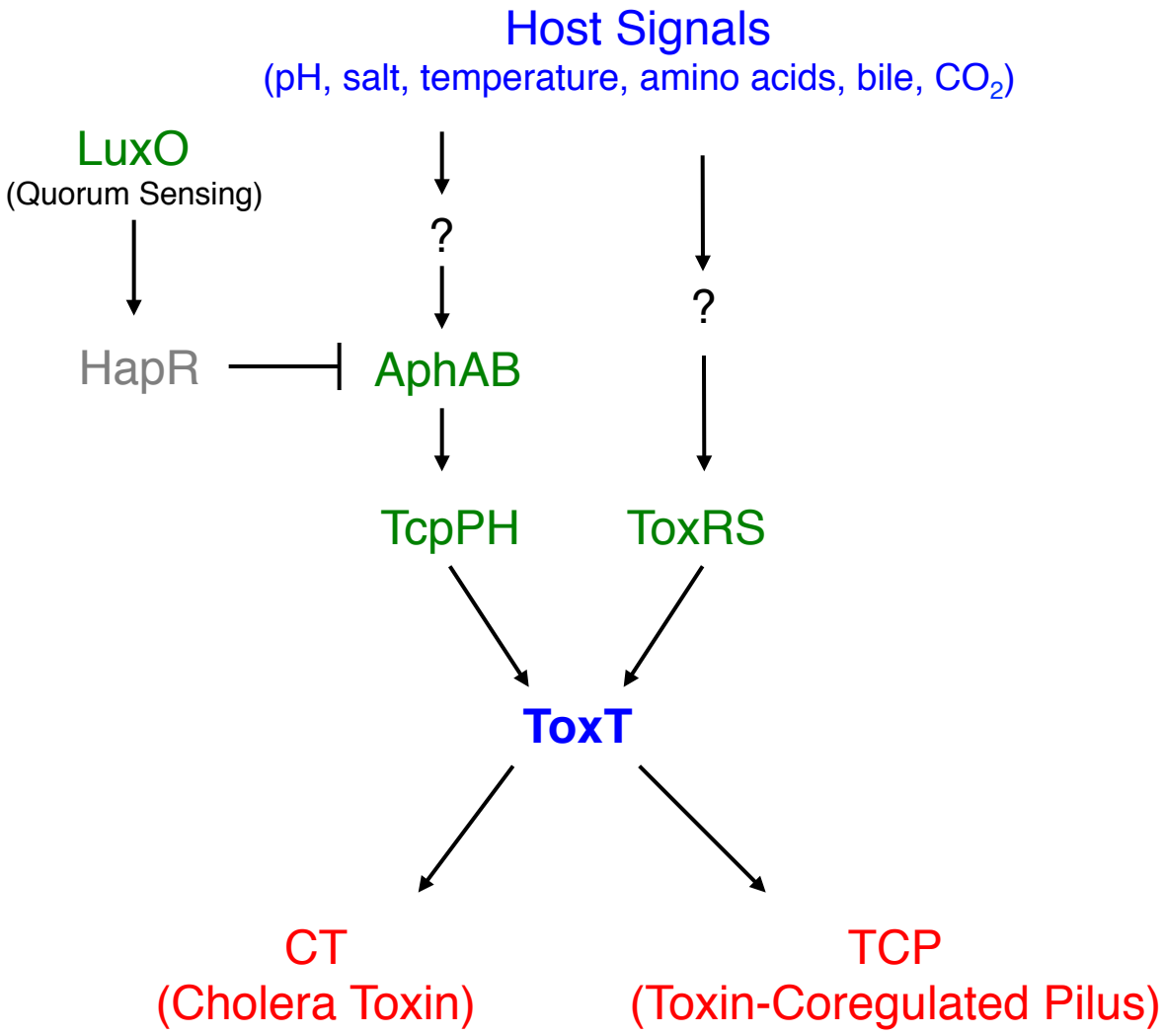




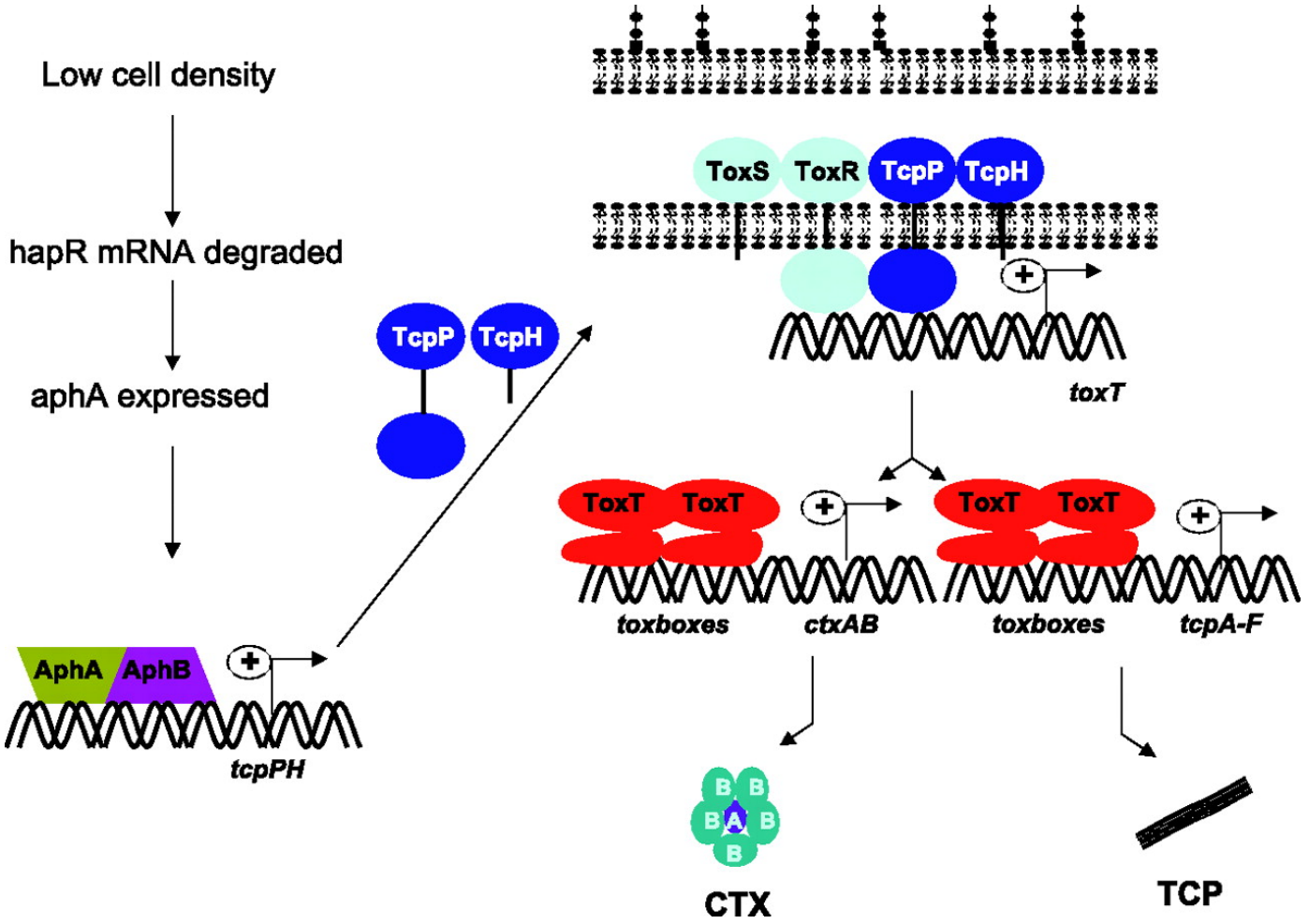
Vibrio cholerae clones



Virulence inducing cascade in Vibrio cholerae

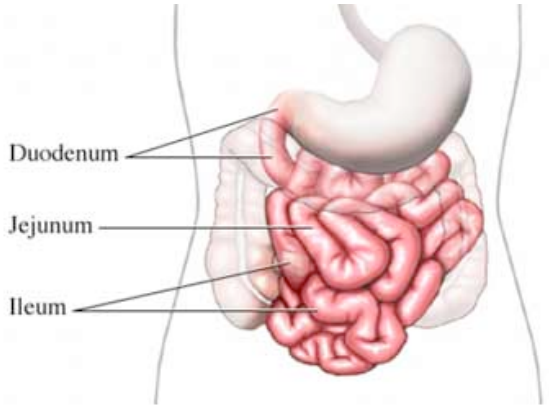


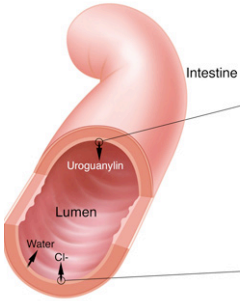
Pathway by which cell density regulates expression of the major *V. cholerae* virulence factors CT and TCP

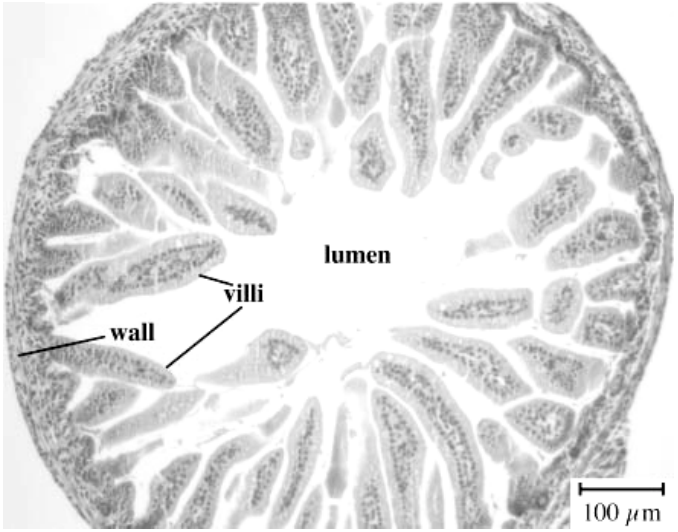


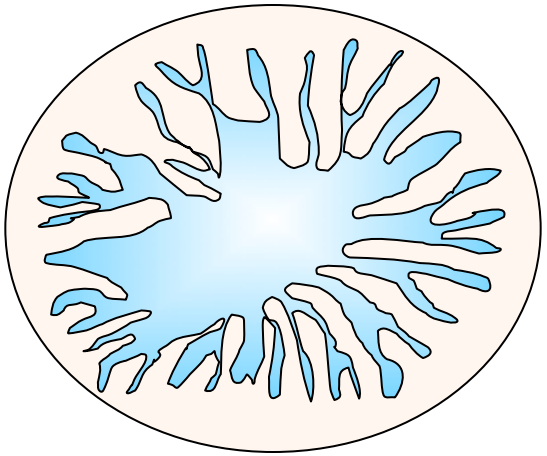
Matson, J. S. et al. 2007. Infect. Immun. 75(12):5542-5549

O_2 gradients encountered by Vibrio cholerae









In vitro cholera toxin production assays

C6706 Strain

AND

Induction-"Standing phase"

(Oxygen tension is lowered)

Growth-"Shaking phase"

(AKI media, 37˚C)

O395 Strain

pH 6.5/ 30˚C/ Shaking

OR

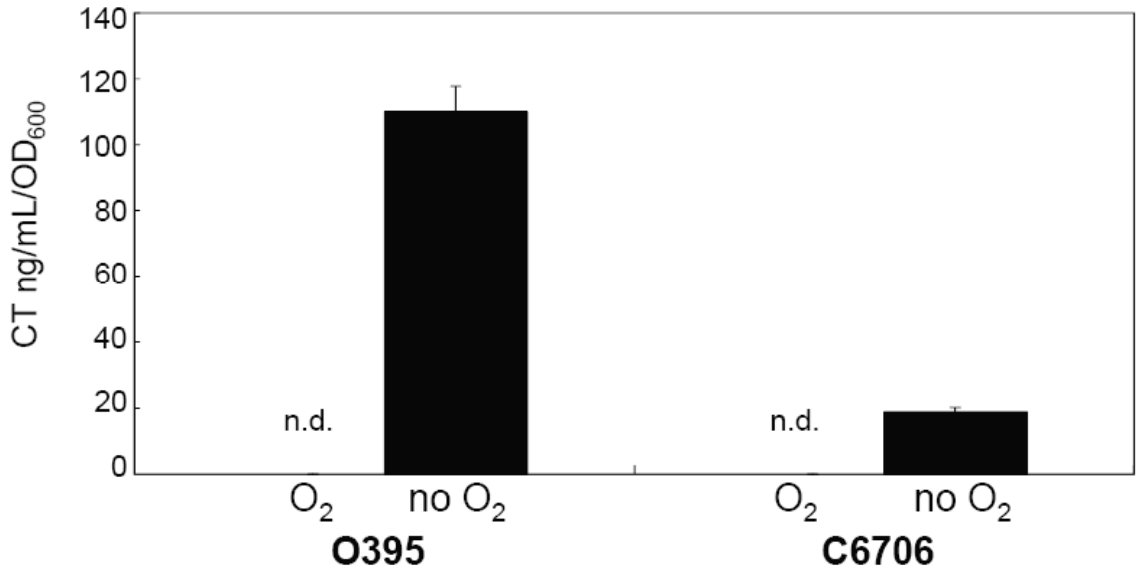
pH 6.5/ 37˚C

Non shaking

Microaerobic/Anaerobic

(LB media)

Low oxygen induces cholera toxin production by Vibrio cholerae



Both the classical O395 and El Tor C6705 strains produce cholera toxin under microaerobic, but not aerobic, conditions.

Prolific replication of V. cholerae in the small bowel during infection

Environmental Aquatic Reservoirs
Estuaries, Ponds and Rivers

Input
~ 100,000 bacteria

Human
Small bowel

Low-oxygen environment
Alternative electron acceptors?

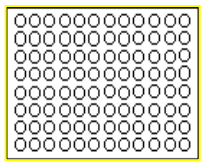
?

Output
~ 20,000,000,000 bacteria

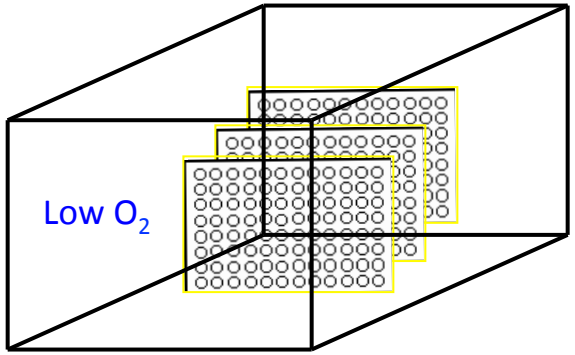
?

Environmental Aquatic Reservoirs

A modified AKI induction method for cholera toxin production



Grow *V. cholerae* in LB media, shaking @ 37˚C for two hours



Inoculate 1:400 into AKI media, incubate at 37˚C for 4.5 hours non-shaking, microaerobic/ anaerobic chamber

After 4.5 hrs:

Read OD_{600}

CT-ELISA

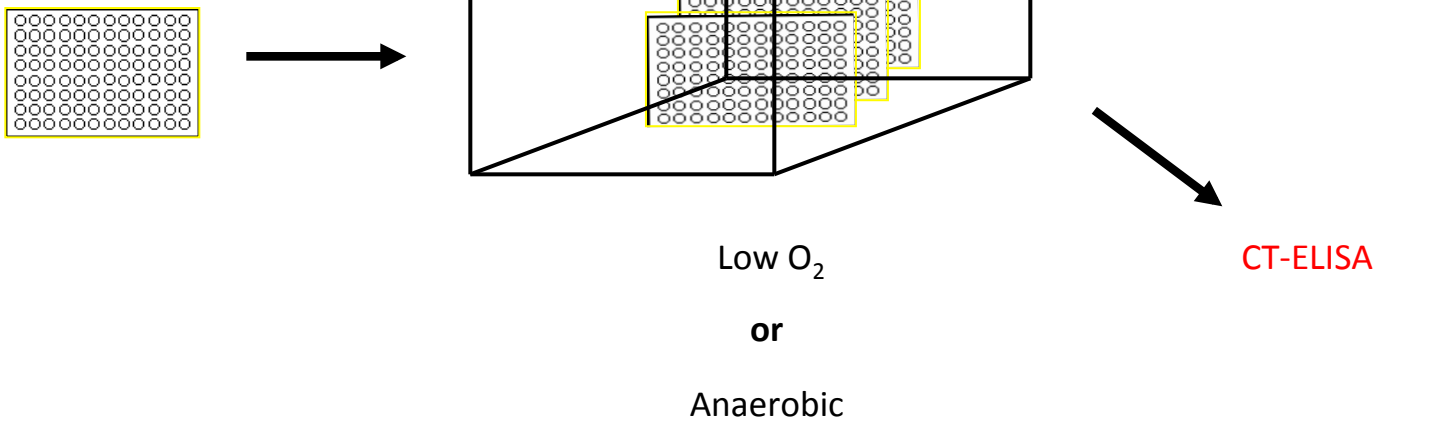
7% O_2 and 5-10% CO_2 = "Microaerobic induction conditions"

Hypotheses

Low oxygen tension encountered by *Vibrio cholerae* in the host induces cholera toxin production and disease

Cholera toxin production is spatiotemporally regulated in the small intestine in response to oxygen and other alternative electron acceptors

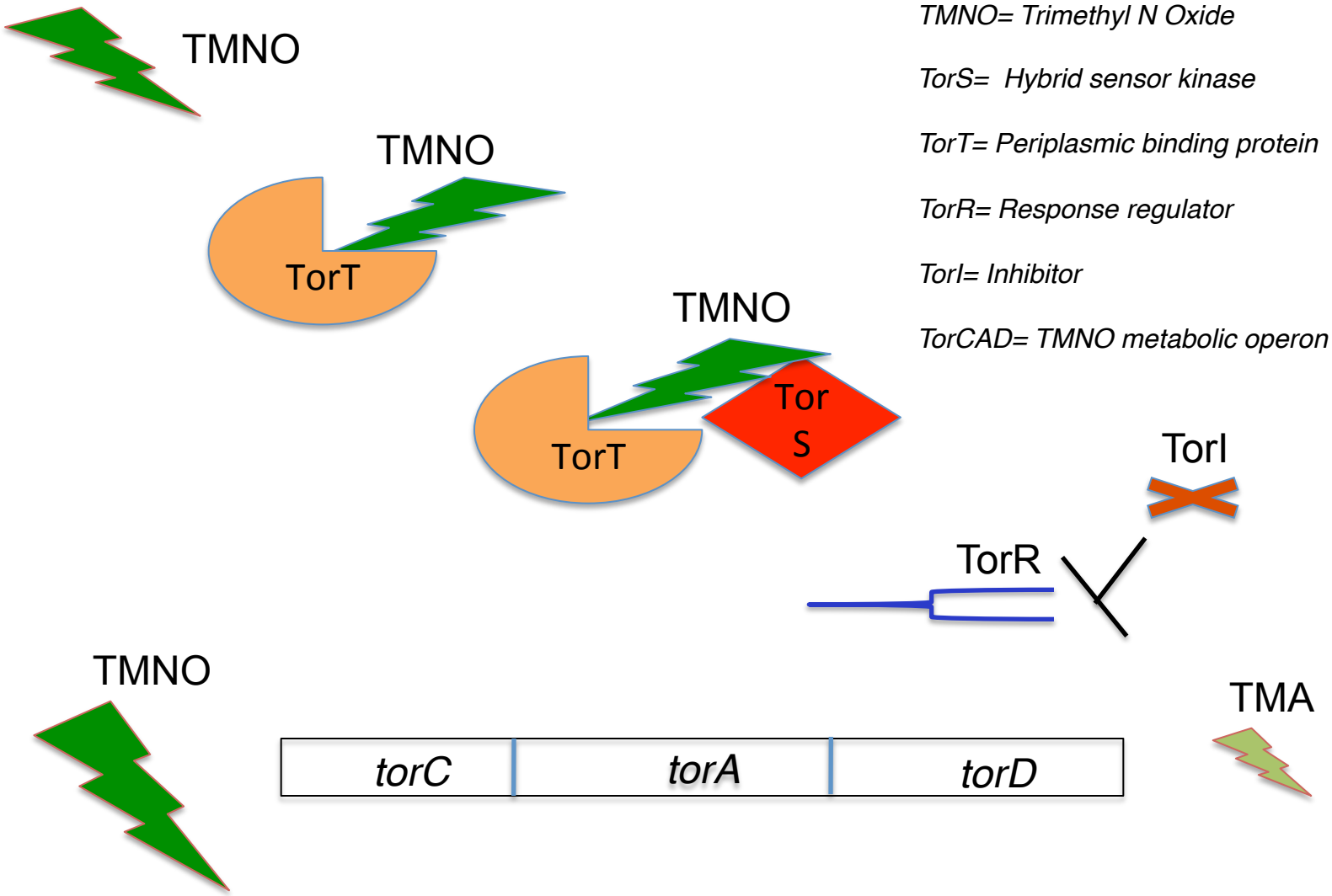
Screen to identify two-component systems regulating cholera toxin production under microaerobic and/or anaerobic conditions



V. cholerae two-component systems identified in the screen

Two – Component System	Sensor/ Regulator
TorS (VCA0709) (Anaerobic metabolism)	Sensor
Kdp (VCAO531) (K^+ transport and turgor)	Sensor
VCAO142	Sensor
VCAO239 (Member of the *E. coli* two-component regulatory system *czcS*/ *czcR* involved in control of cobalt, zinc and cadmium homeostasis)	Regulator (orphan)

Tor system in E. coli during anaerobic respiration



TMNO= Trimethyl N Oxide

TorS= Hybrid sensor kinase

TorT= Periplasmic binding protein

TorR= Response regulator

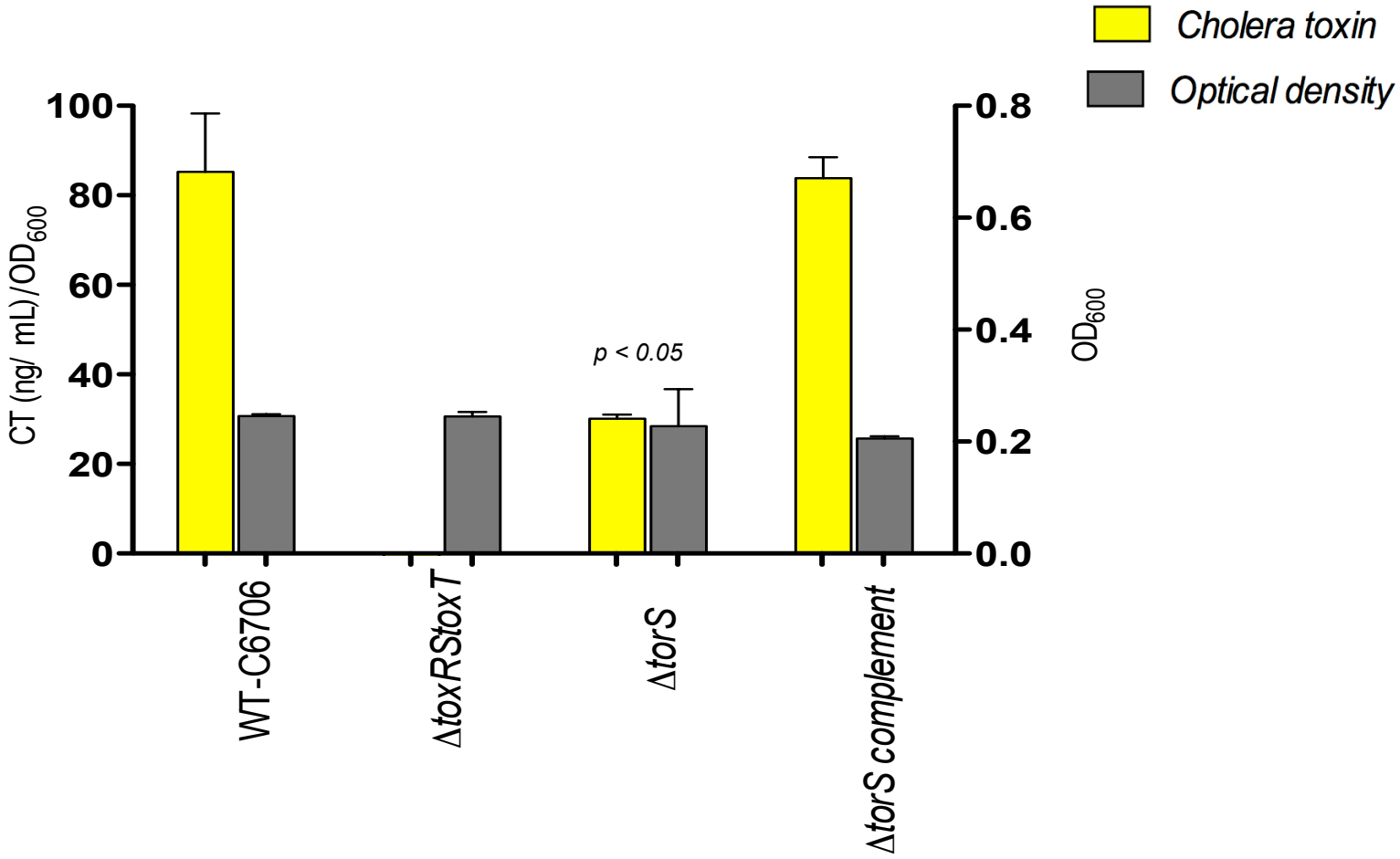
TorI= Inhibitor

TorCAD= TMNO metabolic operon

The Journal of Biological Chemistry, December 15, 2006 , Vol 281, 38189-38199.

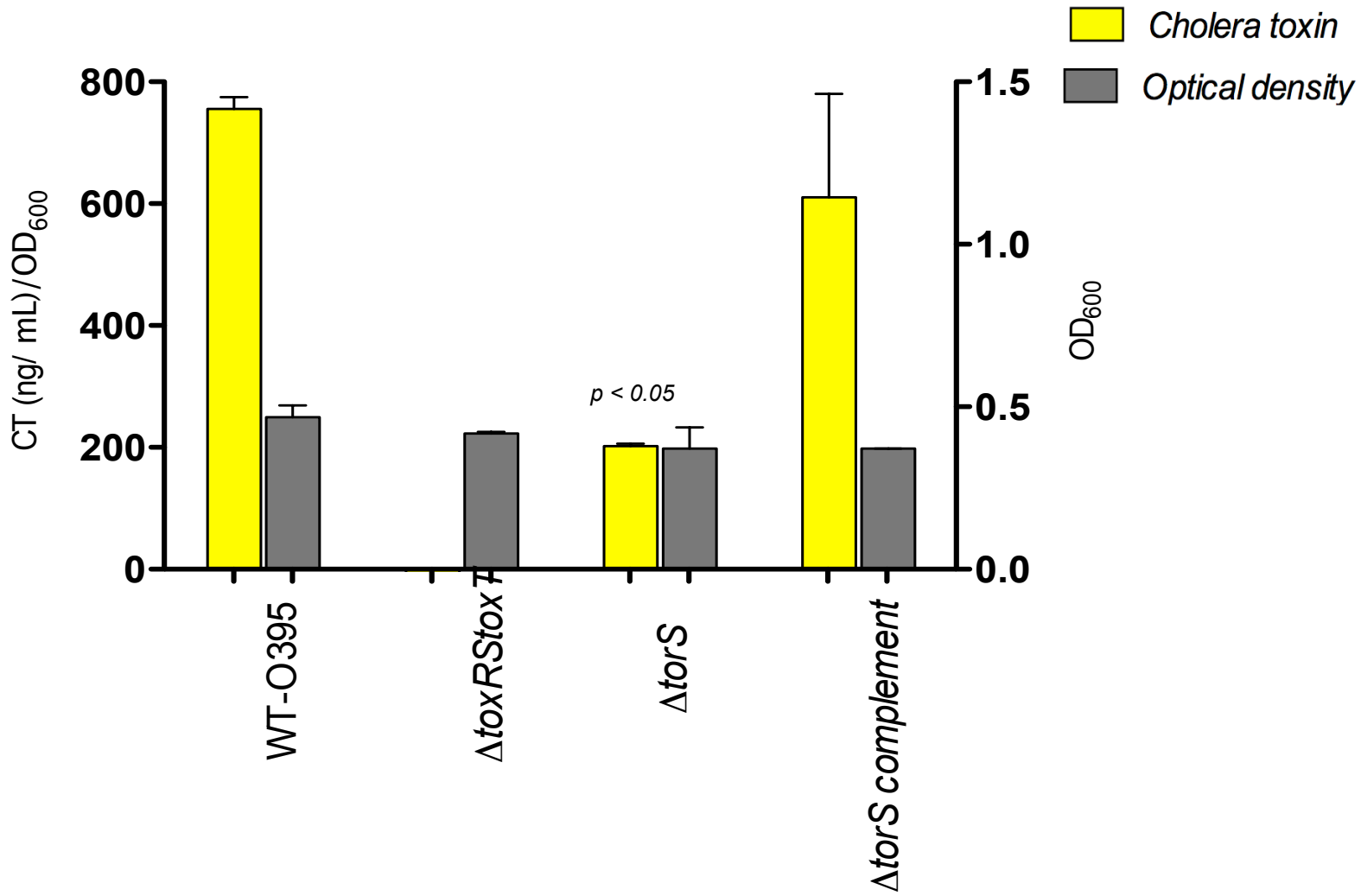
Journal of Bacteriology, April 2001, p. 2691-2695, Vol. 183, No. 8

*torS regulates cholera toxin production in C6706 after microaerobic induction at 37°C/standing in AKI media***



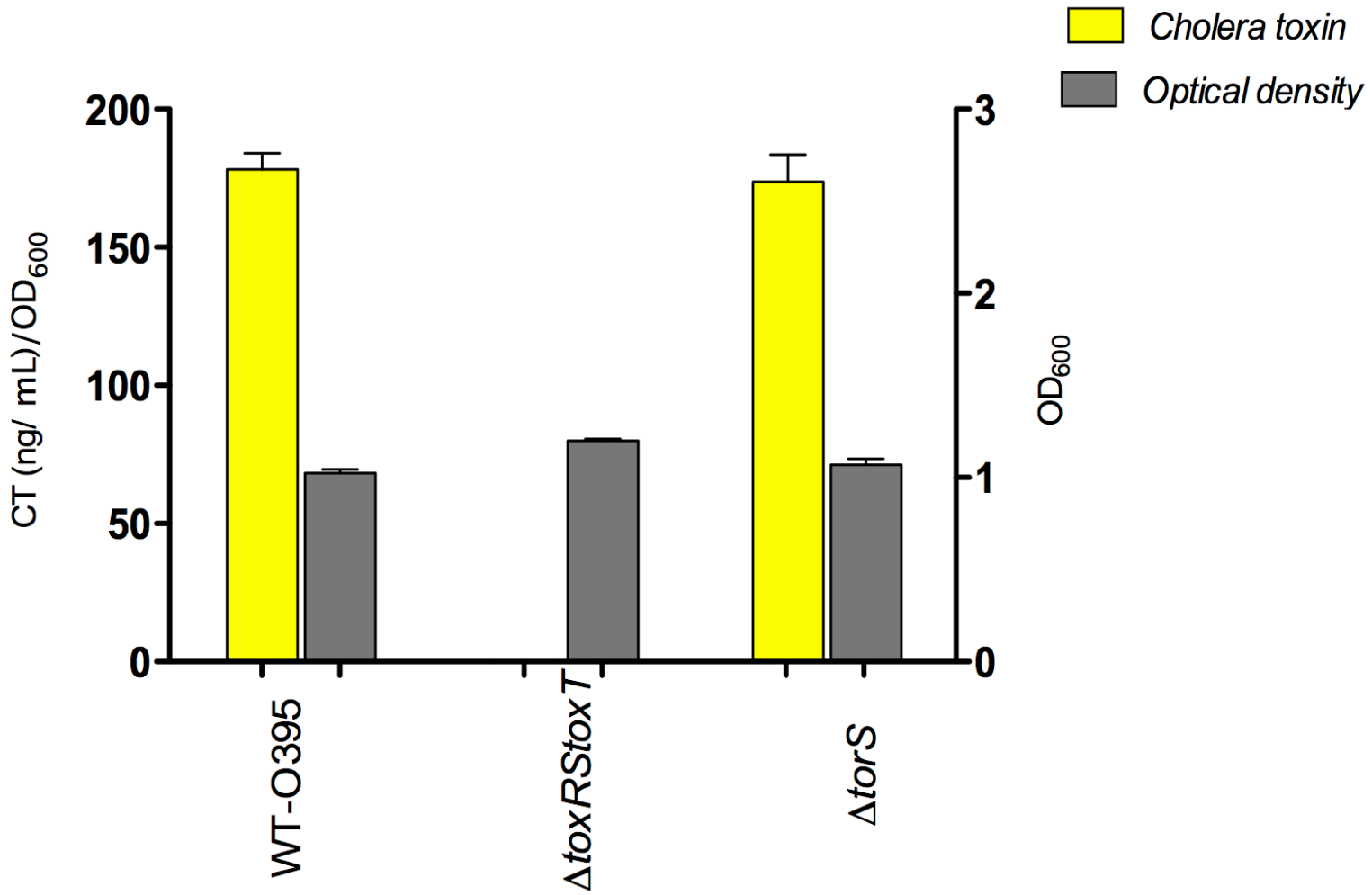
** Representative data from five independent experiments with five technical replicates in each set.

*torS regulates cholera toxin production in O395 strain after microaerobic growth at 37ºC in AKI media***



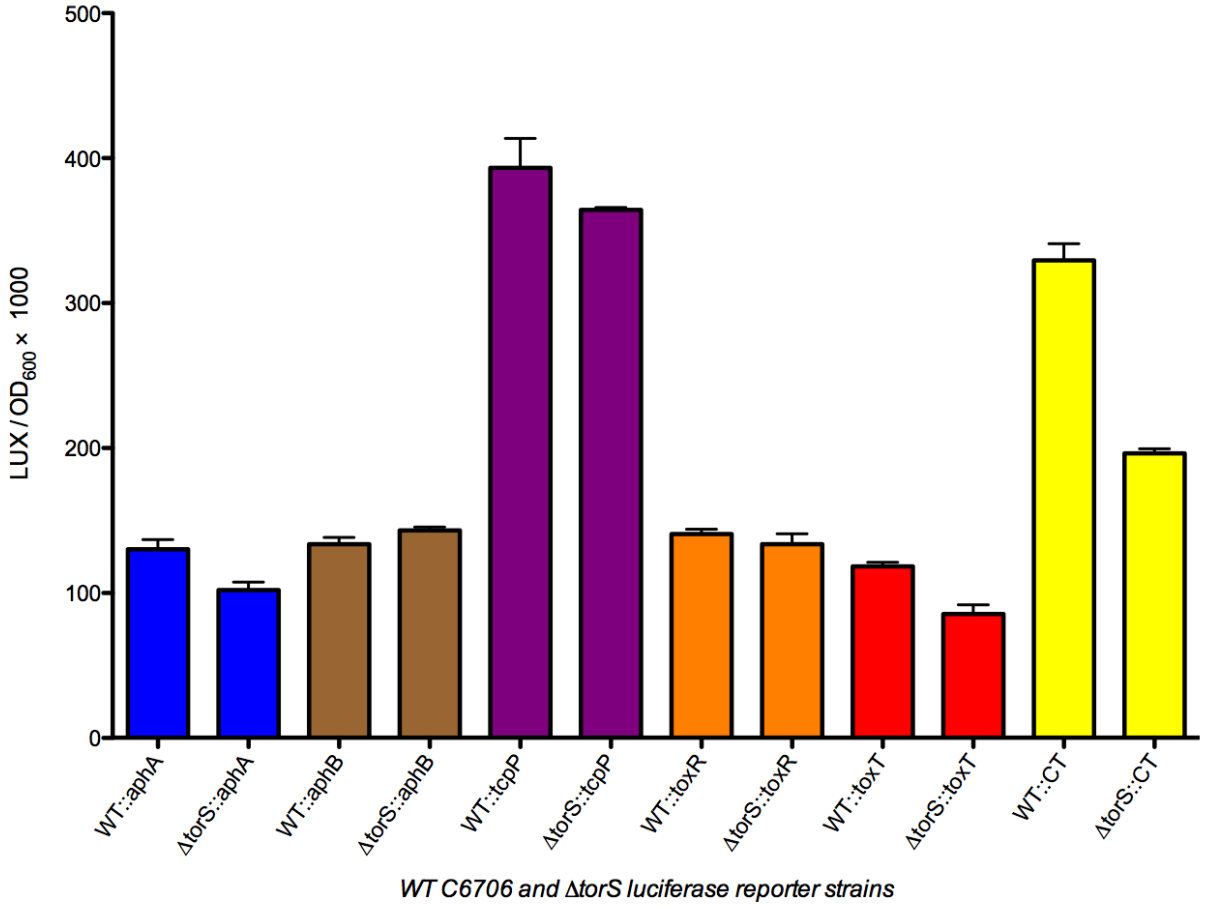
** Representative data from five independent experiments with five technical replicates in each set.

*torS does not affect cholera toxin production in O395 strain after aerobic induction at pH 6.5/ 30° C/ shaking/ LB media***



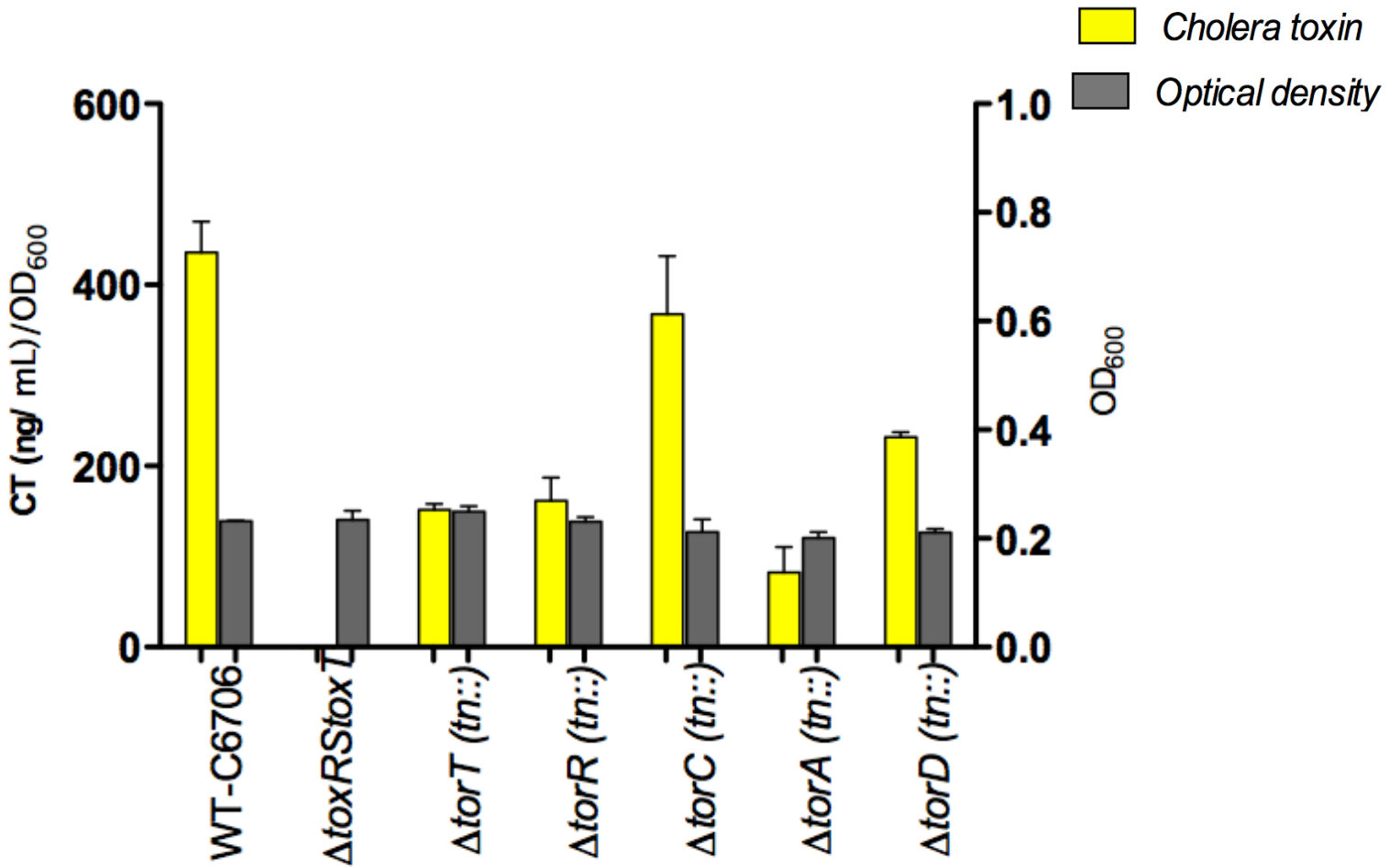
** Representative data from five independent experiments with five technical replicates in each set.

The torS gene may regulate cholera toxin production independent of the canonical virulence-inducing cascade in Vibrio cholerae C6706 strain**



** Representative data from three independent experiments with five technical replicates in each set.

The tor regulon is important for optimal CT production after microaerobic induction at 37°C/standing in AKI media**



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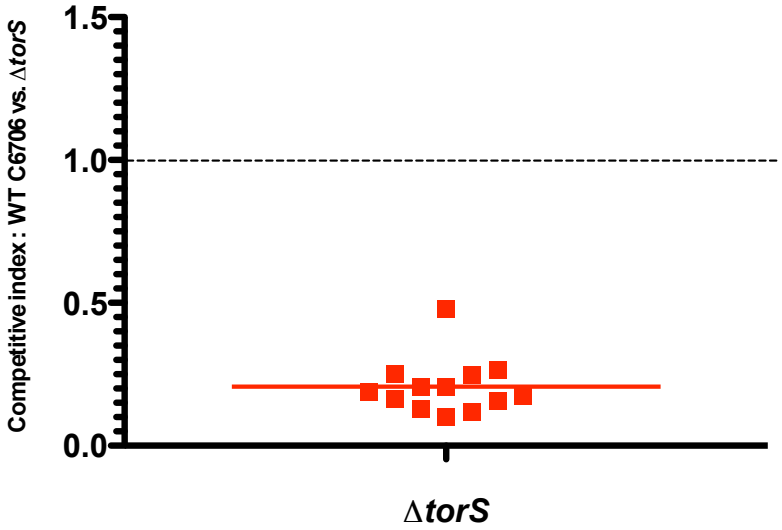
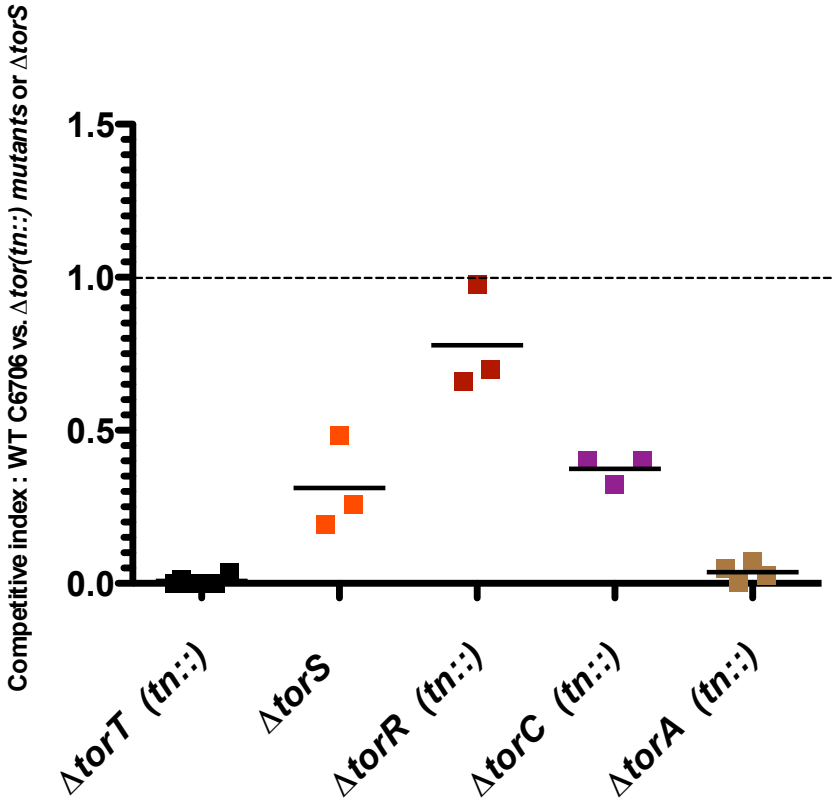
Environmental Aquatic Reservoirs

Essentiality of tor regulon genes for successful colonization of the infant mouse intestine**



Pooled data from three independent experiments

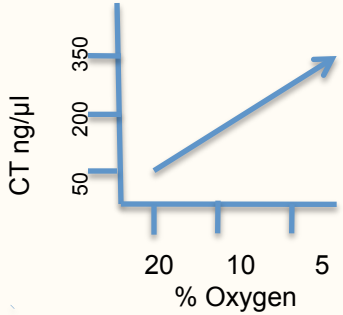


**The tor regulon mutants do not show a growth-defect during in vitro propagation

Summary

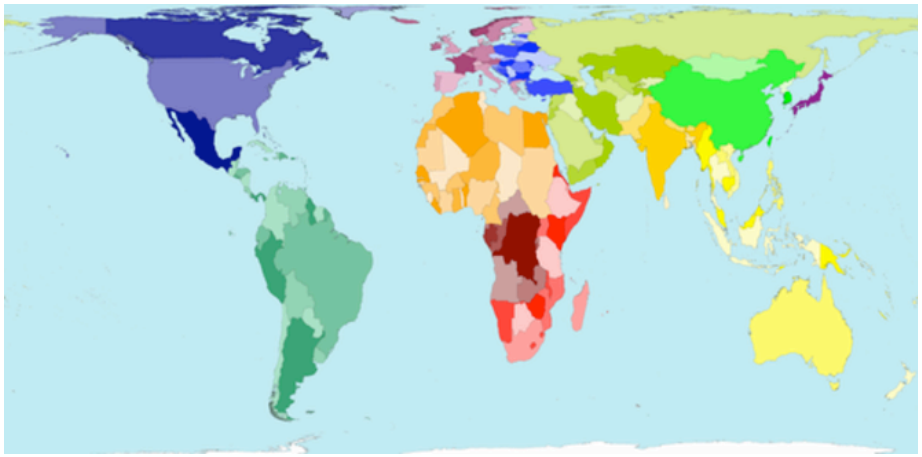
- *V. cholerae* modulates toxin production in response to oxygen availability

low oxygen/anaerobsis serve as signals to turn on toxin production

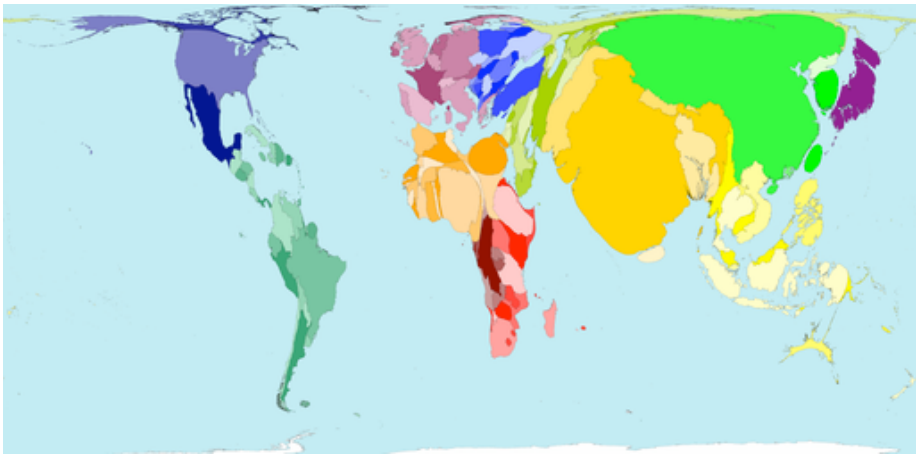


- *torS* is essential for optimal cholera toxin production under low-oxygen conditions
- Mutant strains lacking *torS* or other *tor* regulon genes are severely attenuated *in vivo*
- Availability and the type of alternative electron acceptors play an important role in disease outcome
- *V. cholerae* has fine tuned it's virulence based on the alternative electron acceptors it encounters in the environment

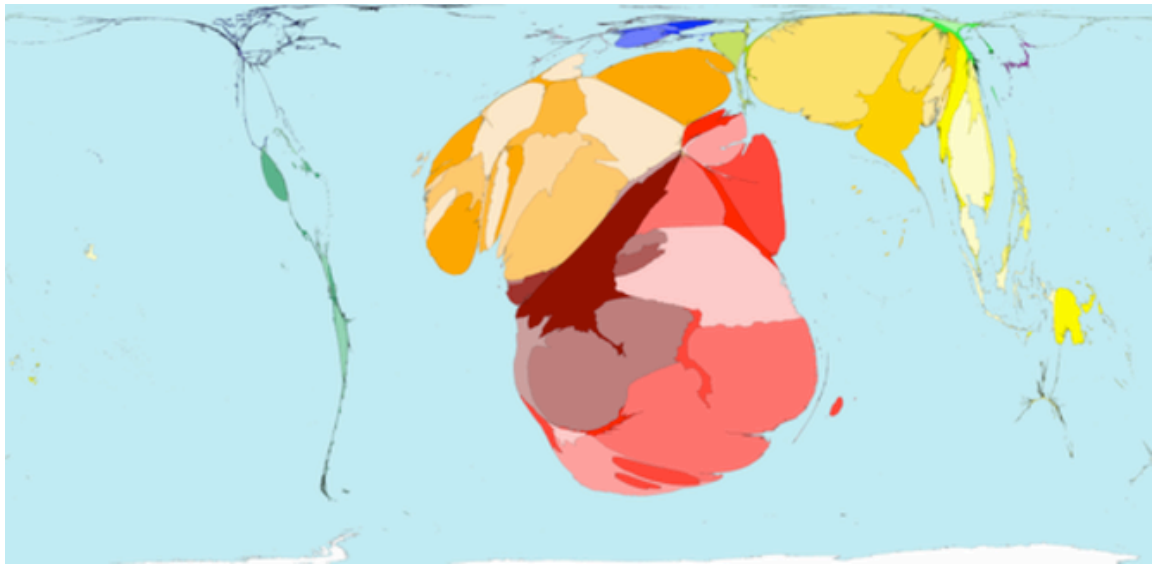
Developing nations and cholera



Population distribution



Cholera cases



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