

Effect of high hydrostatic pressure (HHP) on inactivation of *Vibrio* spp.



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**SEAOHUN International Conference
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Background

□ *Vibrio* spp.

- Gram (-)
- Halophilic bacteria
- Widely occur in marine & estuarine environments





Background

- ***Vibrio* spp.** – the most common bacterial pathogens in seafood associated with human infections

- ***Vibrio* infections:**
 - Foodborne diseases (*V. parahaemolyticus*, *V. cholerae*, *V. vulnificus*)
 - Wound infections & septicaemia (*V. vulnificus*, *V. alginolyticus*)
 - Commonly reported in The US, Asia (Japan, China, Taiwan)
 - Sporadic cases in EU



Background

● Mussels

- The most important bivalves in Europe, mainly cultured in North East Atlantic
- > 34% of the overall EU aquaculture production ([Eurostat, 2017](#))



● Problems

- Mussels – filter feeders → accumulate pathogens (*Vibrio*) during filtration
- Concentration up to 100-fold the amount in surrounding water ([Butt et al., 2004](#))
- Prevalence of *Vibrio* spp. in retail mussels: 41% ([Vu et al., 2018](#))



Background

- **Effective processing methods - HHP:**
 - Inactivate spoilage & pathogenic microorganisms
 - Fruit juices, meat, meat products, shellfish
- **Application of HHP** in inactivation *V. parahaemolyticus* & *V. vulnificus* in oysters & clams ([Kural & Chen, 2008](#); [Mootian et al., 2013](#))



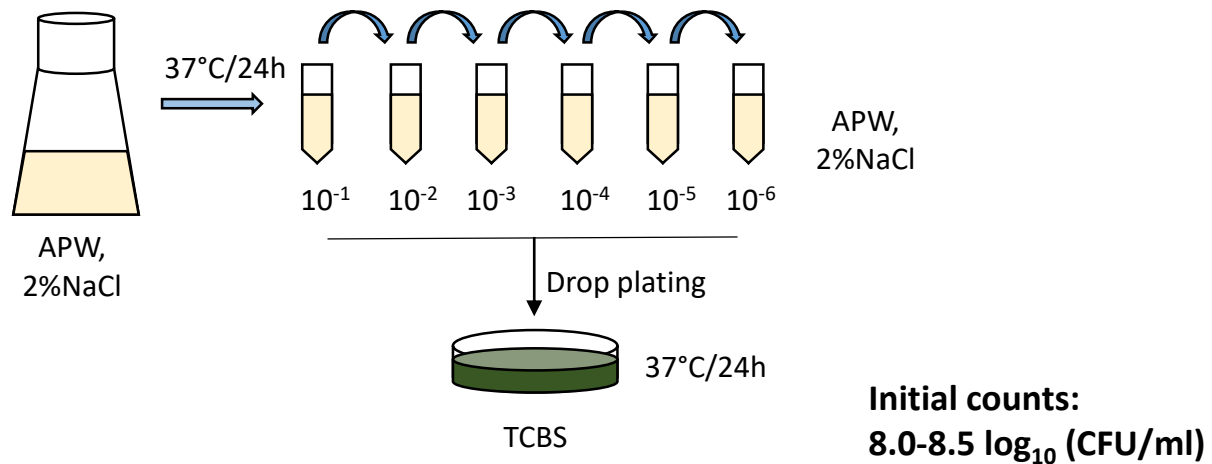
Objectives

- To determine the effect of HHP on inactivation of *Vibrio* spp. in pure culture & mussel homogenates



Bacterial strains & preparation of overnight culture

<i>Vibrio</i> spp.	Strain	Source
<i>V. alginolyticus</i>	ATCC 17749	Clinical strain
<i>V. cholerae</i>	NCTC 4711	Clinical strain
<i>V. parahaemolyticus</i>	RIMD 2210633	Clinical strain
<i>V. vulnificus</i>	V57/10	Clinical strain





HHP treatment of *Vibrio* spp. in pure culture

Overnight *Vibrio* culture
APW, 2%NaCl

Initial counts:
8.0-8.5 log₁₀ (CFU/ml)

1.8 ml cryo-tubes

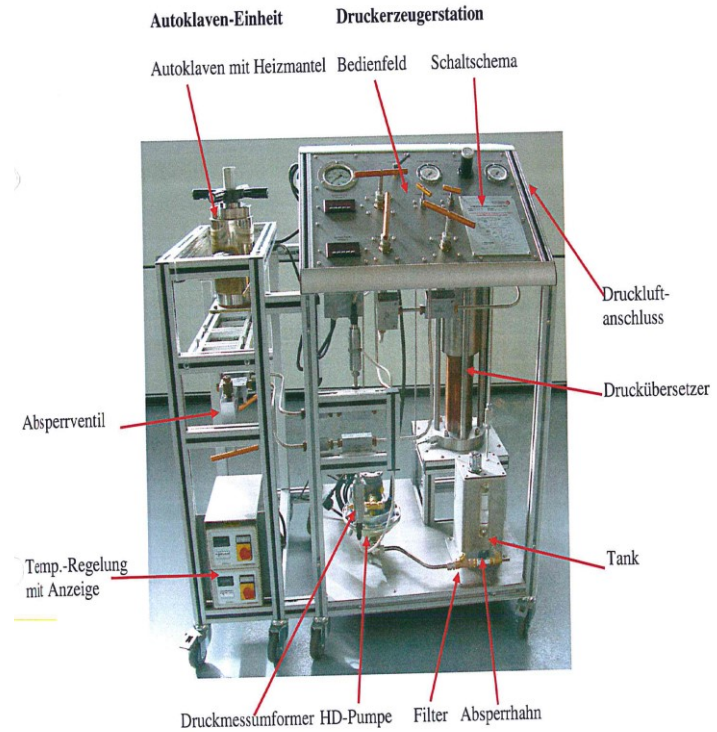
High pressure treatment at 25°C
(5 repetitions each condition)

	1 min	3 min
250 MPa		
350 MPa		
450 MPa		

10⁻¹ 10⁻² 10⁻³ 10⁻⁴ 10⁻⁵ 10⁻⁶

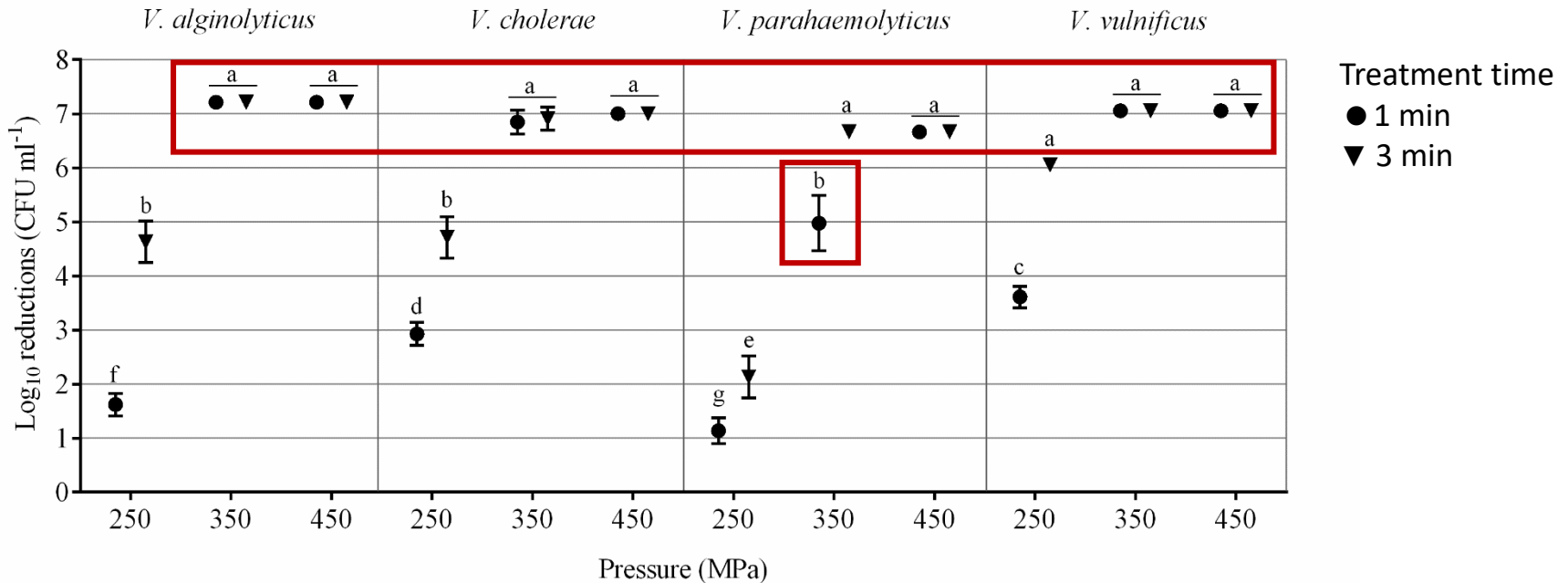
APW, 2%NaCl

Drop plating
37°C/72h
TCBS





HHP treatment of *Vibrio* spp. in pure culture



Note: Groups with different letters are significantly different ($P < 0.05$)

Log reductions of *Vibrio* = $\log_{10} (N_0 / N)$

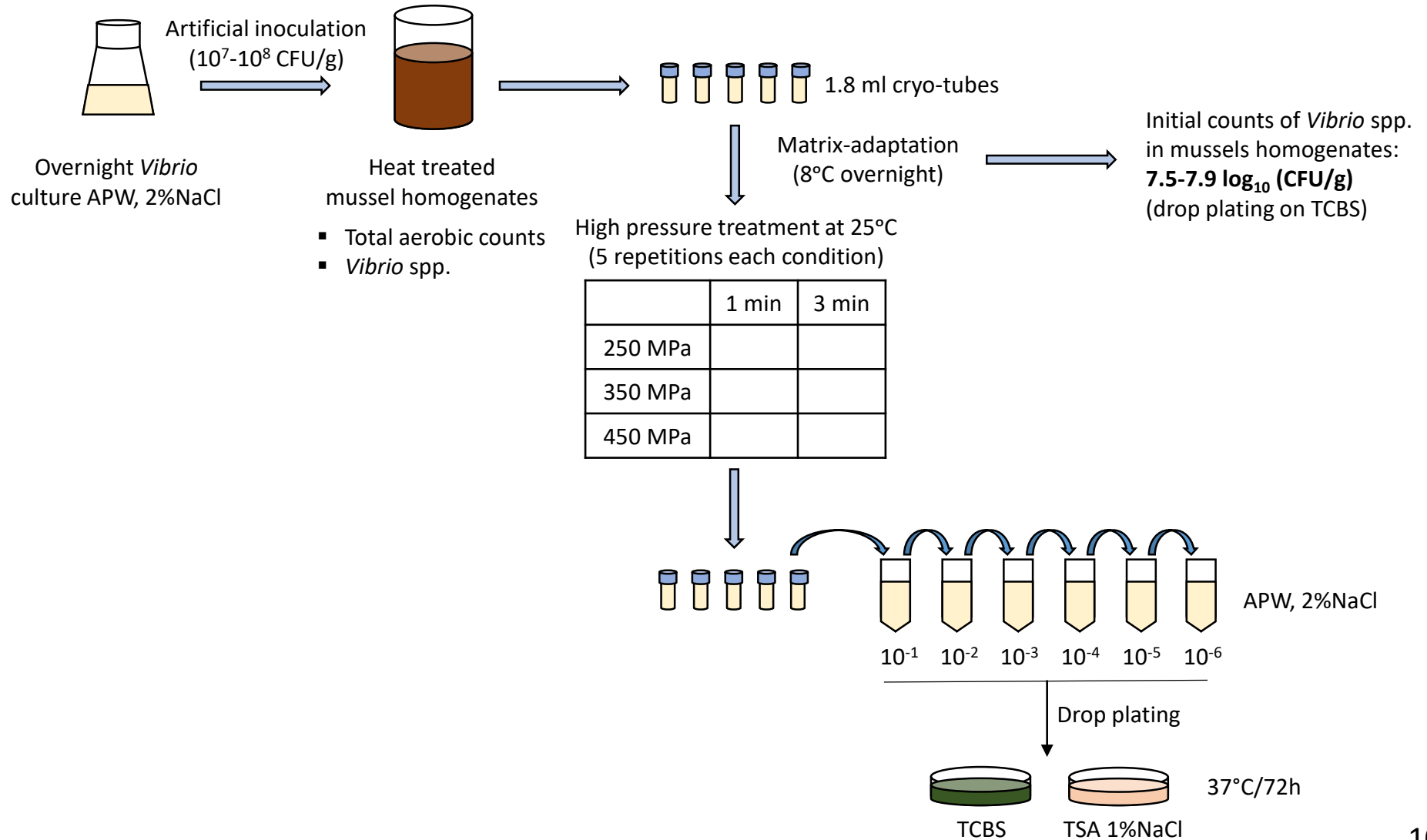
(N_0 = initial count of *Vibrio*, N = number of cell surviving after HHP treatment)

Data are mean values \pm SD

- 350 & 450 MPa for 1 & 3 min completely inactivated *Vibrio* spp. to non-detectable levels (except for *V. parahaemolyticus*)
- *V. vulnificus* - the most susceptible *Vibrio* sp. to HHP



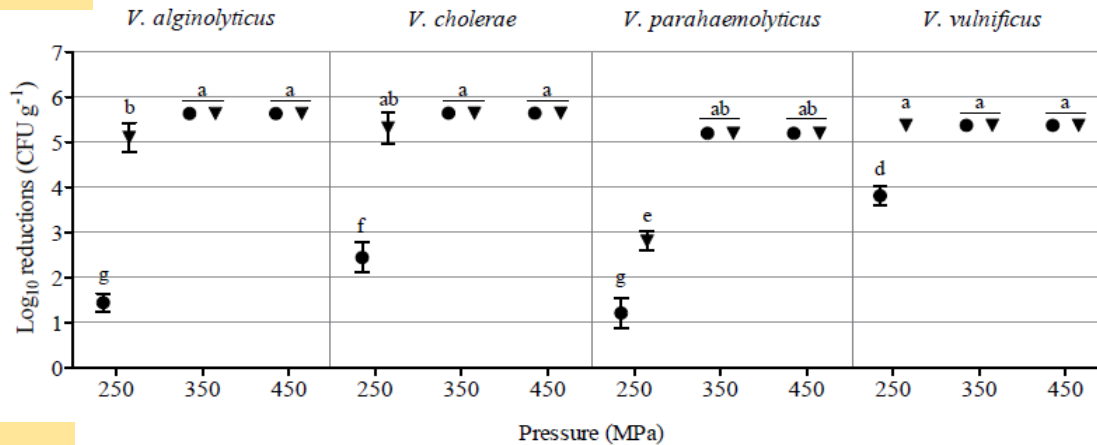
HHP treatment of *Vibrio* spp. in mussel homogenates





HHP treatment of *Vibrio* spp. in mussel homogenates

TCBS agar

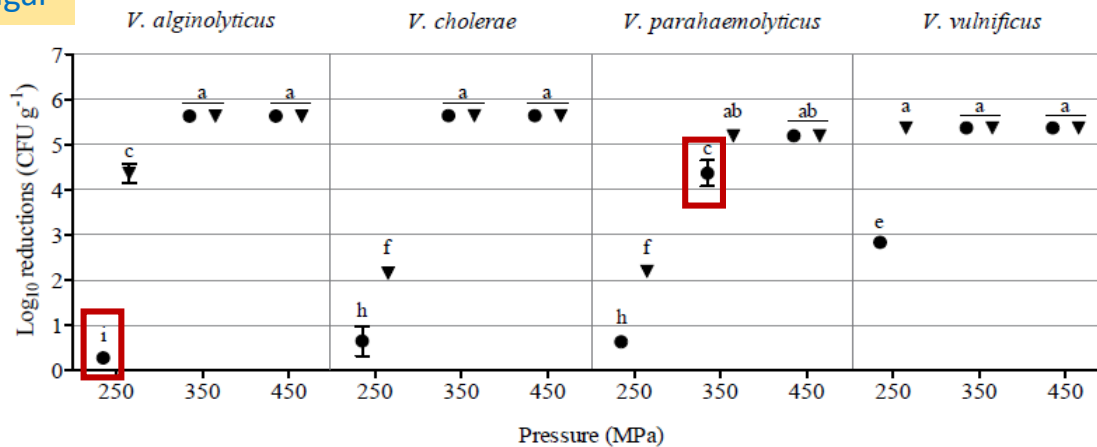


Treatment time

● 1 min

▼ 3 min

TSA agar



- The recovery of *Vibrio* spp. in TSA was significantly higher than in TCBS (particularly at 250 MPa)
- *V. alginolyticus* – the most resistant at 250 MPa for 1 min
- *V. parahaemolyticus* - the most resistant at 350 MPa for 1 min
- *V. vulnificus* - the most susceptible *Vibrio* sp. to HHP

Note: Groups with different letters are significantly different ($P < 0.05$)

Log reductions of *Vibrio* = $\log_{10} (N_0 / N)$

(N_0 = initial count of *Vibrio*, N = number of cell surviving after HHP treatment)

Data are mean values \pm SD



Conclusion

- **To achieve > 5 log reduction of *Vibrio* spp.**
 - 350-450 MPa for ≥ 1 min at 25°C for *V. alginolyticus* & *V. cholerae*
 - 250 MPa for ≥ 3 min or 350-450 MPa for ≥ 1 min for *V. vulnificus*
 - 350 MPa for ≥ 3 min or 450 MPa ≥ 1 min for *V. parahaemolyticus*



Acknowledgment



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BỘ GIÁO DỤC VÀ ĐÀO TẠO
MINISTRY OF EDUCATION AND TRAINING

Thank you for your attention !



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