Tropodithietic acid producing bacteria – A novel tool for improving food safety of molluscan shellfish?

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Publication date:
2012

Document Version
Publisher's PDF, also known as Version of record

DTU Food
National Food Institute

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CONCLUSION

We have showed that *Phaeobacter gallaeciensis* antagonize *Vibrio vulnificus* strains and that it can colonize and persist inside oysters. *P. gallaeciensis* could be a successful candidate for removal of *V. vulnificus* from live mollusks in a new microbiological concept which improve the microbiological safety of bivalves. The concepts of probiotic and bioprotective bacterial cultures are combined by adding a marine, antibacterial bacterium to live mollusks in the depuration step to reduce pathogenic bacteria residing in the animals.

BACKGROUND

Molluscan shellfish are a prime vehicles of food borne diseases as they feed by filtering seawater and thus accumulate human pathogenic bacteria (e.g. *Vibrio*). We **hypothesize** that the pathogens can be removed from oysters by introducing marine, antagonistic bacteria to the live animals. *Phaeobacter gallaeciensis* produces tropodithietic acid (TDA). TDA has a broad antibacterial effect but resistance has not been observed [1]. The **purpose** was to examine the antagonistic effect of a TDA producing bacterium on *Vibrio* strains.

ANTIBACTERIAL EFFECT

Supernatant from *P. gallaeciensis* DSM 17395 was tested in a well diffusion assay and in a minimal inhibitory concentration (MIC) assay against *Vibrio vulnificus* and *Vibrio parahaemolyticus*. Only *V. vulnificus* strains were sensitive to the supernatant, whereas supernatant from a TDA negative mutant MJG-G6 did not inhibit any of the strains (**Table 1**).

Table 1. Sensitivity of *Vibrio* strains to supernatants from *P. gallaeciensis* BS107 and MJG-G6 (TDA negative mutant) determined by well diffusion assay and minimum inhibitory concentration (MIC)

Species	Strain	Clearing zone in well diffusion assay (mm)		MIC value [a)]	
		BS107	MJG-G6	BS107	MJG-G6
Vibrio parahaemolyticus	ATCC 17802	0 ± 0	0	>2	>2
	RIMD2210633	0 ± 0	0	>2	>2
Vibrio vulnificus	DSM 10143	14.5 ± 0.7	0	512	>2
	CMCP6	15 ± 1.4	0	128	>2

Values are means from two independent experiments ± stand. dev.
[a)] The highest dilution of the supernatant which inhibited visible growth

CO-CULTURE EXPERIMENT

P. gallaeciensis DSM17395 and MJG-G6 both inhibited growth of *V. vulnificus* CMCP6 in co-culture experiments in Instant Ocean with *Tetraselmis suecica* (**Figure 1**). Numbers of DSM17395 and MJG-G6 increased from 10^6 to $5x10^6$ cfu/ml in both mono- and co-cultures after seven days. CMCP6 remained at 10^3–10^4 cfu/ml. Nutrient competition between strains could explain inhibition of *V. vulnificus* by both TDA producer and TDA negative mutant.

IN VIVO EXPERIMENT: COLONIZATION

Oysters (*Ostrea edulis*) were inoculated with *P. gallaeciensis* DSM 17395 (10^7 cfu/ml Instant Ocean) which resulted in 10^6 cfu/g oyster after 1 day and this level remained throughout the experiment (4 days) (**Figure 2**).

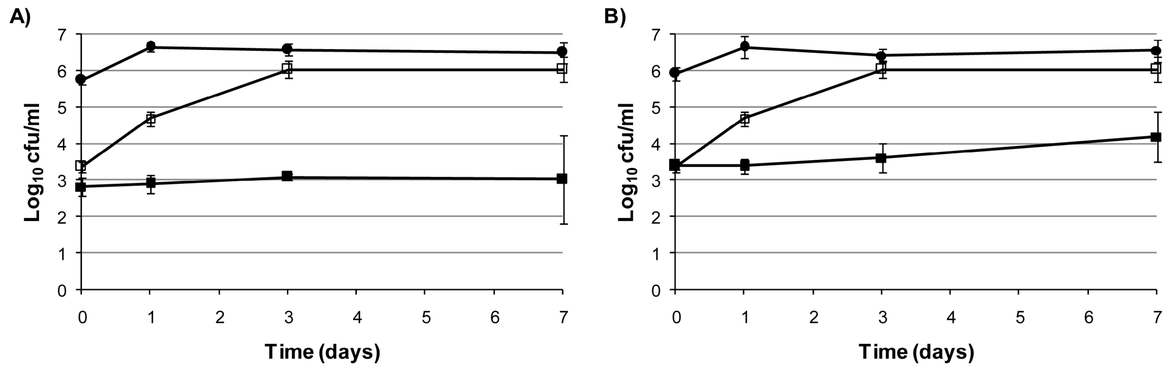

Figure 1. Mono- (□) and in co-culture (■) of *V. vulnificus* CMCP6 in Instant Ocean (3%) with *T. suecica* (10^4 cells/ml) at 15°C with A) *P. gallaeciensis* DSM 17395 or B) *P. gallaeciensis* MJG-G6 (TDA negative mutant) (●). Values are means from two independent experiments with two replicates ± stand. dev.

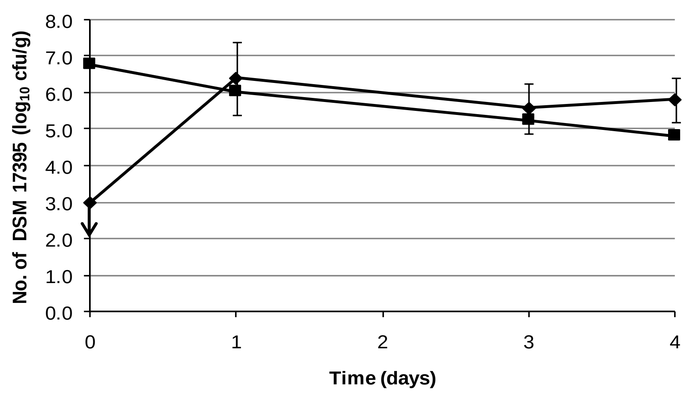

Figure 2. Accumulation of *P. gallaeciensis* DSM 17395 in oysters (◆) and levels in Instant Ocean (3%) (■) at 15°C. Oysters were feed *T. suecica* (10^4 cells/ml) on day 0. Values are means from three replicates ± stand. dev. Arrow: Detection limited.

IN VIVO EXPERIMENT: PERSISTENCE

Oysters were exposed to *P. gallaeciensis* DSM 17395 (10^7 cfu/ml Instant Ocean) for 1 day and moved to fresh Instant Ocean (3%). After 6 days a decrease in numbers of DSM 17395 from 10^6 to 10^4 cfu/g was seen (**Figure 3**). Thus, DSM 17395 persisted in the oysters.

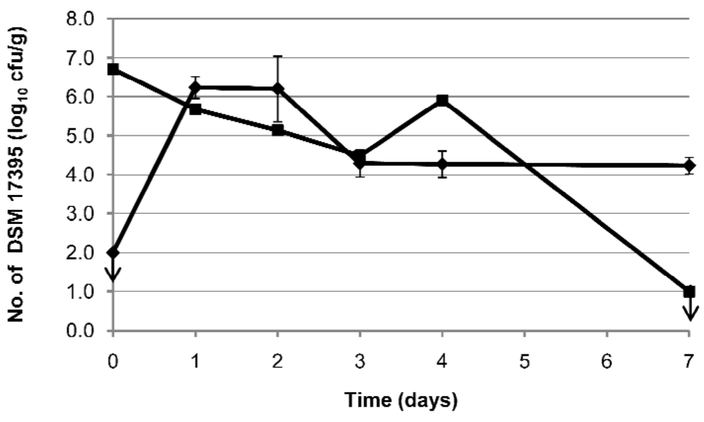

Figure 3. Persistence of *P. gallaeciensis* DSM 17395 in oysters (◆) and levels in Instant Ocean (3%) (■) at 15°C. Day 0-1: DSM 17395 treatment. Day 1-7: Instant Ocean. Oysters were feed *T. suecica* (10^4 cells/ml) on day 0 and 1. Values are means from three replicates ± stand. dev. Arrow: Detection limited.

AFFILIATION
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REFERENCES
[1] Porsby et al. 2011. AAC *55:* 1332-1337

ACKNOWLEDGMENT
Work was supported by Danish Council for Independent Research, Technology and Production Sciences"(11-104551)