

Halobacteriovorax isolated from the Adriatic Sea to challenge *Salmonella*

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Abstract

In the present study, we searched *Halobacteriovorax* strain preying upon *Salmonella* from the seawater of the Adriatic Sea. The *Halobacteriovorax* strain, named M7, was identified using 16S rRNA analysis. The M7 strain predation efficiency was tested against different *Salmonella* and non-*Salmonella* strains, all isolated from food matrices obtained from the Adriatic Sea. Finally, the M7 strain was exposed to *Salmonella enterica* subsp. *enterica* serovar Napoli in challenge tests to evaluate the killing of this specific prey over time. Double-layer agar plating technique was used to enumerate *Halobacteriovorax* and to evaluate its host specificity and predation efficiency. In the 10³ predator/10³ prey challenge test, M7 caused a decrease of *Salmonella* by about 2 log at 24 h compared to the control. In the 10⁷ predator/10⁴ prey challenge test, M7 caused a decrease of *Salmonella* by about 5 log at 24 h compared to the control, and good levels of decrease were obtained even at shorter times. *Halobacteriovorax* strains active against *Salmonella* are rarely present in the Adriatic Sea, Italy seawater. However, the isolate M7 showed high predatory efficiency towards a wide range of *Salmonella* strains. The presence of *Salmonella* in bivalves affects food safety since current decontamination processes are not always effective. M7 may represent a potential candidate for reducing and controlling *Salmonella* contamination in bivalves from harvesting to trade.

Keywords: *Bdellovibrio* and like organisms; bivalves; *Halobacteriovorax*; *Salmonella*

Introduction

Salmonella is the EU's second most common cause of human gastroenteritis (EFSA & ECDC, 2016). Although *Salmonella* is generally not found in aquatic environments, several *Salmonella* serovars are widely distributed in seawater. In more detail, a high prevalence in bivalves, shrimp, clams, and various fish species was reported (Novoslavskij *et al.*, 2016; Rubini *et al.*, 2018; Zahli *et al.*, 2021). Moreover, clinically relevant serovars of *Salmonella* can survive in seawater and within

bivalves for significant periods after just one exposure event (Morrison *et al.*, 2011). However, foodborne illnesses from molluscs' consumption are primarily due to viruses and *Vibrio* spp. strains (Andino and Hanning, 2015; Butt *et al.*, 2004; Iwamoto *et al.*, 2010; Potasman *et al.*, 2002; Rippey, 1994). *Salmonella* infections have been increasingly reported in the last two decades (Amagliani *et al.*, 2012; EFSA & ECDC, 2016). In Italy, mussel farming (*Mytilus galloprovincialis*) has always played the most important role in marine aquaculture because of its high productivity, areal exploitation, and number of farms

(FAO FISHSTAT, 2005). Concerning food safety criteria laid down in EC Regulation 2073/2005 concerning bivalves to be placed on the market, the absence of *Salmonella* in 25 g of flesh and an upper limit of 230 MPN (MPN=Most Probable Number) *E. coli*/100 g sample material are mandatory. Depuration is a controlled process that relies on the ability of bivalves to purge their gastrointestinal contents by filtering clean seawater. Depuration is considered a very effective procedure for eliminating *E. coli* (Baker, 2016) but is far less efficient against *Salmonella* (Barile *et al.*, 2009; Morrison *et al.*, 2011). On the other, innovative post-harvest treatments are expensive, kill bivalves and do not satisfy those consumers who prefer live bivalves (Baker *et al.*, 2016). Biological control may be integrated into conventional systems to increase the efficacy of conventional depuration towards *Salmonella*. *Bdellovibrio* and like organisms (BALOs) are aerobic Gram-negative bacteria in freshwater, seawater, and soil. They belong to the Deltaproteobacteria class, and they are divided into four genera: *Bdellovibrio*, *Bacteriolyticum*, *Peredibacter*, and *Bacteriovorax*, the latter, recently renamed *Halobacteriovorax* (Koval *et al.*, 2015). BALOs prey upon other Gram-negative bacteria, entering the periplasmic space of the host and utilizing its cytoplasmic nutrients for replication (Bratanis *et al.*, 2020). Recent studies reported that *Halobacteriovorax* is capable of containing *V. parahaemolyticus* levels in seawater and oysters at a laboratory scale (Chen *et al.*, 2011; Li *et al.*, 2011; Richards *et al.*, 2012; Williams *et al.*, 2016; Richards *et al.*, 2016; Ottaviani *et al.*, 2018). Moreover, *Halobacteriovorax* has been reported to control *V. parahaemolyticus* load in bivalves during depuration (Li *et al.*, 2011; Richards *et al.*, 2012; Ottaviani *et al.*, 2020b). In the Adriatic Sea of Italy, a *Halobacteriovorax* strain preying specifically upon *V. parahaemolyticus* in subsurface seawater at levels never higher than 10³ PFU per mL was found (Ottaviani *et al.*, 2018, 2020b).

Previous studies reported BALOs' predation upon *Salmonella* strains in marine environments in China and the USA (Lu and Cai, 2010; Richards *et al.*, 2016). Nevertheless, there are currently no studies on the presence in the Adriatic Sea of *Halobacteriovorax* preying specifically on *Salmonella*. The present study evaluated seawater from Central Adriatic, Italy's mussel farming area, for *Halobacteriovorax* preying upon *Salmonella*. Moreover, host specificity and predation efficiency of the isolate *Halobacteriovorax* named M7 towards *Salmonella* and non-*Salmonella* strains, all isolated from bivalves of the Adriatic Sea, were screened. Finally, M7 was tested in challenge predator/prey experiments to monitor predator/prey reduction at selected time points.

Materials and Methods

Prey strains collection

The National Reference Laboratory provided stains used as prey and shown in Table 1 for bacterial contamination of bivalves (IZS Umbria Marche, Ancona, Italy). All strains were isolated from bivalves collected from a mussel growing area site of the Central Adriatic of Italy.

The six repeated experiments on each prey strain gave consistent results.

Isolation of *Halobacteriovorax* from seawater

Water sampling

The analysis was performed monthly from June to December 2018 on seawater collected from a mussel-growing site in Central Adriatic, Italy. At the time of collection, the temperature and salinity of the water samples were monitored. Salinity remained steady between

Table 1. Preys assayed in this study with *Halobacteriovorax* M7.

Prey strains	Origin	Susceptibility to M7
<i>E. coli</i>	Mussels, Adriatic Sea, Italy	+ *
<i>E. coli</i>	Mussels, Adriatic Sea, Italy	+
<i>Salmonella enterica</i> subsp <i>enterica</i> ser. <i>Napoli</i>	Mussels, Adriatic Sea, Italy	+
<i>Salmonella enterica</i> subsp <i>enterica</i> ser. <i>Derby</i>	Mussels, Adriatic Sea, Italy	+
<i>Salmonella enterica</i> subsp <i>enterica</i> ser. <i>Typhimurium</i>	Mussels, Adriatic Sea, Italy	+
<i>Salmonella enterica</i> ser. <i>Typhimurium</i> monophasic variant 1, 4 [5], 12:1	Mussels, Adriatic Sea, Italy	+
<i>V. parahaemolyticus</i>	Mussels, Adriatic Sea, Italy	—§
<i>V. parahaemolyticus</i>	Mussels, Adriatic Sea, Italy	—
Non O1/O139 <i>V. cholerae</i>	Mussels, Adriatic Sea, Italy	—
Non O1/O139 <i>V. cholerae</i>	Mussels, Adriatic Sea, Italy	—

*Positive.

§Negative.

38 and 39 ppt. Seawater was immediately transported to the laboratory in an insulated cooler at ambient temperature. After delivery to the laboratory, the seawater sample was analyzed within 4 h.

Halobacteriovorax isolation

A strain of *Salmonella enterica* subsp. *enterica* ser. Napoli was used as the primary host for isolating *Halobacteriovorax* from seawater. Isolation of presumptive *Halobacteriovorax* in 7.5 mL aliquots of 0.45 µm filtered seawater was performed on a layer of *Salmonella* host cells grown in Pp20 agar (polypeptide peptone supplemented with Bacto agar) by double-layer agar plating technique (Richards *et al.* 2012; 2016). The plates were incubated at 26°C, and plaques developed between 3 and 10 days and progressively increased in size were taken to be confirmed as *Halobacteriovorax* by 16S rRNA analysis.

Halobacteriovorax identification

Genomic DNA of potential *Halobacteriovorax* strains isolated from seawater was extracted for molecular identification by a standardized protocol (Ottaviani *et al.*, 2018, 2020a, 2020b). For this purpose, five individual plaques appearing on the plates of each presumptive positive sample at the highest dilution were removed and resuspended in 100 µL of sterile double-distilled water and vortexed at high speed. The liquid phase was transferred to a new tube and boiled for 3 min. The 16S rRNA was amplified using specific primers for the *Bacteriovorax* 16S rRNA gene (Bac676F primer: 5'-ATT TCG CAT GTA GGG GTA-3'; Bac1442R primer: 5'-GCC ACG GTT CAG GTA AG-3') (Davidov *et al.*, 2006; Richards *et al.*, 2013). The reaction mix, in a final volume of 50 µL, consisted of 5X PCR Buffer (GoTaq® Flexi DNA Polymerase, Promega, Madison, USA), 25 mM of MgCl (GoTaq® Flexi DNA Polymerase, Promega, Madison, USA), 2.5 mM of dNTPs, 25 µM of Primer BAC676-F, 25 µM of Primer BAC1442-R, 5U/µL of Taq (GoTaq® Flexi DNA Polymerase, Promega, Madison, USA) and 4 µL of sample. The PCR conditions were as follows: 1 cycle at 94°C for 1 min for initial denaturation, 45 cycles at 94°C for 1 min, 56°C for 1 min, and 72°C for 1 min for denaturation, annealing and extension. The PCR products were electrophoresed on 1% agarose gel and were detected using a UV transilluminator. Samples showing a band of 700 bp were considered *Halobacteriovorax*. PCR products from a single band were purified for each positive sample using the High Pure PCR Product Purification kit Roche Diagnostics (GmbH, Mannheim, Germany). Sequencing analysis was performed using the reverse primer BAC1442R and ABI Prism® BigDye® Terminator v1.1 Cycle Sequencing kit (Applied Biosystems™, Life Technologies, USA), according to the manufacturer's instructions. Sequenced products were analyzed in an automated capillary sequencer ABI Prism® 310 Genetic Analyzer (Applied Biosystems™, USA). Nucleotide

sequences were manually edited, aligned, and analyzed using CLC genomics workbench V.12 (Qiagen Bioinformatics).

Host specificity and predatory efficiency of M7

Two- to three-day enrichments were filtered through a 0.45- µm-pore-size Millex HV syringe filter (Millipore Corp., Billerica, MA) to remove primary prey, allowing the passage of the smaller predator. Host specificity and predator efficiency were determined by monitoring its ability to form clear lytic haloes with double layer agar plating technique on a layer of prey at 26°C (Ottaviani *et al.*, 2018; Richards *et al.*, 2012; 2016).

Preliminary study to evaluate the optimal predator/prey ratio for M7/*Salmonella* challenge experiments

To choose the most effective predator/prey ratio, test flasks with 50 mL ASW (ASW=Artificial Seawater) were inoculated with the following predator/prey concentrations: 10³ PFU/ 10³ CFU per mL, (PFU=plaque-forming unit; CFU=colony-forming unit) 10⁴ PFU/ 10³ CFU per mL, 10⁵ PFU/ 10⁴ CFU per mL, 10⁶ PFU/ 10⁴ CFU per mL, 10⁷ PFU/ 10⁴ CFU per mL, 10⁷ PFU/ 10⁵ CFU per mL, 10⁷ PFU/ 10⁶ CFU per mL. The same prey concentrations were inoculated into ASW, without M7, as a control for each test microcosm. Cultures were incubated at 26°C on a shaker for 6 h. At 0 and 6 h, the prey counts in test and control microcosms were performed. Each experiment was replicated twice. The difference in the prey counts of duplicate experiments was consistently within 0.5 log, the repeatability limit we had defined based on previous studies (Ottaviani *et al.*, 2018). The average of the counts was calculated, and the log was transformed. Finally, the log difference of the test and control counts for each predator-prey ratio was calculated.

Challenging M7/*Salmonella enterica* subsp. *enterica* ser. Napoli

Two challenge experiments were performed using those predator/prey ratios that in the preliminary study had allowed the maximum prey reductions to be obtained.

First, the test flask contained 50 mL sterilized ASW with predator (average concentration 1×10³ PFU per mL) and prey (average concentration 1×10³ CFU per mL). The second test flask contained 50 mL sterilized ASW with predator (average concentration 1×10⁷ PFU per mL) and prey (average concentration 1×10⁴ CFU per mL). The same prey concentrations were inoculated into ASW, without predator, as control. Cultures were incubated at 26°C on a shaker for 48 h. The prey in test and control microcosms was monitored by bacterial plate counts at

0, 3, 6, 24, and 48 h. The double-layer agar plating technique enumerated the Predator at 0, 3, 6, 24, and 48 h (Richards *et al.*, 2012).

Statistical analysis

Each experiment was repeated in three separate trials, each in duplicate (n=6).

Results of microbiological analyses were reported as mean values (log-transformed) \pm standard deviation. The significant differences in predator and prey counts were determined by the student's t-test (t) with error probability (p) <0.05 .

Results

Isolation of Halobacteriovorax

Plaques were observed in double-layered agar assays from two of the examined seawater samples, one collected in July and the other in August 2018. However, only five plaques that emerged in the July 2018 seawater sample were confirmed by PCR as Halobacteriovorax. The 16S rRNA sequences obtained from the five plaques were identical, and they were considered a new isolate. The new strain, M7, was submitted to GenBank with the accession number MT159667.1.

Host specificity and predatory efficiency of M7

M7 was also tested to verify its host specificity and predatory efficiency against *Salmonella* and non-*Salmonella* strains isolated from bivalves farmed in the Adriatic Sea, Italy (Table 1). The six repeated experiments on each prey strain gave consistent results. M7 could attack all *Salmonella* and *E. coli*-tested strains. The lysis plaques became visible for all *Salmonella* and *E. coli* strains after 72 h. Then they expanded, reaching a maximum after 5 days of incubation at 26°C with a diameter between 7 and 9 mm in diameter, similar to that obtained with primary prey. M7 did not show lytic activity towards the *Vibrio* strains used for the test, as the plaques were never detected, even by extending the incubation to 10 days.

Preliminary study to evaluate the optimal predator/prey ratio for the M7/Salmonella challenge experiments

The difference in prey counts of duplicate experiments was consistently within 0.5 log. The highest prey reductions were obtained with 10^7 PFU/ 10^4 CFU and 10^3 PFU/ 10^3 CFU per mL predator/prey ratio (Table 2).

Table 2. Effectiveness of *Halobacteriovorax* M7 at reducing the level of *Salmonella enterica* subsp. *enterica* ser. Napoli ratio after 6 h at 26 °C.

PFU predator / CFU prey per ml	Differences in the test and control prey count (Log transformed) at 6 h
$10^3/10^3$	1.50
$10^4/10^3$	1.00
$10^5/10^4$	1.28
$10^6/10^4$	1.24
$10^7/10^4$	4.21
$10^7/10^5$	1.23
$10^7/10^6$	0.11

Challenging M7/Salmonella enterica subsp. enterica ser. Napoli

Results of challenge experiment 1 with 10^3 PFU/ 10^3 CFU per mL predator/prey ratio are shown in Figure 1. In the test, predator concentration remained at the same experimentally added level from 0 to 6 h. It increased by about 1 log, from 10^3 to 10^4 PFU per mL, between 24 and 48 h. In the test, prey concentration decreased by about 1 log, from 10^3 to 10^2 CFU per mL, between 0 and 6 h, and then increased until it reached the maximum level of 10^9 CFU per mL at 48 h. In control, prey concentration increased between 0 and 48 h, reaching the maximum level of 10^9 CFU per mL. Lower *Salmonella* level was observed in the test than in the control from 3 at 24 h, with a significant difference of about 1.5 log at 6 h ($t=26.0309$; $p<0.0001$) until reaching the greatest significant difference by about 1.7 log at 24 h ($t=7.3545$; $p<0.0001$).

The results of challenge experiment 2 with 10^7 PFU/ 10^4 CFU per mL predator/prey ratio are shown in Figure 2. In the test, predator concentration remained at the same experimentally added level from 0 to 6 h. It increased by about 1 log, from 10^7 to 10^8 PFU per mL, between 24 and 48 h. In the test, prey concentration decreased by 4 logs, from 10^4 to <10 CFU per mL, between 0 and 6 h, and then increased to about 10^4 CFU per mL to 24 h until it reached the maximum level of about 10^8 CFU per mL at 48 h. In control, prey concentration increased between 0 and 48 h reaching the maximum level of about 10^8 CFU per mL. Lower *Salmonella* level was observed in the test than in control from 3 at 24 h with a significant difference of about 2.5 log already at 3 h ($t=5.1285$; $p=0.0004$), which increases again to 6 h (about 4 log) ($t=12.7742$; <0.0001) until reaching the greatest significant difference by about 5 log at 24 h ($t=26.9984$; $p<0.0001$).

Using a predator/prey ratio of 10^7 PFU/ 10^4 CFU per mL, Halobacteriovorax M7 was able to contain prey level growth more efficiently than using the predator/prey ratio

of 103 PFU/103 CFU per mL, with a significant reduction with respect to the control already in the first hours.

Discussion

Indigenous BALOs against pathogenic vibrios are present in seawater from mussel farming areas of Central Adriatic, Italy. Probably, BALOs play a physiological role as natural modulators (Ottaviani et al., 2018; 2020a). The seasonal trend of BALOs in the Adriatic Sea is coherent with previous studies in the Atlantic and Pacific Oceans. However, our BALOs against vibrios natural levels are higher than those (Richards et al., 2013). Only one previous work has reported the isolation of *Halobacteriovorax* using *Salmonella* as primary prey from a low-salinity (5-ppt) water sample collected from a tidal river in the United States (Richards et al., 2016). To date, there are no studies about the presence in the marine environment of *Halobacteriovorax* specific against *Salmonella*. In this study, seawater from the mussel growing area of Central Adriatic, Italy, was analyzed for the presence of *Halobacteriovorax*, able to prey on *Salmonella*. A strain of *Salmonella enterica* subsp. *enterica* ser. Napoli was chosen as a primary prey because this serovar has recently caused waterborne and foodborne outbreaks in Italy (Sabbatucci et al., 2018).

Our results demonstrate that, unlike what was found for vibrios, *Halobacteriovorax* preying upon *Salmonella* is rarely found in the Adriatic Sea, at least in the limited period covered by our investigation. This is plausible because, while vibrios are native bacteria of the aquatic environment, the presence of *Salmonella* is sporadic and mainly linked to fecal contamination of marine waters (Novoslavskij et al., 2016; Rubini et al., 2018). The same trend of the prey is presumed to have *Halobacteriovorax* active toward *Vibrio* or *Salmonella*. However, we intend to extend the period and the marine area of study to have more information on the possibility that other strains of *Halobacteriovorax* versus *Salmonella* may be present in the Adriatic Sea. M7 showed host specificity for members of the Enterobacteriaceae, being active against *Salmonella* and *E. coli*. However, it could not prey on *V. cholerae* and *V. parahaemolyticus*, i.e., bacteria belonging to *Vibrionaceae*. This result disagrees with what was previously reported, i.e., that *Halobacteriovorax* strains isolated against *E. coli* and *S. Typhimurium* in marine seawater from Delaware Bay had a broader host range than the strains originally isolated against *V. parahaemolyticus* (Richards et al., 2016). M7 showed excellent predatory efficiency, attacking other different *Salmonella* strains than *Salmonella enterica* subsp. *enterica* ser. Napoli, i.e., *Salmonella enterica* subsp. *enterica* ser. Derby, *Salmonella enterica*

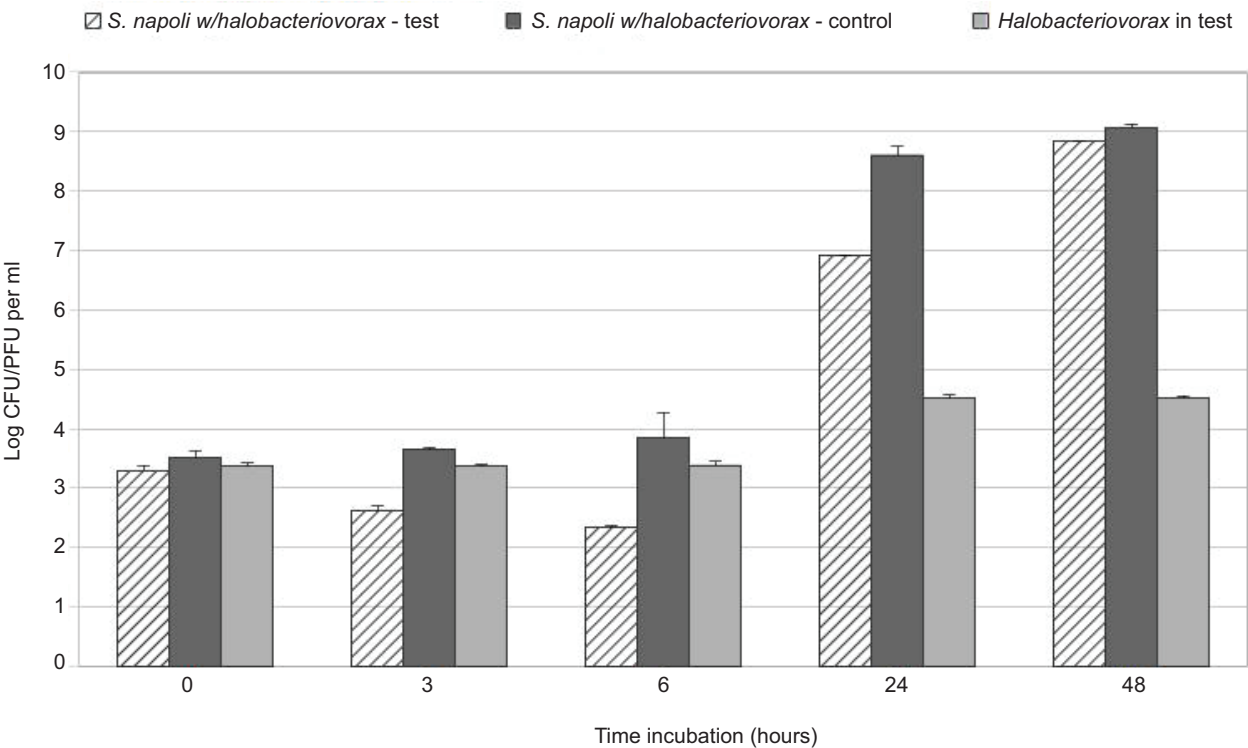


Figure 1. Challenge experiment 1. Trend of *Salmonella enterica* ser. Napoli w/ (with) and w/o (without) *Halobacteriovorax* M7 and growth of *Halobacteriovorax* M7 in the test from 0 to 48 h, using a 10³ PFU/10³ CFU per mL predator/prey ratio.

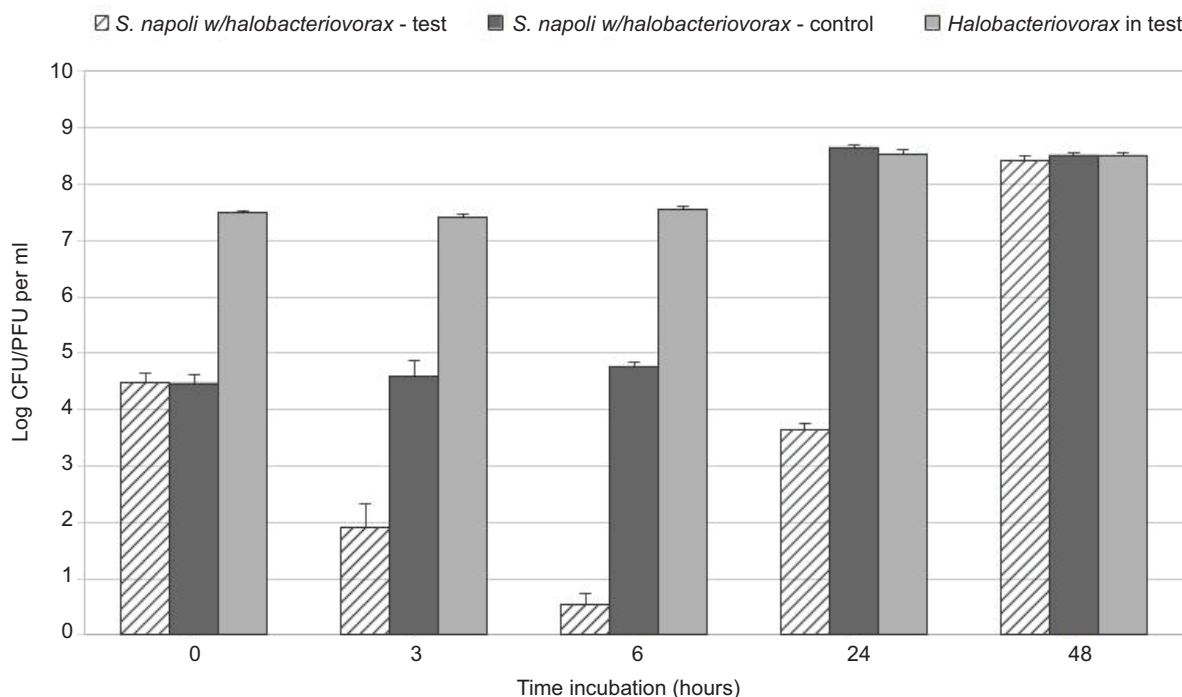


Figure 2. Challenge experiment 2. Trend of *Salmonella enterica* ser. Napoli w/ (with) and w/o (without) *Halobacteriovorax* M7 and growth of *Halobacteriovorax* M7 in the test from 0 to 48 h, using a 10^7 PFU/ 10^4 CFU per mL predator/prey ratio.

subsp. *enterica* ser. Typhimurium, *Salmonella enterica* subsp. *enterica* ser. Typhimurium, monophasic variants 1, 4 [5], and 12:I were isolated from bivalves. In the present study, preys were chosen in the context of a far-reaching project, with the ultimate goal of further applications in seafood hygiene, particularly in bivalve purification. The growth dynamics of M7 in the plaque assay using as primary prey *Salmonella* are comparable to those we reported for another *Halobacteriovorax* strain called HBXCO1, also isolated in the Adriatic Sea (Ottaviani *et al.*, 2018). In that case, however, the plaque assays were carried out using primary prey *V. parahaemolyticus* (Ottaviani *et al.*, 2018). This is probably because both the M7 and HBXCO1 strains belong to the same genus despite preferring taxonomically different prey. In a previous study, we demonstrated that *Halobacteriovorax* against *V. parahaemolyticus* showed maximum predatory efficiency at a predator/prey ratio of 10^3 PFU / 10^4 CFU per mL (Ottaviani *et al.*, 2020a). In the Challenge experiment 1 of this study at a predator/prey ratio of 10^3 PFU / 10^3 CFU per mL M7, despite the low level of prey used, was able to reduce *Salmonella* by 1,5 log after 6 h with the maximum reduction at 24 h by about 2 logs compared to the control. As expected, in Challenge Experiment 2, at a predator/prey ratio of 10^7 PFU / 10^4 CFU per mL, M7 contained more *Salmonella* growth than in Experiment 1, reducing it by 4 logs already after 6 h and by 5 logs at 24 h. Since it has been widely demonstrated that *Halobacteriovorax* cannot grow on eukaryotic cells, they do not represent

a specific risk to human health (Bratanis *et al.*, 2020; Shatzkes *et al.*, 2017). Moreover, its high host specificity and capability to parasitize the bacteria organized on biofilms or in VBNC forms (Bratanis *et al.*, 2020) make *Halobacteriovorax* not susceptible to those common mechanisms of competition or defense that the pathogens can activate on mollusks. *Halobacteriovorax* survives in the bivalve intestine for a sufficient time to exercise their lytic activity against target bacteria in the short (24 h) and medium term (7 days) (Li *et al.*, 2011). In light of all this evidence, *Halobacteriovorax* could find application in the post-harvest decontamination of bivalves from *Salmonella*, substituting or integrating conventional approaches.

Conclusion

This is the first work reporting the isolation and characterization of a *Halobacteriovorax* strain using *Salmonella* as primary prey in seawater. The predatory efficacy of M7 against *Salmonella* strains isolated from mussels makes it a suitable candidate for developing *Salmonella* containment strategies in bivalves and, more generally, in seafood with a short shelf life. Our next goal will be to test M7 at a laboratory scale in a shellfish purification plant on different types of bivalves experimentally contaminated with *Salmonella*, with the ultimate purpose of designing a *Halobacteriovorax*-based post-harvest process capable of containing *Salmonella* in bivalves.

References

- 2073/2005/EC, R. (2005). Commission Regulation (EC) No. 2073/2005 on microbiological criteria for foodstuffs. In E. Union (Ed.), 2073/2005. Brussels, Belgium The European parliament and the council of the European Union.
- Amagliani, G., Brandi, G. and Schiavano, G.F. 2012. Incidence and role of *Salmonella* in seafood safety. Food Res. Inter. 45(2):780–788. <https://doi.org/10.1016/j.foodres.2011.06.022>
- Andino, A. and Hanning, I. 2015. *Salmonella enterica*: survival, colonization, and virulence differences among serovars. The Scientific World Journal. 16. <https://doi.org/10.1155/2015/520179>
- Baker, G.L. 2016. Food safety impacts from post-harvest processing procedures of molluscan shellfish. Foods. 5(2):29. <https://doi.org/10.3390/foods5020029>
- Barile, N.B., Scopa, M., Nerone, E., Mascilongo, G., Recchi, S., Cappabianca, S., et al. 2009. Study of the efficacy of a closed cycle depuration system on bivalve molluscs. Vet. Ital. 45(4):555–560.
- Bratanis, E., Andersson, T., Lood, R. and Bukowska-Faniband, E. 2020. Biotechnological potential of *Bdellovibrio* and like organisms and their secreted enzymes. Frontiers in Microbiol. 11:662. <https://doi.org/10.3389/fmicb.2020.00662>
- Butt, A.A., Aldridge, K.E., Sanders C.V. Infections related to the ingestion of seafood Part I: viral and bacterial infections. The Lancet Infect. Dis. 4(4):201–212. [https://doi.org/10.1016/S1473-3099\(04\)00969-7](https://doi.org/10.1016/S1473-3099(04)00969-7)
- Chen, H., Athar, R., Zheng, G. and Williams, H.N. 2011. Prey bacteria shape community structure of their predators. ISME Journal. 5:1314–1342. <https://doi.org/10.1038/ismej.2011.4>
- Davidov, Y., Friedjung, A. and Jurkevitch, E. 2006. Structure analysis of a soil community of predatory bacteria using culture dependent and culture-independent methods reveals a hitherto undetected diversity of *Bdellovibrio*-and like organisms. Enviro. Microbiol. 8(9):1667–1673. <https://doi.org/10.1111/j.1462-2920.2006.01052.x>
- EFSA & ECDC. 2016. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2015. EFSA Journal. 14(12):4634. <https://doi.org/10.2903/j.efsa.2016.4634>
- FAO. 2005 Regional review on aquaculture development 6. Western-European Region – 2005. FAO Fisheries Circular No. 1017/6 FIMA/C1017/6 ISSN 0429-9329.
- Iwamoto, M., Ayers, T., Mahon, B.E., and Swerdlow, D.L. 2010. Epidemiology of seafood-associated infections in the United States. Clin. Microbiol. Rev. 23:399–410. <https://doi.org/10.1128/cmr.00059-09>
- Koval, S.F., Williams, H.N., and Stine, O.C. 2015. Reclassification of *Bacteriovorax marinus* as *Halobacteriovorax marinus* gen. nov., comb.nov. and *Bacteriovorax litoralis* as *Halobacteriovorax litoralis* comb. nov.; description of *Halobacteriovoraceae* fam. nov. in the class Deltaproteobacteria. Inter. J of Syst. and Evol. Microbiol. 65:593–597. <https://doi.org/10.1099/ijs.0.070201-0>
- Li, H., Liu, C., Chen, L., Zhang, X. and Cai, J. 2011. Biological characterization of two marine *Bdellovibrio*-and-like organisms isolated from Daya bay of Shenzhen, China and their application in the elimination of *Vibrio parahaemolyticus* in oyster. Inter J of Food Microbiol. 151:36–43. <https://doi.org/10.1016/j.ijfoodmicro.2011.07.036>
- Lu, F. and Cai, J. 2010. The protective effect of *Bdellovibrio*-and-like-organisms (BALO) on tilapia fish fillets against *Salmonella enterica* ssp. *enterica* serovar Typhimurium. Letters in Applied Microbiol. 51:625–631. <https://doi.org/10.1111/j.1472-765X.2010.02943.x>
- Morrison, C.M., Armstrong, A.E., Evans, S., Mild, R.M., Langdon, C.J., Joens, L.A. 2011. Survival of *Salmonella* Newport in oysters. Inter. J of Food Microbiol. 148:93–98. <https://doi.org/10.1016/j.ijfoodmicro.2011.05.006>
- Novoslavskij, A., Terentjeva, M., Eizenberga, I., Valciņa, O., Bartkevičs, V., Bērziņš, A. 2016. Major foodborne pathogens in fish and fish products: a review. Annals of Microbiol. 66:1–15. <https://doi.org/10.1007/s13213-015-1102-5>
- Ottaviani, D., Chierichetti, S., Angelico, G., Forte, C., Rocchegiani, E., Manuali, E., et al. 2018. *Halobacteriovorax* isolated from marine water of the Adriatic Sea, Italy, as an effective predator of *Vibrio parahaemolyticus*, non-O1/O139 *V. cholerae*, *V. vulnificus*. J of Appl. Microbiol. 4:1199–1207. <https://doi.org/10.1111/jam.14027>
- Ottaviani, D., Pieralisi, S., Chierichetti, S., Rocchegiani, E., Hattab, J., Mosca, F., et al. 2020a. *Vibrio parahaemolyticus* control in mussels by a *Halobacteriovorax* isolated from the Adriatic sea, Italy. Food Microbiol. 92:103600. <https://doi.org/10.1016/j.fm.2020.103600>
- Ottaviani, D., Pieralisi, S., Rocchegiani, E., Latini, M., Leoni, F., Mosca, F., et al. 2020b. *Vibrio parahaemolyticus*-specific *Halobacteriovorax* from seawater of a mussel harvesting area in the Adriatic Sea: abundance, diversity, efficiency and relationship with the prey natural level. Frontiers in Microbiol. 11:1575. <https://doi.org/10.3389/fmicb.2020.01575>
- Potasman, I., Paz, A. and Odeh, M. 2002. Infectious outbreaks associated with bivalve shellfish consumption: a world-wide perspective. Clin. Infec. Dis. 35:921–928. <https://doi.org/10.1086/342330>
- Richards, G.P., Fay, J.P., Dickens, K.A., Parent, M.A., Soroka, D.S. and Boyd, E.F. 2012. Predatory bacteria as natural modulators of *Vibrio parahaemolyticus* and *Vibrio vulnificus* in seawater and oysters. Applied and Enviro. Microbiol. 78:7455–7466. <https://doi.org/10.1128/AEM.01594-12>
- Richards, G.P., Watson, M.A., Boyd, E.F., Burkhardt, W.I.I.I., Lau, R., Uknalis, J., et al. 2013. Seasonal levels of the *Vibrio* predator *Bacteriovorax* in Atlantic, Pacific and Gulf Coast seawater. Inter. J of Microbiol. 2013:375371. <https://doi.org/10.1155/2013/375371>
- Richards, G.P., Fay, J.P., Uknalis, J., Olanya, O.M. and Watson, M.A. 2016. Purification and host specificity of predatory *Halobacteriovorax* isolates from seawater. Applied and Enviro. Microbiol. 82:922–927. <https://doi.org/10.1128/AEM.03136-15>
- Rippey, S.R. 1994. Infectious diseases associated with molluscan shellfish consumption. Clin. Microbiol. Rev. 7(4):419–425. <https://doi.org/10.1128/CMR.7.4.419>
- Rubini, S., Galletti, G., D'Incau, M., Govoni, G., Boschetti, L., Berardelli, C., et al. 2018. Occurrence of *Salmonella enterica* subsp. *enterica* in bivalve molluscs and associations with *Escherichia*

- coli* in molluscs and faecal coliforms in seawater. Food Control. 84:429–435. <https://doi.org/10.1016/j.foodcont.2017.08.035>
- Sabbatucci, M., Dionisi, A.M., Pezzotti, P., Lucarelli, C., Barco, L., Mancin, M. and Luzzi, I. 2018. Molecular and epidemiologic analysis of reemergent *Salmonella* enterica Serovar Napoli, Italy, 2011-2015. Emerg. Infect. Dise. 24(3):562–565. <https://doi.org/10.3201/eid2403.171178>
- Shatzkes, K., Connell, N.D. and Kadouri, D.E. 2017. Predatory bacteria: a new therapeutic approach for a post-antibiotic era. Future Microbiol. 12:469–472. <https://doi.org/10.2217/fmb-2017-0021>
- Williams, H.N., Lymperopoulou, D.S., Athar, R., Chauhan, A., Dickerson, T.L., Chen, H., et al. 2016. *Halobacteriovorax*, an underestimated predator on bacteria: potential impact relative to viruses on bacterial mortality. The ISME Journal. 10:491–499; <https://doi.org/10.1038/ismej.2015.129>
- Zahli, R., Soliveri, J., Abrini, J. Copa-Patiño, J.L., Nadia, A., Scheu, A-K., et al. 2021. Prevalence, typing and antimicrobial resistance of *Salmonella* isolates from commercial shellfish in the North coast of Morocco. World J Microbiol. and Biotechnol. 37:170. <https://doi.org/10.1007/s11274-021-03136-w>