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Halobacteriovorax to Kill Multi-Drug Resistant *Escherichia coli* and *Salmonella*

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INTRODUCTION

The excessive and often inappropriate use of antibiotics in medicine, agriculture and other activities has facilitated the selection of Antibiotic-Resistant (AMR) pathogens, many of which are Multidrug-Resistant (MDR). The interconnection between humans, animals and their environmental ecosystems inevitably leads to the transmission of antibiotic resistance. This study aimed to isolate *Halobacteriovorax* from aquatic environment with predatory potential against MDR pathogens, particularly *Escherichia coli* and *Salmonella* of different origins.

MATERIALS AND METHODS

The isolation was carried out in brackish water near the mouth of the Musone river, Marche region (Fig.1), using as primary prey a Multi-Antibiotic Resistant (MDR) strain of *E. coli*. One *Halobacteriovorax* isolate, named HE7 was identified by PCR analysis performed on a fragment of the 16S rRNA gene of the *Halobacteriovoraceae* using the primers Bac676F and Bac1442R. Sequencing analysis was also performed on PCR product of 700 bp from a unique plaque (Fig.2) [1].

Fig.1 Sampling technique

The HE7 strain predation efficiency was tested against other MDR *E.coli* and *Salmonella* strains of different origin. HE7 strain was tested in challenge predator/ primary prey experiments to monitor predator/prey reduction at selected time points. Double-layer agar plating technique was used to detect *Halobacteriovorax* and to evaluate its host specificity and predation efficiency [2].

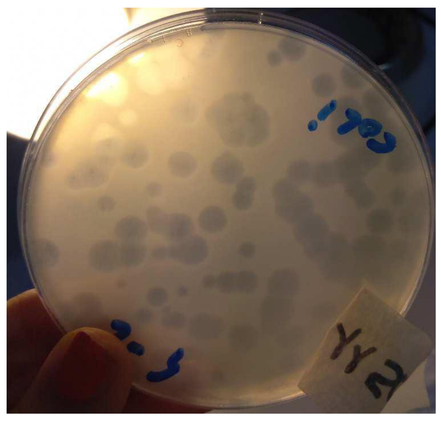

Fig.2 Plaque assay of HE7 with *E.coli*

BVB (BdelloVibrio Broth) tubes were prepared and divided into two series:
TEST - tubes contaminated with 0.5 ml O/N enrichment in BVB of HE7 (filtered through a 0.20 µm syringe filter) + 0.5 ml O/N enrichment of the prey in BHI (Brain Heart Infusion), starting with two predator/prey ratio $10^7/10^7$ and $10^7/10^3$ PFU/CFU/ml. **CONTROL -** tubes prepared in the same condition of test without *B. stolpii*. The tubes were incubated at 25°C and analysed at 0, 3, 6, 24 h to assess the level of prey and predator.

DISCUSSION AND CONCLUSIONS

To date, exploration of predatory bacteria as an alternative to antibiotics has been mostly focused on *Bdellovibrio bacteriovorus*. To the best of our knowledge, this is the first work testing in vitro the efficacy of the genus *Halobacteriovorax* against MDR *E.coli* and *Salmonella* isolates. The results of this study highlight that HE7, but more generally *Halobacteriovorax* could find application alone or in an integrated context of antimicrobial strategies as alternative to antibiotics.

REFERENCES

[1] Davidov Y, Huchon D, Koval SF, Jurkevitch E. (2006). A new alpha-proteobacterial clade of Bdellovibrio-like predators: implications for the mitochondrial endosymbiotic theory. Environ Microbiol., 8(12):2179-88.
[2] Pieralisi, S., Canonico, C., Di Lullo, S., Angelico, G., Cardinali, G., Rocchegiani, E., Maiolatesi, D., & Ottaviani, D. (2023). Effectiveness of Bdellovibrio bacteriovorus to contain Escherichia coli on milk and temperature impact on predation dynamics. Italian Journal of Food Science, 35(2), 80-87.

RESULTS

The 16SrRNA sequence of HE7 showed a 99,85% (671/672 bp) identity to the 16SrRNA sequence of the strain F2 di *Bacteriovorax* sp (GenBank accession number AY294218) (Fig.3). HE7 showed in vitro predatory activity against all MDR strains of *E. coli* and *Salmonella* tested (Fig.4). In the 10^7 predator/10^3 primary prey and 10^7 predator/10^7 primary prey challenges HE7, after 6 h, determined the total killing and 8 log reduction of the prey, respectively, maintaining this effect for up to 24 h (Figs.5 and 6).

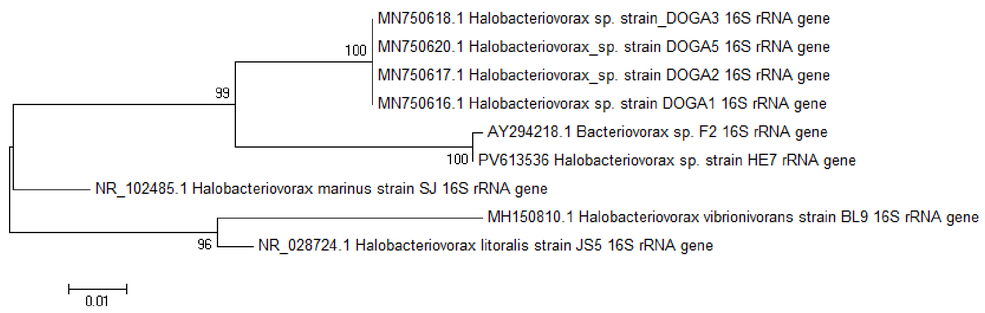

Fig.3 Maximum-likelihood phylogenetic tree, based on 16S rRNA gene sequence comparisons, showing the position of strain HE7 and related type strains. Numbers at branch nodes are bootstrap values (per 1000 trials).

Laboratory identification	Species	Origin	MDR Patterns
E7 (primary prey)	*E. coli*	*Chamelea gallina*	ESBL blaCTX-M-55 AMP FOT CIP CHL NAL TMP TET SMX FEP
E3	*E. coli*	*Chamelea gallina*	ESBL blaCTX-M-1 AMP FOT TMP TET SMX FEP
S3	*Salmonella Infantis*	Human urine	ESBL blaCTX-M-1 AMP FOT KAN NAL TET SMX SXT
S9	*Salmonella Havana*	Ring test	AmpC-phenotype FOX TAZ

Fig.4 Predator activity of HE7 against MDR *E.coli* and *Salmonella* strains.

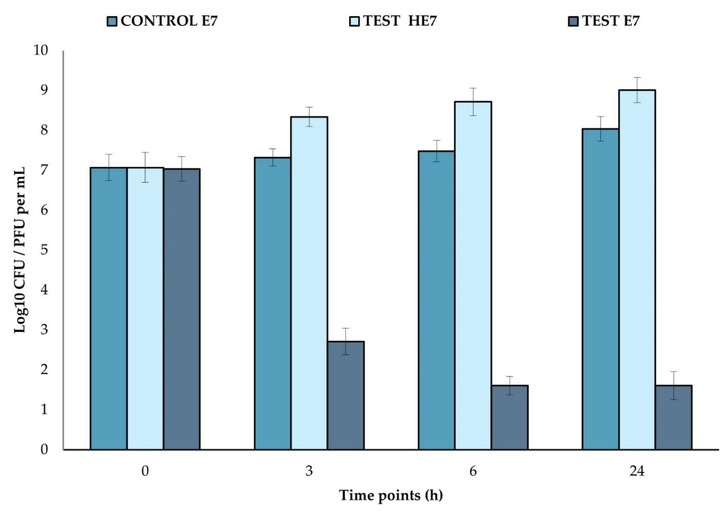

Fig.5 Results of challenge experiment with a ratio of 10^7 PFU/10^7 CFU per mL of *Halobacteriovorax* HE7 and *E. coli* E7 respectively, showing the reduction of E7 in test (with HE7) respect to control (without HE7) in DNB.

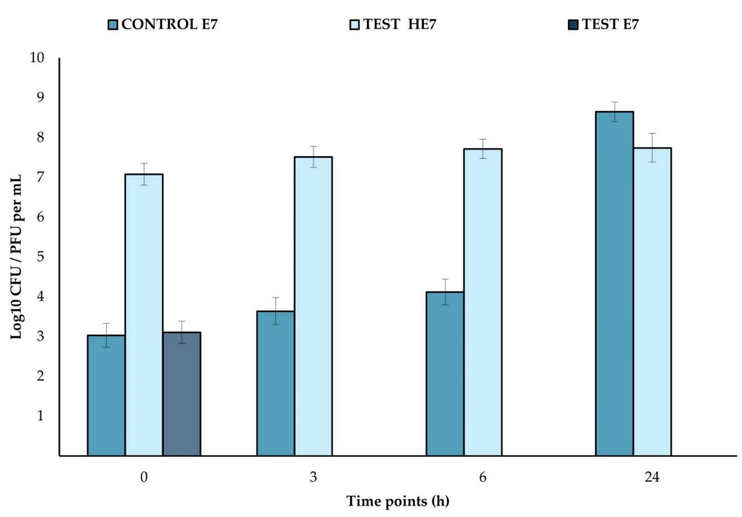

Fig.6 Results of challenge experiment with a ratio of 10^7 PFU/10^3 CFU per mL of *Halobacteriovorax* HE7 and *E. coli* E7 respectively, showing the reduction of E7 in test (with HE7) respect to control (without HE7) in DNB.

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