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Evaluation of a Flow-Through Depuration System to Eliminate the Human Pathogen *Vibrio Vulnificus* from Oysters

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Abstract

The efficacy of a flow-through depuration system in eliminating the human pathogen *Vibrio vulnificus* from eastern oysters (*Crassostrea virginica*) collected from the North Gulf of Mexico coast was evaluated in this study. Depuration experiments were conducted with artificially inoculated oysters using laboratory-grown strains of *V. vulnificus* as well as with naturally contaminated oysters. Determination of *V. vulnificus* numbers in oyster tissues was conducted at 0, 1, 2, 3 and 6 days of depuration. Results showed that the depuration of *V vulnificus* is possible using a flow-through system. Numbers of *V. vulnificus* in laboratory-inoculated oysters were reduced from >100,000 Most Probable Number (MPN)/g of oyster tissue to 23 MPN/g after six days of depuration. As expected depuration results of naturally contaminated oysters were more variable. Depuration at low temperature (15°C) had very little success in reducing the numbers of *V. vulnificus* in oyster tissues. On the contrary, when flow rate was increased from 11 L/m to 68 L/m, numbers of *V. vulnificus* in oysters were reduced from a starting concentration of 110,000 MPN/g to 3 MPN/g in six days. Nevertheless, a high-flow rate was not enough to eliminate *V. vulnificus* from oysters consistently. *Vibrio vulnificus* was effectively eliminated from oysters only when incoming water salinity was higher than 30 parts per thousand (ppt). Depuration did not select for pathogenic *V. vulnificus* strains. Pre- and post depuration *V. vulnificus* isolates contained similar proportions of the proposed more virulent type.

Introduction

Vibrio vulnificus is an opportunistic human pathogen that is an indigenous member of estuarine and marine habitats. Numbers of *V. vulnificus* in seawater, sediments, marine invertebrates and fish are not related to pollution or other forms of contamination [1]. Though distributed worldwide, *V. vulnificus* is commonly found in the United States (US) along the Gulf of Mexico coast where temperatures are subtropical and salinities are relatively low allowing for its optimum survival [2,3]. The eastern oyster (*Crassostrea virginica*) also thrives in this geographic region supporting an economically important commercial industry. Oysters are filter feeders that tend to concentrate microbes present in surrounding waters some of which can cause severe illness in humans [4,5]. Since most oysters are eaten alive, raw or poorly cooked, they can act as vectors for pathogenic microbes including *V. vulnificus*. This human pathogen can cause primary septicemia in susceptible individuals with the highest reported mortality rate (>50%) of any food-borne pathogen [6,7].

In 2008, the US eastern oyster industry landings were valued at $82 million with the Gulf of Mexico accounting for over 89% of the total harvest [8]. However, the mandatory warning labels required on Gulf coast oysters due to the high prevalence of *V. vulnificus* in this region has resulted in an overall decline in consumer demand and a reduction of summer price by 50% [9,10]. There are several methods approved by the Federal Drug Administration (FDA) for post-harvest processing of oysters that eliminate *V. vulnificus*: high hydrostatic pressure (HHP), heat/cool pasteurization (HCP), individually quick frozen (IQF), and irradiation. However, all of these methods change the organoleptic properties of oysters and consumers tend to not appreciate them as much [11,12].

Depuration is defined as the transfer of shellfish containing bacteria to a controlled, clean aquatic environment that permits them to open and function in an optimum physiological mode that favors the elimination of bacteria to non-detectable levels favorable for human consumption without requiring further processing [13,14]. The efficacy of this post-harvest process in reducing *V. vulnificus* in oysters inoculated with laboratory grown strains has been proved successful [15-17]. However, there has been little success with depuration of naturally present *V. vulnificus* strains in oysters using closed, re-circulating systems probably due to recontamination of the oysters [15-17]. The present study was conducted to i) determine the efficacy of a flow-through depuration system to eliminate *V. vulnificus* from eastern oysters and 2) assess if the selecting pressured imposed by depuration selects for certain strains of *V. vulnificus* that may harbor a higher pathogenic potential for humans [18].

Materials and Methods

Bacterial strains and cultures preparation

Vibrio vulnificus reference strain Vv3, originally isolated from Gulf oysters harvested in Alabama, USA, was used in this study to artificially inoculate oysters [19]. Cells were cultured in 50 mL conical vials containing marine broth (MB) (Difco, Detroit, MI, USA) at 35ºC with shaking for 12 h.

During depuration experiments, putative *V. vulnificus* isolates were collected from modified cellobiose polymyxin colistin agar (mCPC) before and after depuration and maintained in 0.3% marine agar (MA) (Difco, Detroit, MI, USA) at room temperature for further characterization.

Oyster stock

Two year-old oysters were obtained from Auburn University

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Shellfish Lab (AUSL) on Dauphin Island, AL. The oysters were grown in 16 mm mesh bags on an adjustable long line system (BST Oyster Supply, Cowel, South Australia) in an intertidal area below a boat dock about 46 cm off the bottom at the following coordinates: 30º 15" 04.68" N, 88º 04" 47.28" W.

Oyster depuration

For each depuration experiment, sixty oysters were harvested and cleaned of any fouling organisms and transferred to the depuration system. Oysters were placed on mesh trays suspended 13 cm off the bottom in a 522 L raceway tank with a water volume of around 300 L. Seawater was piped in from the Gulf of Mexico with a 213 m PVC pipeline extending 107 m offshore. Incoming seawater was filtered through a 200µm bag filter (Aquatic Ecosystems, Inc., Apopka, FL, USA) then treated with a 110 W commercial UV sterilizer (Tropical Marine Centre, Ltd., Chorleywood, UK) (only in the test tank). Oysters were depurated using a water flow-through system with flow rate maintained at 11 L/m (unless specified otherwise) for 6 days at the AUSL. The approximate water replacement time of the system was 30 min. Oysters were fed once a day with marine microalgae concentrate (Shellfish Diet 1800, Reed Mariculture, Campbell, CA, USA), requiring cessation of water flow for 1 h per feeding. The tanks were drained daily and cleaned of feces and pseudofeces with tapwater. Filter bags were also cleaned daily by thoroughly rinsing with tap water. Salinity and temperature were measured twice a day with a YSI model 85 probe (YSI Inc., Yellow Springs, OH, USA). Mortality was checked every morning before cleaning. The control tank was a replica of the test tank without the bag filter and UV filter (incoming seawater in the control tank was not treated at all). Populations of *V. vulnificus* in oysters were analyzed at 0, 1, 2, 3 and 6 days during the depuration process.

Depuration of artificially inoculated oysters: Oysters were collected as described above during the month of February (Experiments 1 and 2) when numbers of *V. vulnificus* are typically non-detectable in shellfish. Before oysters were placed in depuration tanks, a subsample was taken for bacteriological analysis (see below). Oysters were found negative for the presence of native *V. vulnificus* strains. Artificially inoculation was carried out according to Limthammahisorn et al., [19]. Briefly, 150 oysters were placed into 38 L aquaria with 27 L of autoclaved seawater at 25°C and were allowed to acclimate for 24 h. *Vibrio vulnificus* Vv3 strain was incubated overnight at 35°C in marine broth. Cultures were spectrophotometrically quantified and a final concentration of $3.7x10^5$ Colony Forming Units (CFU)/ml of *V. vulnificus* was achieved in each tank (approximately 10^{10} *V. vulnificus* cells were added to each tank). Oysters were artificially contaminated with *V. vulnificus* by self-inoculation filtration at 25°C. After inoculation, oysters were placed into depuration tanks and depurated as described above.

Depuration of naturally contaminated oysters: In Experiments 3 to 7, oysters were collected when water temperature was above 25°C and therefore they were presumed to contain *V. vulnificus*. Oysters were collected as described above and tested for the presence of *V. vulnificus* before starting depuration. Initial levels of *V. vulnificus* varied but were above 10^3 MPN/g in all cases. Experiment 3 used the same flow-rate and as Experiments 1 and 2 and established the baseline for depurating naturally contaminated oysters. In Experiment 4, oysters were depurated under cold temperature by using a chiller unit (Delta Star chiller, AquaLogic Inc., San Diego, CA, USA) that was attached to the test tank to maintain incoming seawater at 15ºC. Water temperature in the control tank was not modified and remained at 25.0±2.1°C for the duration of the experiment. In experiments 5, 6 and 7 the depuration system was tested under different flow rates. In Experiment 5, flow rate was increased up to 48 L/min (from the 11 L/min flow rate used in previous experiments) while flow rate was increased to a maximum of 68 L/min in Experiments 6 and 7.

Bacteriological analysis

Numbers of *V. vulnificus* in oysters were determined at day 0 (before oysters were placed in the tanks) and at 1, 2, 3 and 6 days of during the depuration process. Twelve oysters were analyzed each time according to 3-tube Most Probable Number (MPN) method described in the U.S. Food and Drug Administration's Bacteriological Analytical Manual (BAM) [20] using mCPC. Numbers of *V. vulnificus* are given as MPN/g of oyster tissue (or a log MPN/g).

Characterization of *V. vulnificus* isolates obtained before and after depuration

In order to determine if depuration selects for specific *V. vulnificus* types, isolates were recovered pre- and post-depuration during Experiment 6. A total of 97 putative isolates (41 pre-depuration and 56 at 6 days post-depuration) were recovered on mCPC. DNA was extracted according to Pitcher et al. [21] after isolates were plated onto MA plates and incubated at 37ºC for 16 h. DNA was resuspended in 200µL of deionized water (dH_2O) and stored at -20ºC. DNA was quantified using the Nanodrop® ND-1000 Spectrophotometer (Thermo Scientific, Wilmington, DE, USA) and diluted to 20 ng/µL. Confirmation of V. vulnificus presumptive isolates was carried out by specific PCR amplification of the vvh gene as per Panicker and Bej [22]. Only isolates confirmed as *V. vulnificus* by specific PCR were used for genotyping analysis.

Restriction Fragment Length Polymorphism *(RFLP) of the 16S rDNA*. Strains were ascribed to 16S type A or B according to

		Vibrio vulnificus counts (MPN/g)[a]				
Experiment	Date	Pre-depuration	Post-depuration Control tank	Post-depuration Test tank	Temperature (°C)[b]	Salinity (ppt)[c]
1- Inoculated oysters	02/19/2009	>$1.1X10^5$	75	43	18.0±1.7	25.0±2.8
2- Inoculated oysters	02/26/2009	>$1.1X10^5$	23	20	15.0±1.1	27.0±1.7
3- Standard	06/19/2009	$1.4X10^3$	$9.3X10^2$	$2.4X10^3$	28.3±1.7	24.3±2.3
4- Low Temp. 15°C	08/08/2009	$2.9X10^4$	$7.5X10^3$	$9.3X10^3$	25.0±2.1	26.5±3.2
5- Flow rate 46 L/min	09/01/2009	>$1.1X10^5$	$2.1X10^4$	$2.9X10^4$	28.5±0.6	26.0±3.4
6- Flow rate 68 L/min	09/14/2009	$1.1X10^5$	3	3	27.5±1.7	32.2±1.2
7- Flow rate 68 L/min	10/12/2009	$4.6X10^4$	$1.1X10^5$	>$1.1X10^5$	25.6±3.1	9.0±2.4

[a]*Vibrio vulnificus* counts were determined according to Most Probable Number (MPN) method described in the U.S. Food and Drug Administration's Bacteriological Analytical Manual (BAM) [5]

[b]Average seawater temperature recorded in both tanks throughout the depuration process

[c]Average seawater salinity recorded in both tanks throughout the depuration process

Table 1: Summary of depuration trials.

16S-RFLP type	Pre-depuration	Post-depuration	Total
Type A	26	25	51
Type B	15	31	46

Table 2: Number of *Vibrio vulnificus* isolates that belong to 16S-RFLP type **A** and **B**.

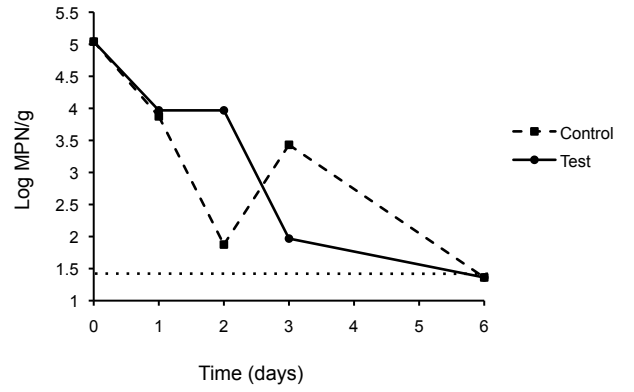

Figure 1: Reduction of *Vibrio vulnificus* in artificially-inoculated oysters (Experiment 1) subjected to flow-through depuration over a 6-day period as determined by Most Probable Number (MPN) analysis. Graph represents log of MPN/gram of oyster meat. Seawater in test tank was UV filtered. Dotted line represents the 30 MPN/g threshold of reduction required by the Federal Drug Administration (FDA) for validation of the system.

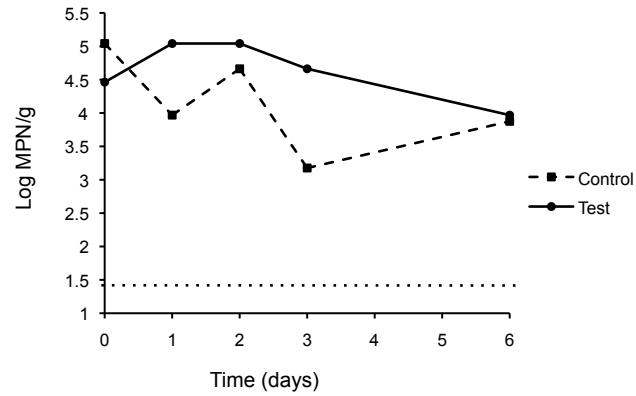

Figure 2: Persistence of *Vibrio vulnificus* in naturally contaminated oyster tissue (Experiment 4) subjected to flow-through depuration at 15°C (test tank) over a 6-day period as determined by Most Probable Number (MPN) analysis. Graph represents log of MPN/gram of oyster meat. Dotted line represents the 30 MPN/g threshold of reduction required by the Federal Drug Administration (FDA) for validation of the system.

Nilsson et al. [18]. Amplification of 16S rDNA was carried out with primers UFUL (5'- GCCTAACACATGCAAGTCGA-3') and UFUR (5'-CGTATTACCGCGGCTGCTGG-3'). Following digestion with *Hae*III, restriction fragments were electrophoresed in a 3% TAE Agarose-1000 (Invitrogen, Carlsbad, CA, USA) gel for 90 min at 80V, stained with ethidium bromide, and photographed under UV light.

Amplified Fragment Length Polymorphism (AFLP): AFLP fingerprintings were determined as previously described by Arias et al. (1997) with the following modifications. PCR amplifications were performed using the MJ Research PTC-200 Thermocycler with the following cycle profile: cycle 1, 60 s at 94°C, 30 s 65°C, and 60 s at 72°C; cycles 2 to 12, 30 s at 94°C, 30 s at annealing temperatures 0.7°C lower than that used for each previous cycle, starting at 64.3°C, and 60 s at 72°C; cycles 13 to 24, 30 s at 94°C, 30 s at 56°C and 60 s at 72°C. After completion of the cycling program, 5µL of AFLP® Blue Stop Solution (LI-COR) was added to the reaction mixtures. Prior to gel loading, the samples were heated for 5 m at 94°C then rapidly cooled on ice to prevent reannealing. The PCR products were electrophoresed on the NEN Global Edition IR2 DNA Analyzer (LI-COR) following manufacturer's instructions.

Data analysis

A depuration experiment was considered successful if numbers of *V. vulnificus* were below the FDA threshold of <30 MPN/g of oysters. Correlation between salinity and *V. vulnificus* numbers at the end of the depuration trials was calculated using the Pearson product moment correlation coefficient. Ascription of *V. vulnificus* strains to ribosomal types 16S-A and 16S-B was done according to the restriction fragments size [18]. AFLP images were processed with BioNumerics v 5.0 (Applied Maths Inc., Austin, TX, USA). Following conversion, normalization, and background subtraction with mathematical algorithms, levels of similarity between fingerprints were calculated with the Pearson product-moment correlation coefficient. Cluster analysis was performed with the unweighted pair-group method using average linkages (UPGMA).

Results

Table 1 summarizes the results obtained from depuration tests through the year 2009. As expected, average temperature and salinity, as well as initial *V. vulnificus* numbers, changed seasonally.

Depuration of artificially contaminated oysters: Oysters were successfully inoculated with *V. vulnificus* by filtering water. Before depuration numbers of *V. vulnificus* in inoculated oyster tissues were $>10^5$ MPN/g of tissue. Figure 1 shows the decline in *V. vulnificus* counts during depuration from Experiment 1. After six days in the depuration tanks, *V. vulnificus* numbers in oysters were reduced by more than 4 orders of magnitude. Similar results were obtained when the experiment was repeated (Experiment 2, see Table 1). Interestingly, the use of a UV filter seemed to have little effect in decontaminating oysters. After 6 days of depuration, oysters in both test and control tanks contained similar numbers of *V. vulnificus*.

Depuration of naturally contaminated oysters: In Experiment 3, oysters harvested from the wild were subjected to depuration using a flow rate of 11 L/min, as in Experiments 1 and 2. Initial *V. vulnificus* counts were at $1.4x10^3$ MPN/g and did not significantly changed in oysters during the depuration trial (Table 1). In Experiment 4, water

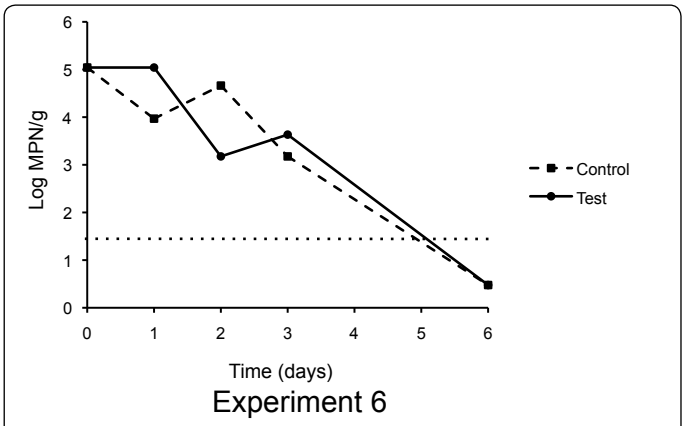

Figure 3: Reduction of *Vibrio vulnificus* in naturally contaminated oyster tissues (Experiment 6) under high flow through (68L/min in test tank). Graph represents log of MPN/gram of oyster meat. Dotted line represents the 30 MPN/g threshold of reduction required by the Federal Drug Administration (FDA) for validation of the system.

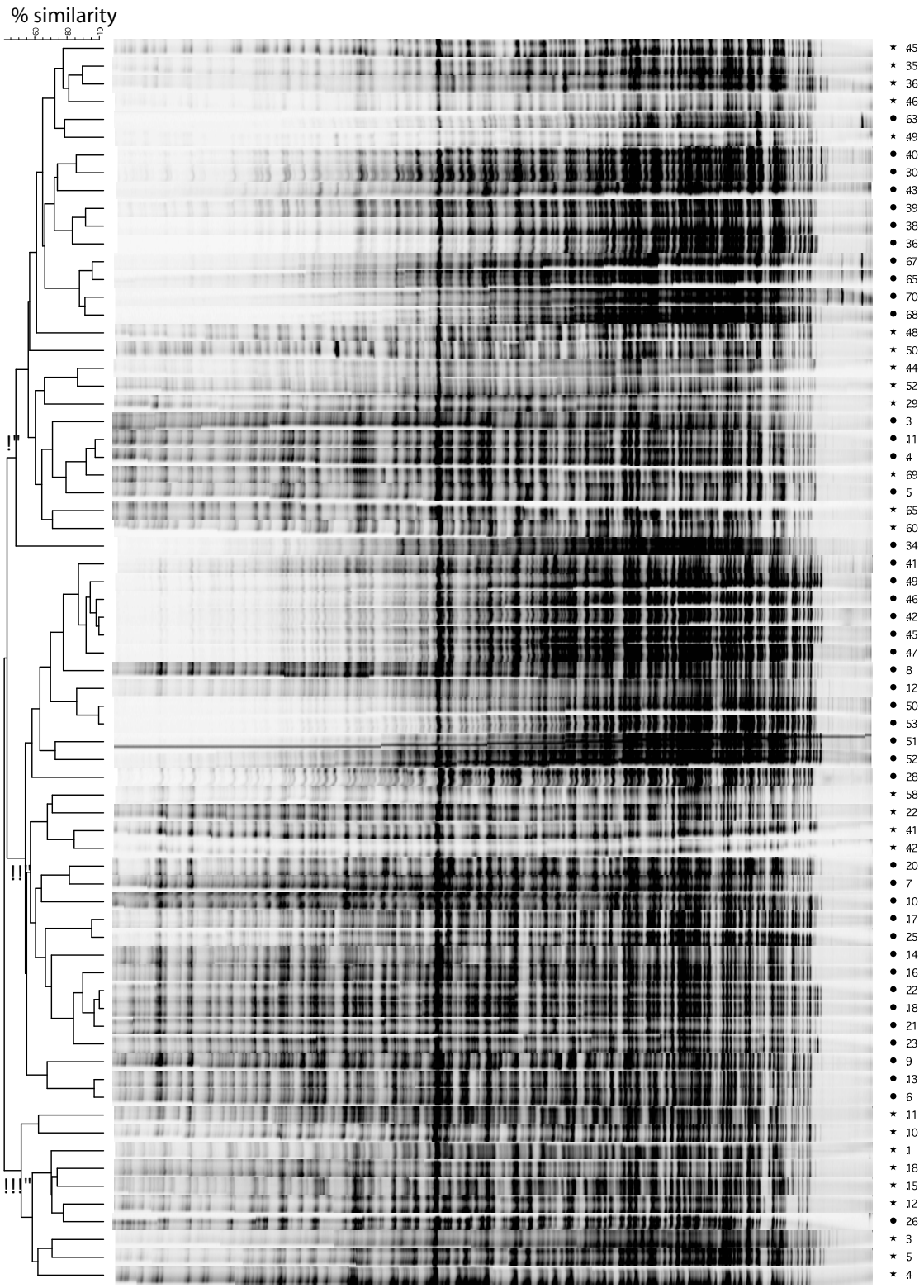

Figure 4: AFLP patterns of *Vibrio vulnificus* isolates recovered from oysters. The dendogram was derived by UPGMA cluster analysis of the AFLP profiles of 70 *V. vulnificus* isolates. The tracks show the processed band patterns after conversion, normalization, and background subtraction. Levels of linkage are expressed as the Pearson product-moment similarity coefficient. Major clusters are noted with roman numerals. Star symbols represent pre-depuration isolates while solid circles represent post-depuration isolates.

temperature was artificially modified by using a chilling unit. We found no major reduction in *V. vulnificus* numbers when oysters were depurated at 15°C (Figure 2). An alternative option for improving depuration efficiency was increasing flow rate. We hypothesized that an increase in flow rate could boost the ventilation rate of the oysters and therefore result in higher reductions of *V. vulnificus* from

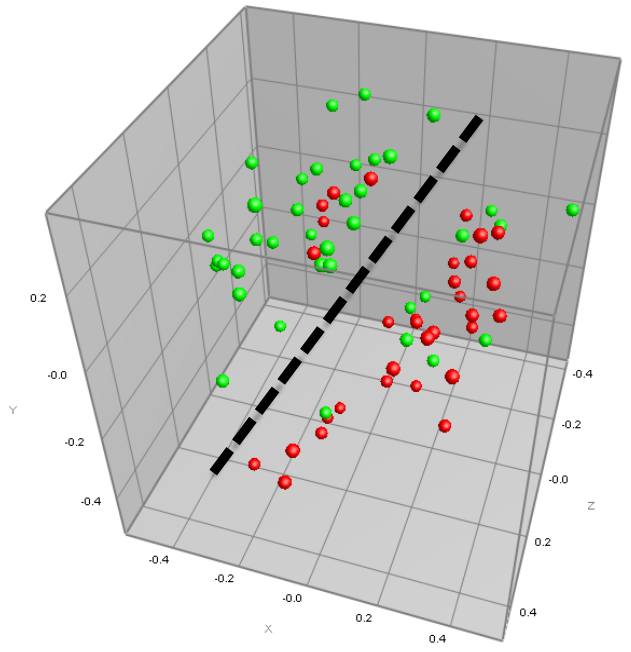

Figure 5: Multidimensional scaling (MDS) plot showing the relatedness of *Vibrio vulnificus* isolates based on AFLP data. Distance between entries represents dissimilarities obtained from the similarity matrix. Dotted line shows the division between the two dominant groups, one containing primarily 16S type A isolates (red) while the other contains mainly 16S type B (green).

oyster tissues. The first arbitrary increase from the standard 11 L/min was to 46 L/min. The results from this test are shown in Table 1, Experiment 5. At day 0 more than $1x10^5$ MPN/g of *V. vulnificus* were present in oyster tissues. By day 6, the numbers had decreased down to $2.9x10^4$ MPN/g. The results of this experiment indicated that a higher flow rate might hold promise for the elimination of *V. vulnificus* from oysters in a flow-through depuration system. Figure 3 shows the results obtained from Experiment 6, in where flow rate was increased to 68 L/min. During this experiment *V. vulnificus* numbers in oyster tissues went from $1.1x10^5$ MPN/g at day 0 down to 3 MPN/g by day 6. This final concentration is below the FDA required <30 MPN/g for validation of a new system for post-harvest processing of oysters. Experiment 7 used the same flow rate as Experiment 6 but was performed a month later and yielded very different results. *Vibrio vulnificus* numbers in oyster were $4.6x10^3$ MPN/g at day 0 and actually increased to over $1x10^5$ MPN/g at day 6. It is noteworthy that the salinity of the incoming seawater was very different between the two experiments. In Experiment 6, salinity remained above 30 ppt during depuration while in Experiment 7 average salinity was below 10 ppt. When final *V. vulnificus* counts were plotted against salinity, a significant correlation was observed (r= 0.79).

Characterization of *Vibrio vulnificus* isolates recovered pre- and post-depuration: To determine if depuration selects for a certain strain of *V. vulnificus* two different typing methods were used with a total of 97 isolates. Isolates were first ascribed to 16S-type A or B using an RFLP-based analysis. As shown in Table 2, a total of 51 strains were 16S type A, while 46 were 16S type B. Type A strains were predominant before depuration (26 out of 41), while 16S type B strains were more abundant after depuration (31 out of 56). However, the increase in the number of 16S type B isolates after depuration was not significant.

The high resolution fingerprinting method AFLP confirmed the high intraspecific diversity of this species. AFLP profiles from 70 isolates (DNA quality from the remainder 27 isolates was not sufficient for AFLP typing) showed complex patterns of more than 80 bands comprised in the 50 to 800 bp range. All isolates clustered at 40% of higher similarity (Figure 4) but three distinct clusters could be inferred from the dendrogram. Cluster I groups 15 isolates obtained before depuration with 16 isolates recovered post-depuration. In contrast, cluster II and III grouped for the most part post-depuration isolates (27 out of 31) and pre-depuration isolates (9 out of 10), respectively. The results of the AFLP cluster analysis are displayed in Figure 5 using a multidimensional scaling (MDS) plot. In this graph, isolates were ascribed to 16S type A and B. Interestingly, AFLP grouping correlated quite well with 16S typing. Two main groups could be inferred each one being dominating by one specific 16S type.

Discussion

Depuration is a common practice to eliminate contaminants, mainly of fecal origin, that accumulate in bivalves when they grow in polluted areas. Bivalves can cleanse themselves of fecal contaminants by active feeding if they are placed in tanks with clean water at the right temperature and salinity. However, depuration of naturally occurring bacteria such as *V. vulnificus* presents additional challenges since both bacteria and oysters coexist in the environment and are adapted to each other. Previous studies have evaluated the potential of depuration to eliminate *V. vulnificus* from oysters using re-circulating systems [15-17,23] but there has been little investigation into the possibilities of using a flow-through depuration system. Our study was intended to be a 'proof of concept' for using a flow-through depuration system for *V. vulnificus* depuration. Flow-through offers several advantages over re-circulating systems including lower risk of bacteria accumulation in the system (that could serve as reservoir and consequently recolonize the oysters), reduce risk of water quality problems (established biofilters are not required) and no need for artificial feeding since incoming seawater provides enough nutrients for the oysters. Our data showed the ease of eliminating this bacterium from artificially-inoculated oysters using a flow-through system. Oysters collected during winter months are naturally free of *V. vulnificus* but can quickly uptake *V. vulnificus* cells from water containing the bacterium. However, *V. vulnificus* cells never become established in oyster tissues and are readily cleared [16,17]. This is most likely due to the loss of pili or other structures needed for bacterial attachment to oyster tissue by culturing the bacterium under laboratory conditions or that *V. vulnificus* cannot displace the microflora present in the oysters during the winter months. Nevertheless, our first two experiments served to established the basic depuration parameters for our system. The first depuration trial using naturally contaminated oysters (Experiment 3) yielded disappointing results with no significant reduction in *V. vulnificus* numbers in either control or test tanks. Experiment 4 tested depuration at low temperatures as it had been previously suggested that moderate low temperatures do not decrease pumping rate in oysters but negatively affect *V. vulnificus* growth [4]. However, no significant changes in *V. vulnificus* counts pre- and post-depuration were observed when oysters were depurated at 15°C. Another basic parameter that influences depuration is flow rate. We tested different flow-rates to investigate if a higher water flow will remove feces or pseudo-feces (that may be contaminated with *V. vulnificus*) faster from the system reducing self-contamination risk. Results from depuration trails under higher flow-rates were disparate. While Experiments 5 and 7 did not showed clear differences between

pre- and post-depuration *V. vulnificus* counts, Experiment 6 results were highly encouraging demonstrating that depuration of naturally occurring *V. vulnificus* is possible in a flow-through system. What made Experiment 6 exceptional was the high salinity encountered during the trial. *Vibrio vulnificus* numbers in the marine environment are related to water temperature but also to salinity. Environmental data suggest that there is a threshold salinity level (at or slightly above 30 ppt) at which point *V. vulnificus* levels drop substantially, regardless of temperature [1]. Our results identified to salinity as the main factor influencing depuration of *V. vulnificus* from oysters. Initial *V. vulnificus* counts were similar in Experiments 4-7 but end point values were dependent on salinity. Experiment 6 (salinity >30 ppt) successfully reduced *V. vulnificus* numbers below the FDA-required level while in Experiment 7 (salinity <10 ppt) numbers of *V. vulnificus* actually increased during depuration.

The majority of *V. vulnificus* clinical isolates are 16S type B therefore this type has been presumptively correlated with virulence in humans. Analysis of bacterial isolates showed that depuration does not select for specific *V. vulnificus* types but, interestingly, we found a higher percent of 16S type B (36.5%) isolates prior to depuration than what has been previously reported [18]. Previous records from this area indicated that only 6% of total *V. vulnificus* environmental isolates were type B [18]. By contrast, Kim and Jeong [24] found that 65% of environmental isolates off the southern coast of Korea were 16S type B [24]. These findings when coupled with the present study indicate geographical location is not the sole determinant of 16S type A/B ratios in oysters. In fact, it is more likely seasonal, which is illustrated in a study by Lin and Schwarz [25] that showed the prevalence of 16S type B isolates were more prevalent during the summer months which also correlates with the higher incidence of disease. When we analyzed the whole genome of our *V. vulnificus* isolates by AFLP, isolates pre- and post-depuration did not cluster together confirming that depuration is not selecting for specific strains. According to our AFLP data, *V. vulnificus* is divided into two distinct populations that coexist in oysters and that seem to be equally susceptible to depuration.

In summary, our results suggest that depuration under high salinity conditions has the potential of eliminate *V. vulnificus* from oysters. Motes and DePaola [26,27] have shown that offshore relaying of oysters to high salinity waters (>32 ppt) effectively reduced levels of *V. vulnificus*. Disadvantages of offshore relaying *versus* an inshore high salinity depuration system include cost of transportation to the offshore site, anchoring permits, and high risk of losing oysters to storms and poachers. On the other hand, maintaining high salinity conditions (>32 ppt) in a flow-through system may not be feasible due to the amount of salt required. Salinity in the coastal areas of the northern Gulf of Mexico can dramatically change overnight making difficult to predict good conditions for inshore depuration. A recirculating system using high salinity could be the best option for future depuration studies and may represent a viable option for the oyster industry to maintain an unprocessed but safe product during the warmer months.

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