Southwest States and Pacific Island Regional Water Program: Joining Forces on a Common Issue

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Introduction

Leptospira interrogans is a zoonotic pathogen hosted commensally by a variety of mammalian species. On the South Pacific island of American Samoa a seroprevalence survey conducted in 2004 indicated that 17% of subjects tested had a positive antibody response to two serovars (Bratislava and icterohaemorrhagiae) that have pigs and rats as maintenance hosts. A source of exposure may be drainage from small scale piggeries that contaminate streams. Resource management agencies, including the Natural Resources Conservation Service in Samoa, have begun to promote passive composting as a means of reducing the risk of water contamination and human exposure. This study examined the efficacy of composting accumulated pig litter to eradicate pathogenic *Leptospira* from spent litter prior to disposal or subsequent application.

In a separate experiment we subjected *L. interrogans* serovar Copenhageni strain M-20 to combined stresses of temperature (25–50 ˚C) and pH (5.2 – 7.9), ranges commonly expected within compost piles, for varying durations of exposure (4 hr to 96 hr) to determine the probability of bacterial survival in response to single or combined stresses. We found that acidic conditions and temperatures in excess of 45 °C were lethal to *L. interrogans* at even the shortest duration of exposure tested (4 hours). These experiments also examined the distribution of temperatures within a demonstration composting pile on the island of Tinian.

Figure 1. A passive composting pile in Tinian, equipped with an aeration system. Hobo® Tidbit temperature dataloggers (inset) were installed in the piles in three horizontal transects (which formed two full vertical transects) which provided information about temperature distributions in the bottom, mid and top fractions of the pile.

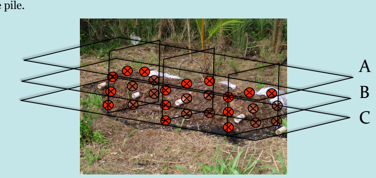

Figure 2. Vertical and horizonal transects included in the temperature sampling network.

Materials and Methods

We conducted experiments to characterize the survival of *Leptospira interrogans* serogroup Icterohaemorrhagiae serovar Copenhageni strain M-20, at the University of Nevada in a range of combinations of pH and temperature conditions, for durations ranging from 4 hours to 30 days.

Directly following exposure, each bacterial suspension was redistributed into proportionally larger individual volumes of EMJH culture medium and incubated at 30 °C for no less than 21 days prior to examination. At the conclusion of the 21 day incubation period each culture was thoroughly inspected for the presence of live spirochetes using darkfield microscopy. The presence of live spirochetes indicated a successful culture and evidence that the bacteria had survived the exposure conditions. The absence of live spirochetes indicated no growth and evidence that the bacteria had not survived the given exposure conditions. The survival data were then analyzed to determine the significance of the applied stress factors on the survival of the bacteria.

The strain of bacteria used was obtained from the National Veterinary Services Laboratory (NVSL) in Ames, Iowa. We used this serovar as a model for both serovars found to be prevalent within the American Samoa population sampled. A fresh parent culture of the bacteria was grown in semi-solid media for *Leptospira* cultures, prepared according to the protocol given by Becton, Dickenson and company for DifcoTM Ellinghausen and McCullough Medium as modified by Johnson and Harris (EMJH). The preparation included 200 μg/ml of 5–flurouracil to suppress contaminant growth and 0.2 % by weight agar noble.

We equipped two passive composting piles on the island of Tinian with 15 Hobo Tidbit dataloggers (Figure 1), distributed in three horizontal and two vertical transects (Figure 2). The dataloggers began reporting information after being installed on 4/09/08, in 6 hour intervals, until 09/09/08. One pile of litter consisted of pig manure, wood chips, and coconut husks (pile 1, depicted in Figure 4) while the other pile was a combination of pig manure, soil, dry grass trimmings, and small quantities of chicken manure (pile 2). We did not record pH in either pile.

Results

The results of exposure experiments indicate that *L. interrogans* has an extremely low probability of survival when exposed to temperatures of 45°C or higher, even for short durations (Figure 3). While duration of exposure was not an important factor, temperature and pH were both significantly associated with survival. Figure 3 displays the results of all experiments, represented by the logistic model below (significant at P<0.001):

$$\hat{p} = \frac{0.071 \times e^{1.09(pH) - 0.18(°C)}}{1 + 0.071 \times e^{1.09(pH) - 0.18(°C)}}$$

in which: $\hat{p}$ represents the proportion of spirochetes that would survive at a specific combination of temperature and pH
pH represents the pH of incubation solutions
ºC represents solution temperature (Celcius).

The data correspond with observations from other research that pathogenic *Leptospira* survive better in slightly alkaline environments (pH>7.0) than in mildly acidic environments (1, 2).

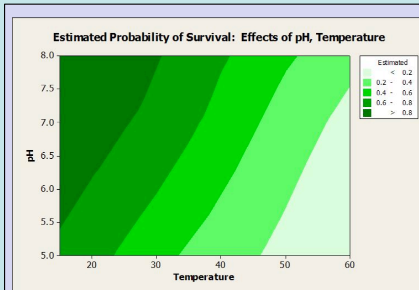

Figure 3. The estimated proportions of spirochetes surviving combinations of pH and temperature following *in situ* exposure, with all durations of exposure represented. The zones represent varying estimates of the probability of survival, based on a logisitic model of survival, using pH, temperature (C) and duration of exposure as predictors. Only pH and temperature were significant and P<0.05.

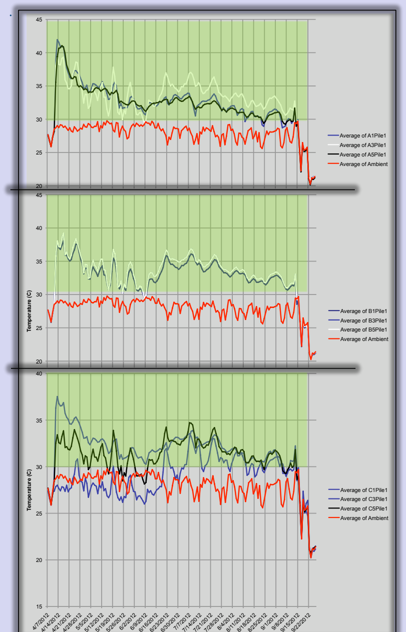

Figure 4: Observed temperatures in transects located at top (A), mid (B) and base (C) of passive composting pile. Green zones represent temperatures at which probability of survival is ≤50% at pH 7.0. Ambient temperatures are shown in red on each graph. Positions 1, 3 and 5 are at the outer edges (1 and 5) and center of each transect.

Results

Sensors from pile 1 registered a maximum temperature of 43.2 °C, with only 4 of the 13 sensors exceeding 40 °C. The second pile achieved high temperatures in isolated locations, with 4 of 13 sensors recording maximum temperatures in excess of 45 °C. However 6 of 13 sensors never exceeded 40 °C. As shown in Figure 4 the locations monitored in the upper transects of pile 1 (transects A and B, Figure 2) reached temperatures that would lead to approximately a 50% survival rate (>30ºC), with the assumption that pH was approximately neutral (~7.0). Temperatures were also higher than those in ambient conditions, indicating that the compost piles generated heat energy, especially during the early phases of the trial. In the lower transect (C, Figure 2), temperatures initially rose to > 30 ºC for several days, but were not as high as those in transects A and B.

Discussion

While there was evidence of elevated temperatures within these passively composted piles, the piles did not achieve temperatures that would be 100% lethal at pH=7.0 (50-60ºC). Given that duration of exposure to temperature and pH combinations was not significantly associated with survival, even short durations of exposure to elevated temperatures (>30ºC) appear to be lethal to spirochetes. Temperatures in excess of 45°C may typically be achieved during the first several weeks of decomposition in well managed compost piles. Products of decomposition include humic and fulvic acids, which may produce mildly acidic pH in piles after the initial sterilizing temperatures begin to decline (4), making composting a potentially effective method for reducing the risk of infection from contaminated pig wastes. Some management (for example turning and monitoring temperatures) will likely lead to a more uniform exposure of the biomass to critical temperatures, compared to the temperature distributions observed in the passive systems. Management could ensure that sufficiently high temperatures are achieved (4) and that all material is exposed for at least a short duration (> 4 hours).

Our estimates of the sterilizing capacity of this type of composting system could be refined by obtaining pH data in future trials, given that the probability of survival in acidic environments is diminished relative to survival in basic environments

References:

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