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Occurrence and Detection of Viable *Listeria* in Food Scrap Compost

Vorhandensein und Nachweis von *Listeria* in Kompost mit Küchenabfällen

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Abstract

Listeria species (*L. innocua, L., ivanovii, L. seeligeri,* and *L. grayi*) were readily detected in food scraps by Nucleic Acid Hybridization (NAH) probes using a standard *Listeria* selective medium (UVM-1) at ambient temperature. Various food scrap compost recipes artificially contaminated with *Listeria* at 10^7 cells per gram wet weight were composted in thermally insulated bench scale reactor vessels. These *Listeria* were not detected when the compost temperature became elevated. Different isolation methods for the *Listeria* showed this result to be a false negative occurring apparently because the heat stressed *Listeria* were unable to survive in the selective medium (UVM-1). Once incubated at 37°C in Universal *Listeria* medium (ULM), the *Listeria* were detectable for a short period in compost at temperatures as high as 64°C.

Zusamenfassung

Vier Listeria-spezies (*L. innocua, L., ivanovii, L. seeligeri,* und *L. grayi*) waren in Küchenabfällen mittels DNA-Sonden problemlos nachweisbar, wenn die Untersuchung bei Umgebungs-temperatur erfolgte. Verschiedene künstlich mit Listerien infizierte Mischungen (10^7 Keime/g Feuchtgewicht) von Küchenabfällen mit Zeitingspapier oder Pflanzenblättern wurden in thermisch isolierten, 4 liter fassenden Laborbehältern kompostiert. Die eingebrachten Listerien wurden nach dem Temperaturansteig nicht mehr nachgewiesen. Verschiedene Isolierungsverfahren zeigten aber, dass es sich dabei um falsch-negative Ergebnisse handelt, da die dem Hitzestress der Kompostierung ausgesetzten Listerien in dem verwendeten Selektivmedium UVM-1 nicht anwuchsen. Erfolgte die Anzüchtung bei 37°C in dem Listerien-Universalmedium ULM, waren die Listerien für einen kurzen Zeitraum bei Temperaturen bis zu 64°C im Kompost nachweisbar.

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Introduction

Standard isolation procedures recommended by the Food and Drug Administration (FDA) or Association of Official Analytical Chemists (AOAC) may not be appropriate for isolating bacteria from thermal environments (8, 20). We have previously demonstrated using NAH probes that *E. coli* could not be isolated from artificially contaminated compost using the standard method of selecting directly with a sodium lauryl sulfate broth. *E. coli* detection was successful if the bacterial community was first grown at 36°C in nutrient broth with 0.5% lactose (8).

One explanation of this observation is that the genome of *E. coli* is expressed differently when in different environments and needs time to acclimate itself to the bile salt. There are examples of this altered expression of bacterial genomes.

It is well known that operons can be regulated by the temperature at which the cells are grown. The degree of chromosomal supercoiling is regulated by the temperature (5) and is important to the expression of virulence genes (4) and genes for mammalian cell invasion (11). Both these functions have been shown only to be expressed only around 37°C.

The cellobiose operon is found in wild type *E. coli, Salmonella* and *Pseudomonas aeruginosa*. It is only expressed at 48°C or higher (7). Thermotolerant and mesothermophilic mutants *E. coli, Salmonella* and *Pseudomonas* grow well at 48°C and 54°C, respectively (6). They use glucose but not cellobiose as an energy source below 42°C and cellobiose but not glucose above 42°C. The cellobiose operon expression is controlled by the temperature of the environment and the operon is expressed at 48°C even in nonreplicating wild type cells. These observations demonstrate that bacteria growing in thermal environments do express their genomes differently than those growing at 36°C. Thus a selective medium for a bacteria which favors growth at 36°C environment may not support grow of these same bacteria in other environments.

It is the intention of this study to determine if a standard method for selection used in food microbiology can be useful in detecting *Listeria* in aerobic thermal compost containing food. Concurrently, the study attempts to assess the public health problem that *Listeria* may present in food composting.

MATERIALS AND METHODS

Food Scrap Samples

Samples of food were obtained from a school cafeteria immediately after lunch, households and the discarded food from a seafood restaurant.

Media

Universal *Listeria* Medium (ULM) consisted of: 5g Proteose peptone, 15g KH_2PO_4; 7g Na_2HPO_4; 5g NaCl; 0.5g glucose; 0.25g $MgSO_4 \cdot 7H_2O$; 0.5g ferric amonium citrate and 0.2g sodium pyurate per liter deionized water (13). The *Listeria* selective broth, Modified *Listeria* Enrichment Broth UVM-1 (UVM-1) broth contained; 5g Proteose peptone; 5g Tryptone; 5g Lab Lemco powder (oxide); 5g yeast extract; 20g NaCl; 1.35g Potassium phosphate monobasic; 12g Sodium phosphate dibasic; 1g Esculin, 1ml of 2% nalidixic acid in 0.1N NaOH, in 1 liter of distilled water (Merck). The *Listeria* were selected on Modified *Listeria* Cultivating Agar (LCA) plates; 52g Brain Heart Infusion Agar; 10g Lithium Chloride; 10g Glycine Anhydride; 15g agar made to 1 liter with distilled water

(Merck). The growth on these LCA selective plates was suspended in phosphate buffered saline and used for assay with NAH probe assays.

Bacterial Strains

The four common *Listeria (L. grayi, L. seeligeri, L. ivanovii* and *L. innocua*) used to contaminate compost in this study were obtained from the American Type Culture Collection (ATCC).

Nucleic Acid Hybridization Probes

The NAH probe for *Listeria* was a commercial kit (12) having a detection limit of 1 cell $25g^{-1}$ of sample (18). This NAH probe has been approved by the AOAC for a *Listeria* presence absence test in foods. The NAH probes were performed as directed by selecting with UVM-1 growing on LCA plates then swabbing from LCA plates and suspending in buffered saline and assaying (12).

Compost Studies

The first trial consisted of food scraps (Küchenabfall) and leaves (ratio: 1:1 as is). Later trials consisted of food and leaves (ratio: 1:1 as is) and (ratio: 1:0.75 as is). Contamination of the material to be composted with *Listeria* was done as follows. Approximately 10^9 cells ml^{-1} each of the four strains, were grown separately in Universal *Listeria* Medium. These cultures were mixed and diluted 1/100 into the compost material to give about 10^7 *Listeria* g^{-1} compost wet weight. Assay of the mixed culture of *Listeria* gave $1.24x10^9$ *Listeria* ml^{-1}. An uncontaminated control compost was also run.

Laboratory Bench Scale Compost

The recipes were placed in 4 liter thermally insulated reactor vessels to permit self-heating. Air enters from a port at the top of the vessel and diffuses into the shallow compost mass. Initially O_2 concentration in the mass drops to approximately 3% and then slowly climbs to ambient towards the end of the process. The composts were sampled by transferring into a clean container, rotating and mixing and removing a sub-sample. Subsequently, they were returned immediately to the reactor vessel. Temperatures in the compost reactors were recorded.

Sampling Method

For food at ambient temperatures a 25g aliquot (wet weight) fresh food waste was suspended directly in 250 ml of UVM-1 medium and incubated at 36°C for 24 hr. With a cotton swab a sample of this growth was spread on a *Listeria* Cultivating Agar (LCA) plate and incubated at 36°C for 24 hr. Growth on the LCA plate was used for the NAH probes. The LCA plate was swabbed with a cotton swab removing as much growth as possible. The growth was suspended in phosphate buffered saline and the NAH probes were performed as directed.

For thermal compost, aliquots of 25g wet weight compost were taken from the lower center of the reactor vessel where the temperature probes were positioned. These samples were suspended in 250 ml of ULM preselective broth and incubated for 24 hrs at 36°C for the nonselective enrichment step. This twenty four hour growth was diluted 1/10 into 90 ml of UVM-1 and incubated 24 hours at 36°C for selective isolation of *Listeria*. From here, the procedure followed is the same as that for food samples.

Confirmation of the NAH Probe

To confirm NAH results, cells from the positive buffered saline samples were streaked on LCA media for isolation. A colony was picked and re-streaked on Biolog Universal

Growing Medium (BUGM) agar. Growth was analyzed phenotypically using the Microlog System. This method was used to confirm positive and negative results.

RESULTS AND DISCUSSION

Isolation of Listeria in Source Ingredients

Listeria directly selected in UVM-1 medium was detected in various freshly sampled food. Results given in Table 1 show that *Listeria* was detected in 3 restaurant samples, 2 household samples and 1 cafeteria sample. All of these positive results were confirmed using the Microlog system. These results indicate that normal selection methods of food microbiology are adequate for *Listeria* when the temperature is ambient.

Method for Isolation of Listeria from Thermal Compost Samples

Listeria contaminated samples of thermal compost 49°C to 51°C (food with newsprint) or 52°C to 53°C (food with leaves), were analyzed by selecting for *Listeria* at 48°C or 53°C with UVM-1 broth as was done with the food samples at ambient temperature. *Listeria* contamination was not detectable with the NAH probe by this recovery method (Table 2). These same thermal compost samples were also first grown for 24 hrs at 36°C in Universal Listeria Medium (ULM) as a nonselective step first. With this method the NAH probe was positive for *Listeria* (Table 2). The results using UVM-1 media for selection from the thermal compost produced a false negative result. Results given in Table 2 show ULM medium nonselective step must be used to recover the *Listeria* from composts with elevated temperatures. Others have observed that the selection of *Salmonellae* from composts by the Environmental Protection Agency (EPA) selection methods, (10), which ask that the compost be placed directly in a selective medium, do not enumerate the total number of *Salmonella* present in the sample (1, 20) has questioned the reliability of present selection methods for detecting indicator organisms and pathogens in waste water and waste water sludge.

Food Scrap Source	**Probe Result [1] with UVM-1 [2]**	**Confirmation[3]**
Restaurant (5 samples)	+(3) - (2)	confirmed
Household (5 samples)	+ (2) - (3)	confirmed
Cafeteria (3 samples)	+ (1) - (2)	confirmed

1. (+) positive (detected) for *Listeri*a species; (-) negative or less than 1 *Listeria* cell $25g^{-1}$ wet weight;
—(+) positiv (nachgewiesen) für *Listeria* spp. (-) negativ (nicht nachgewiesen) in $25g^{-1}$ Frischgewicht

2. Sample placed directly into selective UVM-1 medium at 36°C;
—Probe direkt in selectivem UVM-1 Nährmedium bei 36°C inkubiert

3. Microlog identification of *Listeria* in positive probe samples;
—positiv Probe wurde mit Microlog festgestellt

Table 2. Recovery of *Listeria* from Inoculated Composts as Influenced by the Media (UVM-1 or Universal *Listeria* Medium, ULM) at Different Temperatures

Tabelle 2. Nachweis von *Listeria* in beimpften Komposten, unter Einfluss von UVM-1 oder Universal *Listeria* Medium (ULM) bei vershiedenen Temperaturen

Sample	Maximum Temp °C		UVM-1[1]		Universal *Listeria* Medium (ULM)[2]		Confirmation[3]
			NAH Probe Results[4]				
	---------- Vessel ----------						
	A	B	A	B	A	B	
Food and Newsprint	22	22	+[5]	+	+	+	Confirmed
Food with Newsprint	51	49	-	-	+	+	Confirmed
	---------- Vessel ----------						
	C	D	C	D	C	D	
Food with Leaves	22	22	+	+	+	+	Confirmed
Food with Leaves	53	52	-	-	+	+	Confirmed

1. Sample placed directly into selective UVM-1 medium at 36°C ;
—Probe in selektivem UVM-1 Nährmedium bei 36°C unmittelbar inkubiert
2. Sample first grown on nonselective medium ULM at 36°C then placed into selective UVM-1 at 36°C;
—Probe wurde zuerst in ULM und danach in selektivem UVM-1 bei 36°C angezüchtet
3. Microlog identification of *Listeria* in positive probe samples;
—positiver Nachweis erfolgte mit Microlog
4. Samples were grown on LCA plates suspended in buffered saline;—Probe wurden auf LCA angezüchtet und in gepufferter NaCl suspendiert
5. (+) positive for the *Listeria* species; (-) negative or less than 1 *Listeria* cell $25g^{-1}$ wet weight;
—(+) Nachweis von Listeria spp. (-) negativ <1 Listerienzelle in $25g^{-1}$ Frischgewicht

Detection of Listeria in Composts.

As shown in Table 3, the contamination was detected only at ambient temperature when using the UVM-1 selective medium for immediate isolation. Vessel E but not vessel F showed *Listeria* to be present on day 3 and day 5 as well as in the beginning when ULM medium was used to grow up the community of organisms at 36°C prior to selection for *Listeria* spp. (Table 3). No *Listeria* was ever detected in the non-contaminated vessels.

Comparison of Two Different Compost Runs of Food Waste and Leaves with Differing Moisture Contents

Only when ULM medium was used to recover the *Listeria* from composts with elevated temperatures could the presence of *Listeria* the demonstrated. As shown in Table 4, *Listeria* do survive in two slightly different recipes of food and leaves during the thermal portion of the composting. However, it is important to remove the stress of the heat prior

Table 3. Occurrence of *Listeria* in Food and Leaves Compost (ratio 1:1), directly isolated with UVM-1 or Preselected with ULM Media

Tabelle 3. Nachweis von *Listeria* in Küchenabfällen und Laubblättern (Verhältnis 1:1) und direkt isoliert auf UVM-1 oder vorselektiert in ULM-Medium.

Day	MaximumTemp°C				Contaminated-UVM-1[1]		Contaminated ULM[2]		non-contaminated		Confirmation[3]
	Vessel Analyzed										
	E	F	G	H	E	F	E	F	G	H	
	NAH Probes[4]										
0	22	22	22	22	+[5]	+	+	+	-	-	confirmed
2	34	34	30	38	-	-	-	-	-	-	
3	47	45	46	46	-	-	+	-	-	-	confirmed
4	54	53	53	57	-	-	-	-	ND	ND	
5	61	62	63	65	-	-	+	-	ND	ND	confirmed

1. Sample placed directly into selective UVM-1 medium at 36°C ;
—Probe in selektivem UVM-1 Nährmedium bei 36°C unmittelbar inkubiert
2. Sample first grown on nonselective medium ULM at 36°C then placed into selective UVM-1 at 36°C;
—Probe wurde zuerst in ULM und danach in selektivem UVM-1 bei 36°C angezüchtet
3. Microlog identification of *Listeria* in positive probe samples;
—positiver Nachweis erfolgte mit Microlog
4. Samples were grown on LCA plates suspended in buffered saline;
—Probe wurden auf LCA angezüchtet und in gepufferter NaCl suspendiert
5. (+) positive for the *Listeria* species; (-) negative or less than 1 *Listeria* cell $25g^{-1}$ wet weight;
—(+) Nachweis von Listeria spp. (-) negativ <1 Listerienzelle in $25g^{-1}$ Frischgewicht

to selection for identification in order to avert a false negative result. Heat stressed bacteria may be sensitive to normal food microbiology selection methods. This does not necessarily mean they are not viable but may mean they are expressing their genome in a manner not conducive to producing growth in the selective media.

As can be seen in Tables 3 and 4 *Listeria* are never found consecutively in the daily samples. We attempted to assay for *Listeria* between days 8 and 11 (Table 4, Vessel X). *Listeria* could not be isolated from the NAH probe samples and identified. Standard microbiology identification involves biochemical tests requiring an isolated pure clone. In contrast, the NAH probe will show the presence of *Listeria* if the sample titer is above $1x10^6$ *Listeria* in the bacterial community to be assayed (2, 17). It may be possible that *Listeria* is detectable by NAH probes but not by classical isolation of clones and biochemical methods. The samples on day 8 and 11 were never proven to be false negatives.

We have previously shown that both *Salmonella* and *E. coli* have the ability to mutate becoming strains that grow well to 48°C and 54°C (6). This characteristic is passed on from generation to generation. It has been reported that both *Salmonella* and *Listeria* when exposed to thermal conditions can develop an acquired thermotolerance (3). *E. coli*

Table 4. Occurence of Listeria spp. in Thermal Samples of two Different Food Waste Compost Recipes (X-Y) with Different Isolation Media.

Days Composting	Maximum Temperature		Contaminated Isolated with UVM-1[1]		Contaminated Isolated with ULM[2]		Confirmation [3]
			VESSEL				
	X	Y	X	Y	X	Y	
			NAH Probes [4]				
0	22	22	+ [5]	+	+	+	+ Confirmed
5	54	38	-	-	-	-	
7	65	50	-	-	-	-	
8	64	48	-	-	+	-	+ Confirmed
9	67	45	-	-	-	+	+ Confirmed
10	64	45	-	-	-	-	
11	50	52	-	-	+	+	+ Confirmed
12	32	32	-	-	-	+	+ Confirmed
13	33	30	ND	ND	-	-	
21	22	22	ND	ND	-	-	

1. Sample placed directly into selective UVM-1 medium at 36°C ;
—Probe in selektivem UVM-1 Nährmedium bei 36°C unmittelbar inkubiert
2. Sample first grown on nonselective medium ULM at 36°C then placed into selective UVM-1 at 36°C;
—Probe wurde zuerst in ULM und danach in selektivem UVM-1 bei 36°C angezüchtet
3. Microlog identification of *Listeria* in positive probe samples;
—positiver Nachweis erfolgte mit Microlog
4 . Samples were grown on LCA plates suspended in buffered saline;
—Probe wurden auf LCA angezüchtet und in gepufferter NaCl suspendiert
5. (+) positive for the *Listeria* species; (-) negative or less than 1 *Listeria* cell $25g^{-1}$ wet weight;
—(+) Nachweis von Listeria spp. (-) negativ <1 Listerienzelle in $25g^{-1}$ Frischgewicht

also expresses acquired thermotolerance (14). This type of thermotolerance is not permanent and is not passed on to future generations. Thermal mutants of *Listeria* have never been reported and may not have ever been investigated.

Detection of L*isteria* at 64°C in compost (Table 4) suggests that high temperature alone may not be the only important factor for removal of *Listeria.* (15, 16*)* using standard methods for isolation showed that heat does not fully remove *Listeria* in short-term composting. We have previously reported that in thermal compost *Enterobacteriaceae* and *Pseudomonadaceae* were prominent organisms in 60°C samples. Gram positive nonspore-formers, i.e *Staphyloccus sciurii*, could be found at ambient temperatures. but no gram positive bacteria were observed in the thermal samples except *Bacillus* (9). These observations suggest that gram positive non-sporeformers do not survive well in aerobic compost. Most likely, both temperature and microbial antagonism are factors that act to exclude gram positive non-spore formers.

The low frequency and random occurrence in compost samples of the inoculated *Listeria* as determined by our study with NAH probes suggests that *Listeria* may not be a sig-

nificant hygiene problem when food is composted properly. However, we demonstrate that a special isolation method may be required for *Listeria* where the use of food microbiology techniques can yield a false negative result.

In conclusion, bacterial genomes can be expressed differently when growing in different environments. Therefore, when using a selective medium to isolate a bacterium from an environment, certainty is needed that the chosen medium is appropriate to that environment.

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