Pergamon

0961-9534(95)00002-X

Biomass and Bioenergy Vol. 8, No. 3, pp. 191–195, 1995

0961-9534/95 $9.50 + 0.00

EVIDENCE FOR THE PROMINENCE OF WELL CHARACTERIZED MESOPHILIC BACTERIA IN THERMOPHILIC (50–70°C) COMPOSTING ENVIRONMENTS

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(Received 21 June 1994; revised received 31 October 1994; accepted 15 November 1994)

Abstract—The prominent bacterial strains from both active (50–70°C) and presumably completed (ambient temperature) composts were isolated and identified using a carbon utilization technique with computerized database matching for bacterial identification. Prominent bacteria included *Escherichia coli*, *Serratia. marcesens*, *Citrobacter freundii*, *Klebsiella pneumoniae*, *Enterococcus gallarium*, *Enterobacter cloacae*, *Pseudomonas aeuginosa*, *Pseudomonas pseudoalcaligenes*, *Pseudomonas alcaligenes*, *Actineobacter genospecies* and *Alcaligenes fecalis* ss fecalis. When temperatures at sampling exceeded 60°C, the prominent bacteria found were assumed to be growing at or above this temperature, despite their classification as mesophils. The isolation of these strains from active compost strongly indicates that some mesophils have mechanisms for survival and perhaps replication at elevated temperatures, implicating a role in the composting process.

Keywords—Composting; mesophils; temperature; *E. coli*.

1. INTRODUCTION

Concerns have been raised about the presence of potentially pathogenic mesophilic bacteria in composts.[1–3] In the United States, laws govern the disposal of sludges and require a process to significantly reduce pathogens (PSRP) and/or a process to further reduce pathogens (PFRP) prior to land application.[4,5] With the passage of new EPA rules, the actual monitoring of selected bacterial, viral and ova densities is now required.[5,6] These regulations appear to be based on observations that re-growth of pathogens may occur after elevated temperatures assumed to destroy pathogens have subsided following treatment.[5–8]

The presence of mesophilic bacteria in completed composts has been linked to reinfection by a vector[5] or regrowth after bacteria surviving in the coolest parts of the compost pile remultiply. Only limited observations of pathogen survival throughout the composting cycle have been made.[9] Previous work suggests that antibiotic effects[7] or simple competition among species[7,8] may be an important factor linked to elimination of pathogens. With the advent of aerobic composting as a means of waste disposal, it has become important to determine which factors account for the presence of potentially pathogenic mesophilic bacteria in compost products.

Earlier studies indicate that the well-characterized mesophils may have the capacity to survive the high temperatures of composting. *B. subtilis* and *B. pumilus* produce thermophilic mutants able to grow between 50 and 70°C. *S. typhimurium*, *E. coli* and *P. aeruginosa* all create mutants capable of growth at 54°C.[10,11] These strains all carry genetic information which is expressed at temperatures of 48°C or higher,[12–14] suggesting that these bacteria characterized as mesophils are capable of survival and growth in hot composts. Mutant thermophilic strains of *E. coli* and *S. typhimurium* were previously identified with 16sRNA gene probes.[15] Both *E. coli* and *Salmonella* were shown to survive for days in compost with temperatures of at least 60°C.[15,16]

Modern carbon utilization techniques with computerized database matching can identify bacterial phenotypically by the pattern of respiration on 96 different substrates,[17,18]

Table 1. Prominent bacteria after bin composting of school food scraps

Age at sampling: 90 days	Maximum compost temperature achieved: 76°C	Temperature at sampling: 30–40°C
	Gram negative bacteria *Prvcrobacter immobilis*	Gram positive bacteria *Staphylococcus cohnii* *Staphylococcus hommis* *Bacillus thuringiensis/cereus*

permitting rapid identification of about 1100 bacteria. Moreover, these methods allow for cataloging various strains not previously identified. In this study we used the carbon utilization approach to determine which well-known mesophilic bacteria are present and we infer from this their importance to the composting process.

2. MATERIALS AND METHODS

2.1. *Compost materials*

Samples of fresh and actively composting cow manure, yard waste and food waste were evaluated in this study. Cow manure samples derived from a mixed-herd dairy farm in Orange County, NY, which employs a FAN separator to process liquid manure. Yard waste composts consisting of grass, leaves and wood chips were taken from the Southeastern Oakland County composting facility near Detroit, MI. Samples of food scrap compost were derived from two sources of cafeteria food waste which was either (1) pre-composted in an indoor compost bin facility, or (2) bench-scale composted in a laboratory facility.

2.2. *Bench-scale laboratory composting*

Four liter batches of compost materials were pre-mixed and placed into thermally insulated vessels which permit self-heating similar to that commonly encountered in large-scale composts. At appropriate times the vessels were opened, turned and sampled by transfer to a clean container during the composting process, and the compost reintroduced into the same vessels. By composting and sampling in the laboratory under controlled conditions, the potential for reinfection of the compost product was believed to be minimal.

2.3. *Isolation of prominent mesophilic bacteria present during composting*

A 5 g sample was suspended in 50 ml of sterile distilled water, vortexed, and allowed to soak for about 1 h to remove bacteria adhering to the compost, and vortexed again. A Hygicult paddle for total bacteria count was dipped into the compost–water suspension, and incubated overnight at 53°C. A sample from the paddle was streaked and grown on the Biolog universal growth medium (BUGM) or trypic soy agar at 37°C. Individual clones were picked, restreaked, and grown for 24 h at 37°C and identified.

2.4. *Identification of bacteria*

Identification of all isolates was accomplished with the BIOLOG™ system. This procedure

Table 2. Prominent bacteria after laboratory composting of cafeteria food scraps

Age at Sampling: 80 days	Maximum compost temperature achieved: 68°C	Temperature at sampling: 55°C
	Gram negative bacteria *Salmonella* subspecies 1G *Serratia marcesens* *Alcaligenes faecalis* ss faecalis *Pseudomonas aeruginosa* *Citrobacter freundii*	Gram positive bacteria None found
Age at sampling: 87 days	Maximum compost temperature achieved: 68°C	Temperature at sampling: 34°C
	Gram negative bacteria *Pseudomonas aeruginosa*	Gram positive bacteria *Enterococcus gallinarium* *Staphylococcus scuiri*

Table 3. Prominent bacteria in laboratory composting of cow manure

Age at sampling: 0 days	Maximum compost temperature achieved: 25°C	Temperature at sampling: 25°C
	Gram negative bacteria *E. coli* *Enterobacter cloacae* B *Citrobacter freundii* *Serratia marcesens* *Serratia liquefaciens/grimes* II	Gram positive bacteria *Bacillus sphaericus*
Age at sampling: 4 days	Maximum compost temperature: >60°C	Temperature at sampling: >60°C
	Gram negative bacteria *Pseudomaonas alcaligenes* A *Comanonas testosteroni* *Acinetobacter radiosesistens/genospecies* 12 *Klebsiella pneumoniae* ss ozaenae *Sphingobacterium muzutaii*	Gram positive bacteria *Bacillus pasteurii* *Bacillus sphaericus* *Bacillus brevis*
Age at sampling: 8 days	Maximum compost temperature: >60°C	Temperature at sampling: >60°C
	Gram negative bacteria *E. Coli* *Alcaligenes faecalis* ss faecalis	Gram positive bacteria *Bacillus brevis*
Age at sampling: 16 days	Maximum compost temperature: >60°C	Temperature at sampling: >22°C
	Gram negative bacteria 7 not identified 3 related to *Greenleaf* (see Table 4)	Gram positive bacteria *Bacillus brevis*

relies on the ability of the bacteria to respire on specific substrates distributed in a 96 well plate.[14] Identifications were routinely checked by classical methods. Identifications for both *E. coli* and *Salmonella* were confirmed with specific 16sRNA gene probe assay kits (GeneTrak, Framingham, MA).

3. RESULTS

In Table 1, we show the bacterial composition of compost from the school cafeteria scraps composting project. In Table 2, we give the composition of compost derived from prison cafeteria scraps which has been laboratory composted. The data demonstrate significant differences in bacterial prominence between the two groups of food waste composts. In the school cafeteria waste compost, gram positive bacteria (*Staphylococcus*) predominated. The prison food waste compost (Table 2) indicated several gram negative species at two sampling dates including *Salmonella* and *Pseudomonas aeruginosa*. The school cafeteria waste appeared

Table 4. Prominent bacteria in yard waste compost

Age at sampling: 14 days	Maximum compost temperature: 68°C	Temperature at sampling: 68°C
	Gram negative bacteria *Deleya aesta* *Citrobacter freundii* *E.coli*[1] *Salmonella* subspecies IG	Gram positive bacteria None found
Age at sampling: 21 days	Maximum compost temperature: 68°C	Temperature at sampling: 68°C
	Gram negative bacteria *Enterobacter agglomerans* biogroup B 4 similar unidentified bacteria called *Greenleaf*[2]	Gram positive bacteria None found
Age at sampling: 29 days	Maximum compost temperature: 68°C	Temperature at sampling: 59°C
	Gram negative bacteria *Acinetobacter genospecies* 15 *Pseudomonas pseudoalcaligenes* A *Citrobacter freundii* *Klebsiella pneumoniae* ss pneumoniae	Gram positive bacteria None found

to go to completion (did not reheat significantly) while the prison food waste with contained paper and plastic reheated to 68°C when moistened.

Cow manure compost from laboratory vessels is characterized in Table 3. *E. coli* was found to be prominent in both the uncomposted manure and day-8 compost. At the time of sampling, the day-8 compost had achieved temperatures of 60°C or higher for at least 6 days. After 16 days of laboratory composting we observed uncataloged, gram negative bacteria and *Bacillus brevis*. The compost did not reheat at this stage and appeared to be moderately stable.

In composting yardwaste (Table 4), several gram negative mesophils predominated when temperatures rose to about 60°C. *Citrobacter freundii* was found at both 2 and 4 weeks, suggesting this mesophil might be growing. The *E. coli* strain found after 2 weeks in this compost grew and formed large swarming colonies at 60°C on agar plates; however, it became unable to grow at elevated temperatures after storage on a slant at room temperature for about 6 weeks. It did not grow below 36°C, although it remained viable. This clone was confirmed as *E. coli* by a 16sRNA gene probe.

The gram negative isolates *Sphingobacterium muzutaii* and *Comanonas testosteroni* found in cow manure (Table 4) were able to form colonies under laboratory conditions at 53°C. Additonally, an uncataloged bacteria called *Greenleaf* was able to grow at 53°C. Most of these bacterial isolates lost the ability to grow at 53°C after a short period of storage at room temperature.

4. CONCLUSIONS

Evidence in this study indicates that a variety of bacteria, traditionally characterized as mesophils, survive and are prominent at the high temperatures of active composting. This agrees with our previous results which show that *E. coli*, *S. typhimurlum* and *P. aeruginosa* thermophilic mutants can grow on agar at 54°C.[10,11,19] The controlled conditions of the laboratory-scale in-vessel composting we employed were believed to have eliminated the possibility that mesophils initially present survived in cool areas of the compost, or were reintroduced by a vector. Therefore, survival at high temperatures is sufficient here to explain the presence of these mesophilic bacteria identified in the later stages of composting. From this, it is expected that the hygienic control of the compost would necessitate curing past the active phase of composting. Thus, the results of this study suggest that the U.S. EPA rules currently promulgated which require monitoring of *E. coli* and *Salmonella* in later stages after treatment are appropriate measures of control for composting where pathogen effects may be of concern.

REFERENCES

1. N. Goldstein, What's in store in 1994 with biosolids regulation. *Biocycle*, pp. 48–51 (January 1994).
2. D. A. Stahl, The natural history of microorganisms—new approaches are needed to appreciate how microorganisms fit in the biosphere. *ASM News* **59**, 609–613 (1993).
3. D. Strauch, Hygienische Aspekte der Bioabfallkompostierung, in *Proc. Compost Hygiene Workshop*, Hohenheim University, Stuttgart (1994)(in German).
4. Federal Register, *Solid Waste Disposal Criteria: Final Rule*. Code of Federal Regulations, Parts 257 and 258 (1991).
5. U.S. EPA, Environmental regulations and technology: control of pathogens in municipal wastewater sludges. US EPA Report 625./10-89/006 (1989).
6. Federal Register, *Solid Waste Disposal Criteria: New Rule*, Code of Federal Regulations, Part 503 (1992).
7. J. S. Wiley, Pathogen survival in composting municipal wastes. *J. Water Pollut. Contr. Fed.* **34**, 80–90 (1962).
8. B. B. Wiley and S. C. Westerburg, Survival of human pathogens in composted sewage. *Appl. Microbiol.* **18**, 944–1001 (1969).
9. J. B. Farrell, Fecal pathogen control during composting, in *Composting Science and Engineering*. Ohio University Press (1993).
10. M. L. Droffner and N. Yamamoto, Prolonged environmental stress via a two step process selects mutants of *Escherichia coli*, *Salmonella typhimurium* and *Pseudomonas aeruginosa* that grow at 54°C. *Arch. Microbiol.* **156**, 307–311 (1991).
11. M. L. Droffner and N. Yamamoto, Procedure for isolation of *Escherichia coli*, *Salmonella* and *Pseudomonas* mutants capable of growth at 54°C. *J. Microbiol. Methods* **14**, 201–206 (1991).
12. M. L. Droffner and N. Yamamoto, Demonstration of *cel* operon expression in *Escherichia*, *Salmonella* and *Pseudomonas* at elevated temperatures refractory to their growth. *Appl. Environ. Microbiol.* **18**, 944–1001 (1992).
13. M. L. Droffner and N. Yamamoto, Role of nalidixic acid in isolation of *Salmonella typhimurium* strains capable of growth at 48°C. *Cur. Microbiol.* **25**, 257–260 (1992).
14. K. S. Srivenugopal, D. Lockshon and D. R. Morris, *Escherichia coli* DNA topoi somerase II: purification and characterization of a new type I enzyme. *Biochemistry* **23**, 1899–1906 (1984).
15. W. F. Brinton and M. L. Droffner, Microbial approaches to characterization of composting processes. *Compost Sci. Util.* **12**, 12–17 (1994).
16. M. L. Droffner and W. F. Brinton, Survival of *E. coli* and *Salmonella* populations in aerobic thermophilic composts as measured with DNA gene probes, submitted to *Int. J. Environ. Med.* (1995).

17. J. Krieger, Technique identified bacteria that degrade wastes. *Science/Technology*, p. 36 (1992).
18. C. E. Stager and J. Davis, Automated systems for identification of microorganisms. *Clin. Microbiol. Rev.* **5**, 302–327 (1992).
19. M. L. Droffner and N. Yamamoto, Isolation of thermophilic mutants of *B. subtilis* and *B. pumilus* and transformation of the thermophilic trait to mesophilic strains. *J. Gen. Microbiol.* **131**, 2791–2794 (1985).