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TAXONOMIC AND METABOLIC MICROBIAL DIVERSITY DURING COMPOSTING

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Summary

A great variety and high numbers of aerobic thermophilic heterotrophic and/or autotrophic bacteria growing at temperatures between 60-80°C have been isolated from thermogenic (temperatures > 60°C) composts in several composting facilities in Switzerland. They include strains related to *Thermus aquaticus, Bacillus schlegelii*, *Hydrogenobacter spp.*, and of course heterotrophic sporeforming *Bacilli*. This contrasts with the generally held belief that thermogenic composts (> 60°C) support only a very low diversity of heterotrophic thermophiles. This biodiversity suggests efficient decomposition of organic matter at temperatures above 60°C, and a good thermo-hygienization.
During the terminal cooling or maturation phase of composts high numbers and a great metabolic diversity of mesophilic bacteria was observed, including nitrogen-fixers, sulfur-oxidizers, hydrogen-oxidizers, nitrifyiers, and producers of extracellular polysaccharides or bacterial humin. This microbial diversity plays an essential role for compost stabilization. It is suggested that mature compost application improves soil chemistry and microbiology, and can thus be regarded beneficial for agriculture.

1. Introduction

Among the various processes used to manage organic wastes (landfill, incineration), only the biological process of composting can bring about a stabilization of the waste, making its return to the environment as soil fertilizer and conditioner possible. Composting is a self-heating, aerobic solid phase biodegradative process of organic waste materials. The composting process at the microbial level involves several interrelated factors, i.e. metabolic heat generation, temperature, ventilation (oxygen input), moisture content, and available nutrients (12,14,18). The temperature both reflects prior microbial activity and the current rate of activity. The initial rapid increase of temperature involves a rapid transition from a mesophilic to a thermophilic microflora. The compost ecosystem then tends to limit itself due to inhibitory high temperatures, resulting from excessive heat accumulation. If a good management is continuously provided (i.e. regular aeration or frequent turning), the thermogenic stage continues until the heat production becomes lower than heat dissipation, due to the exhaustion of easily metabolizable substrates. During the terminal cooling or maturing phase, the amount of readily available nutrient becomes a limiting factor, causing a decline in microbial activity and heat output. During these temperature changes various microbial groups succeed each other, each of which being adapted to a particular environment.
We present here the results of the research in our laboratory on different microbiological aspects of the composting process. The purpose of this report is to provide a better understanding of the taxonomic and functional diversity of microorganisms, particularly aerobic bacteria, during the composting process.

2. Microbial diversity during the composting process

A large variety of mesophilic, thermotolerant and thermophilic aerobic microorganisms (including bacteria, actinomycetes, yeasts, molds and various other fungi) have been extensively reported in composting and other self-heating organic materials at temperatures between 20-60°C (2,14,17,18,20,27,28,31,32,34). Many factors determine the microbial community during composting. Under aerobic conditions, temperature is a major factor determining the type of microorganism, species diversity, and the rate of metabolic activities. At an early phase of the composting process (temperatures between 20-40°C) mesophilic/thermotolerant fungi, principally yeasts and molds, and acid producing

bacteria are the dominant active degraders of fresh organic waste. Actinomycetes develop far more slowly than most bacteria and fungi and are rather ineffective competitors when nutrient levels are high. Mesophilic microorganisms are partially killed or are poorly active during the initial thermogenic stage (temperatures between 40-60°C), where the number and species diversity of thermophilic/thermotolerant bacteria, actinomycetes and fungi increase (14,18,34). The optimal temperature for thermophilic fungi is 40-55°C, with a maximum at 60-62°C. Fungi are killed or are present transiently as spores at temperatures above 60°C (4, 18). Thermophilic actinomycetes are generally more tolerant than fungi to high temperatures and their number and species increases markedly at their optimum growth temperatures of 50-55°C (2,17,18,34). At temperatures above 60°C their number and the species diversity decreases, and their importance in the degradation process becomes negligible. Thermophilic bacteria are very active at 50-60°C, and at temperatures above 60°C the degradation process is performed essentially by these microorganisms (18,20,27,30,31). All research focused on enumeration and isolation of aerobic thermophilic bacteria from composts has been performed essentially on rich organic complex media.

2.1. Aerobic thermophilic bacteria isolated from thermogenic composts

A high diversity of obligately heterotrophic *Bacilli* was isolated from thermogenic composts at temperatures between 50-60°C (20,31,32). However, bacterial species diversity dropped markedly at temperatures above 60°C, and at the highest temperatures studied (65-69°C) only strains related to *Bacillus stearothermophilus* were identified (31,32). The present state of knowledge on microbial diversity at higher temperatures remains surprisingly poor. We report here for the first time the presence of taxonomic and metabolic bacterial diversity in hot composts (60-80°C). We examined composts samples (mainly garden and kitchen waste, and sewage sludge) from 10 different compost facilities throughout Switzerland, representing different industrial composting systems (classical open air windrow, semi-closed aerated and/or turned boxes, and closed automatically aerated bioreactors of 24 m^3 and 200 m^3). The compost facilities were located at 30-250 km distance from the authors laboratory. None of the compost facilities studied used seeding with a commercial compost-starter containing thermophilic bacteria.
Our results showed the presence of different taxonomic and metabolic aerobic thermophilic bacteria, related to the following genera or species :

Hydrogenobacter spp. : 10^4-10^6 cells / g CDW, growth at 60-80°C, optimum 70-75°C
Bacillus schlegelii : 10^5-10^7 cells / g CDW, growth at 55-75°C, optimum 65-70°C
Thermus aquaticus : 10^3-10^{10} cells / g CDW, growth at 40-80°C, optimum 70°C
Heterotrophic Bacilli : 10^7-10^{11} cells / g CDW, growth at 35-70°C, optimum 50 or 60°C
(g CDW = g compost dry weight)

These strains can be splitted into four groups :

2.1.1 Obligately autotrophic sulfur-and hydrogen-oxidizers *(Hydrogenobacter spp.)*

All strains isolated (14 strains) were rods 0.5 x 1.5-6 µm in size, no spore-formers, gram stain negative, variable in motility, and penicillin G sensitive proving to belong to the Bacteria domain. These bacteria were able to grow under microaerophilic conditions with either hydrogen, crystalline elemental sulfur or thiosulfate as sole energy and electron donor, and with CO_2 as carbon source. They were not able to grow on the simple and complex organic substrates tested. Thus, we regard them as obligate autotrophic strains. These strains had DNA % mol guanine + cytosine contents (35-39.4) similar to those published for the reference strains related to *Hydrogenobacter spp.*, isolated at this time only from geothermal areas (3). Almost all strains shared a high DNA:DNA homology (71-92%) among each other, and a similar homology with *Hydrogenobacter* reference strain T3, belonging to a DNA:DNA homology group found in geothermal springs in Italy and in the USA. One strain showed no significant homology with strain T3, but a high homology (86%) with *Hydrogenobacter* reference strain MF-3, belonging to another DNA:DNA homology group (3).

2.1.2. Facultatively autotrophic sulfur- and hydrogen-oxidizers *(Bacillus schlegelii)*

Nine of the strains isolated (total 10 strains) were rods 0.6 x 2.5-5 µm in size, formed spherical (0.8-1 µm in diameter) and terminal endospores, were gram stain variable, variable in motility, and penicillin G sensitive, proving to belong to the Bacteria domain. One strain formed no spores. Metabolism was strictly

aerobic. These bacteria were able to grow autotrophically under microaerophilic conditions with hydrogen, but they were not able to grow autotrophically on reduced inorganic sulfur compounds (9). However, all strains possessed constitutive rhodanese, thiosulfate- and sulfite-oxidizing activities. Under mixotrophic growth conditions (e.g. pyruvate or acetate or hydrogen + thiosulfate) these sulfur-oxidizing activities were strongly increased (Beffa et al unpublished). They were able to grow on amino and organic acids, but not on sugars. All strains isolated had similar DNA % mol guanine+cytosine content (60-64) and shared high DNA:DNA homology (74-84 %) with the reference strain of *B. schlegelii*. This is the first report of the isolation of *B. schlegelii* from hot composts. Previous studies reported the presence of *B. chlegelii* in several geothermal and nongeothermal (fresh-water lake sediments, glacier ice, air) environments, and all strains isolated were related to the same genospecies (3).

2.1.3. Obligately heterotrophic non spore-formers related to the genus *Thermus*

High numbers (10^7-10^{10} cells/g CDW) of obligately aerobic thermophilic bacteria related to the genus *Thermus* (12 strains) were isolated in our laboratory from hot composts (> 65°C). Their number was lower at the beginning and at the end of the composting process. Isolation was done by incubation at 75°C in unshaked culture containing 0.8 % nutrient broth (NB) + 0.2 % yeast extract (YE). Best growth was obtained in basal mineral medium supplemented with organic compounds (e.g. acetate, pyruvate, glucose, starch, or NB + YE). Enrichment at 60°C and 65°C on the same media yielded mostly spore-forming bacteria. Preliminary results (4 strains) showed positive growth at high concentrations of organic compounds (5 % NB + 0.2 % YE, 4 % acetate + 0.2 % YE, 2.5 % starch + 0.2 % YE). All strains isolated were non-motile, non-sporeformers, rods and filaments 0.5-0.8 µm diameter, and lacking pigments. The length of the cell increased strongly as a function of incubation temperature.
Four strains isolated from the dominant heterotrophic population in hot composts showed similar % mol G+C (58-63 %) and high DNA:DNA homology with the reference strain of *Thermus aquaticus* (60-79 %), and poor DNA:DNA homology (< 29 %) with the reference strain of *Thermus ruber*. DNA:DNA homology with the strain *Thermus thermophilus* has not been studied yet. The physiological and taxonomical properties of the strains isolated and tested clearly correspond to the characteristics reported for the species *Thermus aquaticus* (13,35).

Two nonspore-forming strains of thermophilic bacteria have previously been isolated at 60°C from sewage sludge compost (20). Their optimum temperature ranged from 60-65°C, and they were tentatively identified as *Thermus spp.*. No taxonomical studies and numbers of these bacteria in compost have been reported.

2.1.4. Obligately heterotrophic oval endospore-formers *Bacilli*

Thermophilic heterotrophic *Bacilli* are dominant at temperatures between 50-65°C. More than 30 strains were isolated in our laboratory, showing a wide variation in colony morphology. Several strains were motile, spreading on agar plates. High heterogeneity and several different taxonomically distinct groups has been previously reported (30, 32). On the basis of their growth temperature optimum, 2 groups could be distinguished, with optima at 50°C and 60°C, respectively. Several strains growing fast at 50°C were unable to grow at 65°C. The upper limit of growth temperature for all strains isolated from hot compost was 70°C. Growth characteristics and genomic studies of the strains growing at 65-70°C gave strong evidence for these strains belonging to the *B. stearothermophilus* complex (30), as previously reports (31).
We have also isolated 3 strains of facultatively aerobic heterotrophic *Bacilli* growing anaerobically at 65°C on an organic medium with nitrate as terminal respiratory electron acceptor.

2.2. Microbial diversity during the cooling or maturing phase

The degree of maturity of the compost critically affects its successful utilization in agriculture. Immature composts induce high microbial activity in the soil for some time after their incorporation, causing oxygen deficiency and a variety of indirect toxicity problems to plant roots (22,36). Compost maturation varies considerably in function of the system and the composting management. Chemical and biological stability of the end-product seems to be very difficult to define with only a single analytical methods, whether chemical (22) or biological (36). Benefits of compost application in agriculture and its role in biological control of plant diseases have been previously reported (1,16,21).
A decisive factor for the maturation of composts is the microbial populations involved in the nutrient cycles (i.e. C, N, S, P). Knowledge of the microbial composition of mature composts is important to predict its potential impact on soil fertility and other biological parameters.

The present-state of knowledge on microbial diversity, particularly bacteria, during the maturing phase of composting is surprisingly poor. During this phase the diversity and the number of mesophilic/thermotolerant actinomycetes and fungi, attacking and/or degrading natural complex polymers (i.e. lignin, hemi-cellulose, cellulose) increases strongly (14,18,34). During the cooling phase, the bacterial population decreases of 1 or 2 logarithmic order in comparison with the numbers present during the thermogenic phase (10^8-10^{11} cells/g CDW), but its taxonomic and metabolic diversity increases markedly.
The microbiological characterization of four samples of composted urban refuse (15) showed 10^8-10^9 viable microorganisms per g CDW. The bacteria represented 80 % of the total counts, and a small proportion was spore-forming. Actinomycetes and fungi were present in numbers between 10^7-10^8 cells/g CDW and 10^5-10^8 cells/g CDW, respectively. Algae were absent. Most of the population (10^4-10^8 cells/g CDW) involved in the carbon cycle had proteolytic, ammonificant, amylolytic, and aerobic cellulolytic capacities, followed by free-living nitrogen fixers (*Azotobacter*, 10^3-10^5 cells/g CDW), denitrifiers (10^4-10^6 cells/g CDW), sulfate reducers (10^4 cells/g CDW), and sulfur oxidizers (10^4 cells/g CDW). An important feature was the scarcity or absence of ammonium- and nitrite-oxidizers.

Microbial diversity in maturing composts in a classical green waste windrow system

We report here the preliminary results of microbial diversity, particularly bacteria, in maturing composts from a classical windrow open air industrial composting system using an intensive management. The starting material consisted of 70 % green waste, 10 % kitchen waste, and 20 % shredded wood, with a good structure and porosity. The initial carbon/nitrogen ratio was 25-28. The compost was considered to be satisfactory mature after about 10-12 weeks. Sampling was performed on the surface and in the center. Prior to sampling the following parameters were measured in the heaps: Temperature, oxygen-, ammonia-, sulfide-, methane-, and carbon dioxide-concentration. The temperature of all samples were comprised between 22-43°C.
Enrichment and quantification were performed at 25°C on a rich organic complex medium (nutrient broth supplemented with yeast extract) and in a basal mineral medium supplemented with the appropriate energetic or assimilative compounds (8-11).
To ascertain the metabolic functions of mixed populations (directly from enrichments) and of isolated strains some electrode-respirometry measurements for hydrogen-, thiosulfate-, sulfur-, ammonium-, nitrite-, and organic-oxidizing activities were carried out (8-11). Respiratory chain inhibitors were used to detect respiratory chain linked reactions. Nitrogen-fixing activity (nitrogenase) was confirmed by gas chromatography measurements (Carlo Erba, Porapack N column, FID detector) of the reduction of acetylene to ethylene. Controls were made without acetylene and with NH_4Cl (1 g/l) to exclude any non-nitrogenase-dependent ethylene production. Extracellular polysaccharide production was confirmed by sugar analysis after acid hydrolysis of the bacterial polymers produced. Colorimetric analyses of nitrite and nitrate were performed to measure non respiratory chain linked reactions.

Our results confirm an increase of bacterial metabolic diversity during the terminal maturing phase of the composting process. Several bacterial functions important for compost maturation that are absent or not detected in the thermogenic phase appear during the cooling phase, such as autotrophic and heterotrophic nitrogen fixation, nitrification, production of large amount of exopolysaccharides or bacterial humic compounds.

Heterotrophs : High numbers of heterotrophic bacteria were isolated by enrichment on organic media. Surprisingly, metabolic studies revealed that several heterotrophic strains isolated were not simple organic oxidizers, but possessed other metabolic properties, such as nitrogen-fixation, autotrophic or heterotrophic sulfur-oxidation, exopolysaccharide production, nitrite production from ammonium under heterotrophic conditions, hydrogen-oxidation, and growth on methanol and ethanol. Spore-forming heterotrophic bacteria were not dominant and represented < 1 % of the total viable heterotrophic bacteria isolated from composts.
Hydrogen-oxidizers : All strains isolated under autotrophic conditions on hydrogen were also heterotrophic strains, and the majority were able to fix nitrogen under autotrophic (hydrogen) and heterotrophic (pyruvate) conditions. Several strains hade yellow pigments, and were related to the genera *Hydrogenophaga* (old name *Pseudomona*s) and *Xanthobacter*.
Sulfur-oxidizers : The majority of the strains isolated were facultatively autotrophic, growing on different organic substrates and on thiosulfate and elemental sulfur as unique electron donors. Two species have been characterized: *Thiobacillus versutus* (10) and *Paracoccus denitrificans*.

Nitrifyiers : Autotrophic nitrifying bacteria growing on ammonium or nitrite were not isolated. The nitrification capacity, principally related to the production of nitrite from ammonium, was related to heterotrophic strains.
Nitrogen-fixers : High numbers of autotrophic and heterotrophic nitrogen-fixing bacteria were isolated. Several strains formed exopolysaccharides, and oxidized inorganic sulfur compounds under heterotrophic growth conditions .
Arthrobacter-like bacteria: These strains were isolated on the selective medium according to (23). High numbers of *Arthrobacters* were principally isolated from maturing composts at low temperatures (< 30°C). All strains isolated presened the typical *Arthrobacter* morphology changes during the growth cycle. Nitrification seemed positive in 2 strains.

Table 1 : Metabolic diversity of microorganisms isolated under aerobic conditions from maturing composts after 12 weeks of composting. The minimal and maximal cells numbers are reported. The two last positive dilutions of the enrichments were used for isolation and preliminary biochemical characterizations. nd = not determined.

Type of metabolism or microorganism	Number of different strains or species	Cells numbers (g compost dry weight)
Bacteria :		
Heterotrophic (no spore-forming)	18	10^7 - 10^9
Heterotrophic (spore-forming)	3	10^5 - 10^6
Hydrogen-oxidizing (facultatively autotrophic)	6	10^5 - 5 x 10^6
Hydrogen-oxidizing (obligately autotrophic)	0	< 10
Sulfur-oxidizing (obligately autotrophic)	2	10^3 - 10^4
Sulfur-oxidizing (facultatively autotrophic)	7	10^4 - 10^7
Sulfur-oxidizing (obligately heterotrophic)	3	10^5 - 10^7
Nitrifying (obligately autotrophic)	0	< 10
Nitrifying (obligately heterotrophic)	4	5 x 10^4 - 10^6
N_2-fixing (autotrophic on hydrogen)	5	5 x 10^4 - 5 x 10^6
N_2-fixing (heterotrophic on pyruvate)	7	10^5 - 10^7
Exopolysaccharide producers (on glucose)	10	10^6 - 10^8
Arthrobacter-like	4	10^5 - 10^7
Thermophilic sporeforming bacteria (growth at > 40°C)	nd (> 3)	10^6 - 10^8
Fungi :		
Total molds	14	10^4 - 10^5
Aspergillus fumigatus	nd	10^2 - < 10^4

Other microorganisms : A low number but a high diversity of molds (isolated on malt extract agar with the antibiotics novobiocin and streptomycin) were present in maturing compost, and *Aspergillus fumigatus* represented < 5 % of the total counts. High numbers of thermophilic sporeforming bacteria, most probably as inactive spores, were present. Actinomycete numbers and diversity will be considered in the future.

Further characterization and confirmation of the taxonomic and metabolic properties of these different strains are being presently performed in our laboratory.

3. Microbial diversity and management

Composting is defined as an aerobic process, and several factors influence the concentration of oxygen in compost, and therefore the microbial population diversity and their type of metabolism (aerobic or anaerobic). The nature and structure of the initial substrates, the particle size, the compost volume, the moisture content, the consumption by the microbial biomass and in particular the compost technology used and the management influence considerably the oxygen concentration in composts (12,14,18,19). Industrial composting is a controlled process. This implies an active or intensive management, i.e. a judicious mixture of the initial substrate, a frequent mixing of the compost, and sufficient aeration and hydration. These conditions are met by composting in closed industrial bioreactors with automatic aeration and/or turning, in classical open air windrows with frequent or daily turnings of the heaps, or in automatic aerated and turned semi-closed boxes. Under these conditions an intense aerobic microbial

degradation can be insured for a long period of time in almost the whole composting mass. The microbial diversity and degradation rate are homogenous.
In contrast, in extensive management composting systems, such as classical open air windrows which are poorly structured and turned, in too dense or hydrated, poorly mixed and aerated composts in boxes or bioreactors, the temperature stratification is heterogeneous, and only a small thermogenic zone is observed (4,6). This is followed generally by the formation of large and less thermogenic anaerobic zones with production of large amounts of methane, carbon monoxide, sulfides, ammonia, and volatile organic acids. Under these conditions the degradation of the organic matter is slow, discontinuous and heterogeneous. Destruction of pathogens is not assured, and nauseating odors can be created.

4. Influence of temperature on the composting process and the hygienization

The composting process can, if not properly managed, induce the proliferation and dispersion of potentially pathogenic and/or allergenic thermotolerant/thermophilic molds and bacteria (4,5,7,24,26). Among the fungi, the mold *Aspergillus fumigatus* is predominant. Because of its cellulolytic and thermotolerant properties (best growth between 30°C and 45°C, and transient resistantany to temperature up to 60°C) it finds ideal growth conditions in compost, particularly in the outer layers of compost heaps (4). Numbers of were high in fresh compost (10^5 to 10^7 CFU/g CDW), remained high in extensive managed composts systems, and diminished towards at the end of the composting process (4,5). An intensive management, with temperatures exceeding 60°C for several weeks was necessary for decreasing significatively *A. fumigatus* numbers, and preventing significative recolonisation of cooling composts by this mold. Organic matter degradation, with regard to the carbon dioxide production and the constant high temperatures observed during several weeks in intensive compost systems, seemed not be significatively affected .

5. Conclusions

A great variety and high numbers of aerobic thermophilic heterotrophic and/or autotrophic bacteria growing optimally at temperatures of 65-75°C have been isolated from thermogenic (> 60°C) composts of several industrial facilities in Switzerland. It seems that bacteria related to *Bacillus schlegelii, Hydrogenobacter spp.* and particularly *Thermus aquaticus* are the principal active microorganisms in these composts (temperatures > 65°C and < 80°C). This is the first report of composts as habitats for thermophilic bacteria known to date only from geothermal manifestations, or other natural hot environments.
Microbial diversity is of course a prerequisite for a satisfactory composting process. High temperatures (> 60°C) are often considered to reduce dramatically the functional biodiversity. It is generally assumed that to obtain efficient and rapid decomposition temperatures should not be allowed to exceed 55-60°C (25,33). However, at these temperatures the thermohygienization towards potentially pathogenic and / or allergenic microorganisms is not guaranteed.
Few studies reported high decomposition rates at 60-75°C (28,29). The metabolic diversity of highly thermophilic bacteria reported in this study suggests that the thermogenic phase of the composting could be performed for a long period of time at temperatures higher than 50-60°C, but not exceeding 75°C. More microbiological and physico-chemical research on industrial and laboratory composting systems are necessary to further elucidate the advantages or inconvenients of high temperatures during the thermogenic phase.
However, we think that an intensive management of the composting process, permitting to reach transiently high temperatures during the thermogenic phase, is necessary to obtain a homogenous and satisfactory aerobic decomposition rate, the reduction or elimination of human, animal and plant pathogens, and the destruction of seeds and phytotoxic compounds. Furthermore it prevents the formation of large anaerobic zones that are sources of nauseating odors.

Our preliminary results demonstrated that a high bacterial diversity of mesophilic bacteria appeared during the cooling or maturing phase of the composting process, e.g. nitrogen fixers, nitrifiers, and producers of large amounts of extracellular polysaccharides. These microbial properties play an essential role for compost maturation and mineralisation. Incorporation of compost to soil could therefore improve soil chemistry and fertility (by nitrogen fixers, nitrifyiers, sulfur-oxidizers), structure (by exopolysaccharides producers) and microbiology.
The breakdown rate of lignocellulose by actinomycetes and fungi, and lignin by fungi with production of complex polymers is usually low during the short processing time of industrial composts. The presence of high numbers of heterotrophic bacteria able to produce (under laboratory conditions) large amounts of exopolysaccharides suggests that complex polymers are abundantly synthetized during the maturing

phase. These bacterial polymers (bacterial humin) and/or bacteria synthesizing them could improve soil structure and water and mineral retention.
Strains described taxonomically could be related to soil-living bacteria (i. e. genera *Arthrobacter, Hydrogenophaga or Pseudomonas, Xantobacter, Thiobacillus*). Bacteria related to the genus *Arthrobacter* form a numerically important fraction of the natural bacterial flora of soils, and their presence and numbers in mature composts could be used as one additional microbiological parameter for compost maturity evaluation.

Multidisciplinary, fundamental and applied laboratory, and particularily field research, involving engineers, agronomists, microbiologists, and pedologists, would be helpful for a better understanding and promotion of the composting process, and for a beneficial use of compost in agricultural and other soils.

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