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Thermophilic methanogenic *Archaea* in compost material: Occurrence, persistence and possible mechanisms for their distribution to other environments

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Abstract

Since compost is widely used as soil amendment and the fact that during the processing of compost material high amounts of microorganisms are released into the air, we investigated whether compost may act as a carrier for thermophilic methanogens to temperate soils.

All eight investigated compost materials showed a clear methane production potential between 0.01 and 0.98 μmol CH_4 g dw^{-1} h^{-1} at 50 °C. Single strand conformation polymorphism (SSCP) and cloning analysis indicated the presence of *Methanosarcina thermophila*, *Methanoculleus thermophilus*, and *Methanobacterium formicicum*.

Bioaerosols collected during the turning of a compost pile showed both a highly similar SSCP profile compared to the corresponding compost material and clear methane production during anoxic incubation in selective medium at 50 °C. Both observations indicated a considerable release of thermophilic methanogens into the air.

To analyse the persistence of compost-borne thermophilic methanogens in temperate oxic soils, we therefore studied their potential activity in compost and compost/soil mixtures, which was brought to a meadow soil, as well as in an agricultural soil fertilised with compost. After 24 h anoxic incubation at 50 °C, all samples containing compost showed a clear methanogenic activity, even 1 year after application.

In combination with the *in vitro* observed resilience of the compost-borne methanogens against desiccation and UV radiation we assume that compost material acts as an effective carrier for the distribution of thermophilic methanogens by fertilisation and wind.

Keywords: Compost; Thermophilic methane production; *Methanosarcina thermophila*; *Methanoculleus thermophilus*; Bioaerosols; UV-light; Desiccation

Introduction

Thermophilic or hyperthermophilic microorganisms have been isolated from environments with elevated temperatures, e.g. hydrothermal vents in freshwater lakes and ocean sediments [10,11,22,23,37], geo- and hydrothermal environments [25,38], or hot springs [2]. However, thermophiles also seem to exist in permanent cold or temperate environments [6,28,29,43], although the environmental conditions are not ideal. Until now, little has been known about the origin of these microorganisms in the aforementioned environments,

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although some thermophiles might be able to grow at mesophilic temperatures because of their large temperature range [43]. This would be one possible explanation for their existence in moderate environments. The input of microorganisms via air from volcanic or geothermic/hydrothermic habitats might be a further explanation for their distribution [6]. Additionally, the fertilisation of arable soil with cattle manure, which is well known to contain anaerobic methanogens, was studied and found to be an inoculum for species of thermophilic methanogenic *Methanoculleus* and *Methanosarcina* [16]. These results confirm the assumption of Fey et al. [13], who detected thermophilic methanogens in paddy soil, that these organisms are widespread in different soil habitats. The strictly anaerobic *Archaea* do not form any resting stages, thus their survival in oxic soils is astonishing. Peters and Conrad [31] enriched anaerobic methanogenic *Archaea* from desert soil, cultivated soil and forest soil. The good survival of methanogens during O_2 exposure was earlier described in different studies [12,24].

During the last decade, the separate collection of organic wastes has become a part of everyday life in Germany, and nowadays up to 7.2 mio t of biowastes are treated in 900 composting plants and biogas reactors. The main application of compost is its recycling through the soil–plant system and therefore the annual 4.5 mio t of composts produced are mainly applied as soil amendment to agricultural soils (www.BMU.de). Beside the beneficial effects of compost on soils, e.g. improvement of structure [3] and fertility [14], these anthropogenic activities influence the microbial community structure and diversity [32,33].

Nevertheless, thermophilic granular sludge and compost material, both of which have been shown as a potential source for thermophilic methanogens [21,36,40], were not considered as carriers of these organisms to oxic and moderate soils. Therefore, we analysed the thermophilic methanogenic community in compost materials from different origins, as well as their potential distribution to oxic soil by fertilisation and by air transport.

Material and methods

Samples

Sample collection of the compost materials (Table 1) took place according to Thummes et al. [39]. To get a representative sample of each investigated compost pile, three sampling points per heap were selected, one in the centre and one at each end, each at a depth of 20 cm. Approximately 500 g of compost material were collected from the three points, and they were pooled, mixed and sieved over 4 mm in the laboratory.

Table 1. Investigated compost material: origin, features of processing, age, pH, and potential methane production rate in anoxic incubations at 36, 50 and 65 °C

Sample	Processed material	Processing method [44]	Turning of piles	Capacity (t/a)	Age of the material sampled	pH	µmol CH_4 gdw^{-1} h^{-1} ± SD		
							36 °C	50 °C	65 °C
CC	1:3.5 Loppings, bio waste	Open windrow composting (initial 3–4 weeks GORE™-Cover)	2–3 × / week	10 000	11 weeks	7.2	0.02 ± 0.005	0.34 ± 0.042	0.32 ± 0.050
NC	1:4 Loppings, bio waste	Tunnel-composting	2 × /week	20 000	20 weeks	8.1	0.001 ± 0.0003	0.01 ± 0.002	0.004 ± 0.0007
TC	Bio waste	Tunnel-composting	No	37 000	12 days	7.7	0.04 ± 0.005	0.12 ± 0.030	2.75 ± 0.135
AC	Bio waste	Box-composting	No	30 000	4 weeks	7.3	0.09 ± 0.008	0.47 ± 0.02	1.33 ± 0.07
HC	Loppings	Open windrow (not aerated)	4 × /a	1200	1 year	7.4	0.0	0.02 ± 0.003	0.0
MC	Loppings	Open windrow (not aerated)	4 × /a	1200	1 year	7.0	0.001 ± 0.0001	0.088 ± 0.005	0.01 ± 0.004
UC	Loppings	Open windrow (not aerated)	5 × /a	1200	2 year	7.4	0.0004 ± 0.0	0.977 ± 0.008	0.018 ± 0.0004
SC	Loppings	Open windrow (not aerated)	5 × /a	1200	2 year	7.6	0.006 ± 0.0	0.048 ± 0.012	0.021 ± 0.001

Bio waste: organic waste from domestic organic (kitchen) waste.Loppings: leaf fall, tree pruning, garden clippings and other cellulose and lignin-rich materials.