Forensic Science International 245 (2014) 101–106

Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

Changes to soil bacterial profiles as a result of *Sus scrofa domesticus* decomposition

Ayodeji O. Olakanye, Tim Thompson, T. Komang Ralebitso-Senior *

School of Science and Engineering, Teesside University, Borough Road, Middlesbrough TS1 3BA, UK

ARTICLE INFO

Article history:
Received 24 April 2014
Received in revised form 28 July 2014
Accepted 4 October 2014
Available online 8 November 2014

Keywords:
Cadaver decomposition
Soil bacterial community
Sus scrofa domesticus
PCR-DGGE

ABSTRACT

The importance of cadaver decomposition knowledge for clandestine grave location cannot be over emphasised. Notwithstanding this, only a limited understanding is available on the resulting soil microbial community dynamics. To address this paucity, a pig leg (*Sus scrofa domesticus*; 5 kg) was buried in freshly weighed (20 kg) sandy loamy soil in a sealed microcosm (40 cm height) in parallel with a soil only control. Both microcosms were perforated nine times at equal distances and maintained outside. Soil samples were collected through these perforations from the top (0–10 cm), middle (10–20 cm) and bottom (20–30 cm) segments every three days for the first two weeks, and then weekly up to 14 weeks. PCR-DGGE gels quantified by 1D Phoretix showed increases in the cumulative soil community richness values of 43, 66 and 106 for the top, middle and bottom segments, respectively, in the presence of *Sus scrofa domesticus*. Shannon–Wiener's (H') and Simpon's (D) indices confirmed corresponding species diversity increases in the middle (H' = 1.58–2.33; D = 0.79–0.91) and bottom (H' = 2.48–3.16; D = 0.85–0.95) depths between days 10 and 71 compared with the control. In contrast, similar evenness was recorded for all segments in both the *Sus scrofa domesticus* and control soils.

1. Introduction

Billions of microorganisms are found in terrestrial habitats with bacteria often the most prevalent [1]. Bacteria and fungi are responsible for almost 90% of organic matter breakdown and play significant roles in the carbon and nitrogen cycles [2]. Less than 1% of microorganisms have, however, been characterised by culture-based methods [1,3]. Despite this, the use of molecular techniques to study soil microbial communities has proven to be very successful [4]. Extraction of nucleic acids from microbial cells in soil samples by molecular techniques and their various applications in polymerase chain reaction (PCR) amplification and hybridisation experiments has allowed researchers to identify and characterise numerous microorganisms in different environments [5,6]. The importance of cadaver decomposition knowledge cannot be over emphasised hence emergent studies have analysed changes in the postmortem microbiome or necrobiome of the abdominal, interior anal and buccal cavities and skin of decomposing carcasses [7–10]. Nonetheless, the majority of research has focused on above ground decomposition [6,11–13]. As a result, very little is known of soil microbial community changes following cadaver decomposition within grave sites [4,14–16].

Cadaver decomposition is a complex process and is affected by enzymatic catabolism, microbial activity and changes in different environmental variables. It begins approximately four minutes after death and progresses through different stages namely: autolysis, putrefaction, and decay [14,16–18]. Of fundamental importance to these is the unique roles played by microorganisms although, overall, decomposition is still largely a "Black Box" [12,19–21]. Characterisation and identification of changes in microbial diversity can facilitate the location of clandestine or secondary graves and provide additional intelligence in cases of complete decomposition (as with juvenile remains). Hence, identification of specific catabolic biochemical and molecular markers in soil microbial communities represents an essential additional tool for forensic practitioners.

The hypothesis for this decomposition study was that soil microbial community changes would result with time and would be identified by targeting specific biochemical and molecular markers and, subsequently, quantified by different ecological measures. Also, soil microbial community changes were anticipated for the

* Corresponding author. Tel.: +44 1642 342525; fax: +44 1642 342401.
E-mail addresses: A.Olakanye@tees.ac.uk (A.O. Olakanye), T.Thompson@tees.ac.uk (T. Thompson), K.Ralebitso-Senior@tees.ac.uk (T. Komang Ralebitso-Senior).

http://dx.doi.org/10.1016/j.forsciint.2014.10.002
0379-0738/

different microcosm depths relative to the buried material. This hypothesis was tested by identifying temporal and spatial soil community profile changes through DNA-based polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis (DGGE) targeting the 16S rRNA gene.

2. Materials and methods

2.1. Sus scrofa domesticus *burial and sampling*

Two sealed microcosms filled with sandy loamy garden soil (20 kg fresh weight) were maintained outside for 98 days with (experimental) and without (control) a 5 kg leg of domesticated pig (*Sus scrofa domesticus*). This species has been used for decomposition studies by various researchers because of its close similarities to the human body [14,22]. The microcosms were 40 cm in height and were divided into three depths (top, middle and bottom). Perforations at equal distances and heights facilitated aeration, moisture migration, sampling and hygiene maintenance. The mean temperature for the study duration was 14.9 °C and the average rainfall was 3.5 mm (Fig. 1). Composite soil samples for pH determination, DNA extraction and PCR-DGGE profiling were collected from the three segments on days 0, 3, 7, 10 and 14 of the first two weeks and then subsequently at weekly intervals.

2.2. Soil characterisation

Soil physicochemical characteristics (Derwentside Environmental Testing Services Ltd., County Durham, U.K.) were Al (28 g kg^{-1}), Ca (18 g kg^{-1}), Mg (9.2 g kg^{-1}), K (5.3 g kg^{-1}), Na (0.37 g kg^{-1}), total organic C (2.8%), total S (0.03%), aqueous extractable NO_3^- (1.5 mg L^{-1}), ortho PO_4^- as P (<0.10 mg kg^{-1}), pH (7.6), calorific value (1.2 MJ kg^{-1}) and electrical conductivity (250 μS cm^{-1}).

2.3. pH measurement

The soil samples were mixed thoroughly with deionised water at a ratio of 1:5 (w/v) prior to pH determination with a pH 213 Microprocessor (Hanna Instruments, Bedfordshire, U.K.) fitted with a Fisher electrode according to the method described by Stokes et al. [13].

2.4. DNA extraction

DNA extractions were made with FastDNA®Spin kits for Soil (MP Biomedicals, U.K.) according to the manufacturer's instructions and stored at −20 °C until needed. Mixtures of 5 μL DNA template and 1 μL 6× loading buffer were analysed on 1.5% (w/v) agarose gels which contained 6 μL SYBR Safe (Invitrogen, U.S.A.). The gels were electrophoresed in 1× TBE buffer for 90 min at 150 V and viewed (AlphaImager HP®, Alpha Innotech, Braintree, U.K.) under UV light.

2.5. Polymerase chain reaction (PCR) – denaturing gradient gel electrophoresis (DGGE)

The V3 region of the bacterial 16S rRNA gene from position 356 to 519 was targeted for amplification according to Manefield et al. [23], with the forward primer (5′ CGCCCGCCGCGCCCCCGCCCCGGCCCGCCGCCCCCGCCCACTCCTACGGGAGGCAGC 3′) and the reverse primer (5′ GTATTACCGCGGCTGCTG 3′). The 25 μL PCR reaction mixture consisted of 12.5 μL of 2× PCR master mix (Promega, Southampton, U.K.), 0.5 μL of the forward and reverse primers (0.2 μM), 1.25 μL BSA (0.5 mg mL^{-1}), 8.25 μL molecular grade water (Promega, Southampton, U.K.) and 2 μL of DNA templates. The thermo-cycling programme (Primus 96 Plus, MWG-Biotech, Ebersberg, Germany) consisted of 1 cycle at 95 °C for 2 min, 35 cycles of: denaturation at 95 °C for 1 min; annealing at 60 °C for 1 min; and

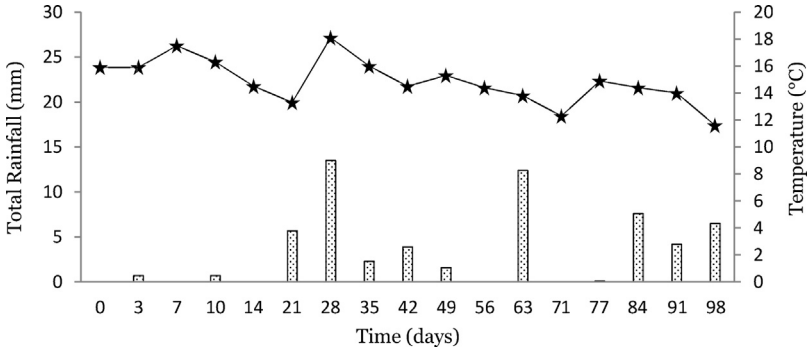

Fig. 1. Average temperature (•) and rainfall ( ) during the 98-day study from June to September 2010 in Middlesbrough, United Kingdom
Source: Data from http://www.worldweatheronline.com.

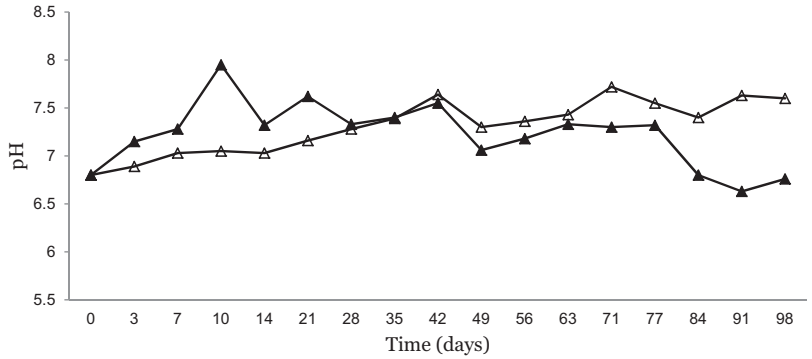

Fig. 2. pH readings for the top 10 cm soil segment in the *Sus scrofa domesticus* (▲) and control (△) microcosms during 98 days of study.