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Editor's Choice

ORIGINAL ARTICLE

The use of PCR-DGGE to determine bacterial fingerprints for poultry and red meat abattoir effluent

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Significance and Impact of the Study: This study was the first to demonstrate the application of denaturing gradient gel electrophoresis (DGGE) to construct bacterial diversity fingerprints for high-throughput abattoir effluents. Proved redundancy of fat removal as PCR inhibitor and change in diversity similarity introduced by nested PCR approach. The importance of limiting excessive handling/processing which could lead to misrepresented diversity profiles was emphasized.

Keywords
abattoir effluent, denaturing gradient gel electrophoresis, Green Drop, molecular fingerprint, source tracking, wastewater.

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Abstract

Strict legislation and chemical composition monitoring of effluent may be useful, but the data generated do not allow for source tracking, and enforcing legislation remains problematic in the South African setting. These difficulties emphasize the necessity for effluent source traceability. Denaturing gradient gel electrophoresis (DGGE) targeting the V3 region of the 16S rRNA gene was considered as fingerprinting technique for effluent originating from abattoirs slaughtering different animal species. The influence of treatment to remove excess fat from effluent prior to molecular analyses and different PCR approaches on the detection of bacterial diversity were considered. Use of a treatment option to remove fat and a nested PCR approach resulted in up to 51% difference in inter-sample diversity similarity. A robust approach with no pre-treatment to remove PCR inhibitors, such as fat, and direct amplification from genomic DNA yielded optimal/maximal bacterial diversity fingerprints. Repeatable fingerprints were obtained for poultry abattoir effluent over a 4-month period, but profiles for the red meat abattoir varied with maximum similarity detected only 33·2%. Genetic material from faecal indicators *Aeromona* spp and *Clostridium* spp were detected. Genera unique to each effluent were present; *Anoxybacillus*, *Patulibacter* and *Oleispira* in poultry abattoir effluent and *Porphyromonas* and *Peptostreptococcus* in red meat abattoir effluent.

Introduction

Whenever food in any form is handled, processed, packaged and stored, there will always be an inherent generation of wastewater. The food industry has one of the highest emissions of organic waste into water resources, accounting for more than a third of the pollution of water by factories and industries (Heilig 1999).

South African industries within municipal limits discharge their wastewater into the city's sewage system and through this joint processing of wastewater, the municipality ultimately accepts responsibility for the final treatment and disposal of wastewater. Local limits are developed (by-laws) to address specific needs and capabilities of individual treatment plants (Hammer and Hammer 2008). Municipalities will often enforce surcharges or penalties on nearby industries if their effluents contain excessive levels of toxic materials that overload the treatment works, but enforcement is problematic, and unmonitored dumping of wastewater is consequently difficult to ascertain. This concern is especially relevant with the current pressure on municipalities to obtain Green Drop certification; a South African quality

assessment initiative for treated wastewater to be released back into the natural environment (DWA 2011).

Monitoring of effluent deposited into wastewater systems concentrates on chemical parameters (biochemical oxygen demand (BOD), suspended solids (SS), pH, conductivity, oxygen absorption (OA), nitrogen and phosphorus) as directed by the South African Water Act (Metcalf 2003). Although these parameters are useful to monitor the quality of effluent, the information generated does not allow for source tracking. A broader understanding of the microbial organization generally associated with wastewater of a specific origin has the potential to address this shortcoming.

Studies using molecular techniques to target microbial diversity during wastewater treatment have demonstrated the usefulness of this approach to identify bio-communities that influence the final effluent quality (Boon *et al*. 2002; Wagner *et al*. 2002; Ibekwe *et al*. 2003; Bramucci and Nagarajan 2006; Conn *et al*. 2012; Stets *et al*. 2014). One such method, denaturing gradient gel electrophoresis (DGGE) is a polymerase chain reaction (PCR)-based approach that generates a population of PCR fragments identical in length, but different in sequence. Since the first report which applied the DGGE tool to analyse complex microbial populations (Muyzer *et al*. 1993), many studies have alluded to the usefulness of DGGE in environmental microbiology (Lopes dos Santos *et al*. 2009). DGGE has been used in different environments including food (Ercolini 2004; Koo *et al*. 2013), water (Conn *et al*. 2012) and wastewater (Ziembinska-Buczynska *et al*. 2014). The fact that it is a culture independent technique makes it exceedingly popular, especially because it provides a much broader assessment of the microbial content of an environment than classical microbiological approaches can. Practically any gene can be targeted for this purpose and new genes are identified on a regular basis, such as the use of the *gryB*, *rpoD* and *sodB* gene targets identified for *Aeromonas* species in water (Calhau *et al*. 2010). However, the 16S rRNA gene remains the most powerful and universal target for environmental ecosystems where bacterial diversity is of interest and limited information is available of such content (Lopes dos Santos *et al*. 2009; Klindworth *et al*. 2013). Using this same approach to generate microbial fingerprint profiles for the effluent originating from individual food industries remains unexplored.

However, each step involved in the molecular analysis, specifically of environmental samples, is a source of bias, which could lead to distorted information. These include sampling procedure, sample pretreatment, DNA extraction method and DNA/RNA template used for PCR approaches (Wintzingerode *et al*. 1997). The current research was therefore a preliminary investigation of the usefulness of DGGE as method to generate bacterial fingerprints able to distinguish between poultry and red meat abattoir effluent; with particular emphasis placed on the influence of pretreatment to remove excess fat and use of different PCR templates on diversity results.

Results and discussion

Influence of pretreatment and PCR approach on bacterial diversity

There are many pitfalls when analysing microbial diversity using PCR-based analyses (Wintzingerode *et al*. 1997; Pontes *et al*. 2007; Harwood *et al*. 2014). Molecular biology is also a fast evolving discipline and many new hurdles in DGGE applications have recently come to light (Ascher *et al*. 2010; Balazs *et al*. 2013). It is therefore crucial to establish whether sample processing prior to DGGE analysis suits the application and produces PCR products of the desired quality and quantity. Since the two abattoirs used different methods to remove fat from their effluent before discharging into the municipal sewage system, it was vital to evaluate the influence of pretreatment to remove excess fat from the effluent samples prior to DNA extraction. This essentially results in excess fat present in the effluent from the poultry abattoir, but not the red meat abattoir. As fat is a known PCR inhibitor (Drake *et al*. 1996), it was important in this case to establish its influence on PCR and the bacterial diversity as a whole.

A schematic representation of poultry abattoir effluent sample similarity related to pretreatment to remove excess fat and PCR approach (direct and nested) is depicted in Fig. 1. This analysis was conducted on the effluent samples from the poultry abattoir only, since effluents from the red meat abattoir did not yield any pellets after the pretreatment protocol to remove excess fat was administered. Clustering of the PCR-DGGE profiles showed grouping into two definite clusters differentiating between not only effluent samples, but also PCR approaches and pretreatment options used. Cluster A grouped diversity profiles obtained from a direct PCR approach (genomic DNA used as template) while cluster B represents diversity profiles generated from a nested PCR approach. Further subclustering also distinguished effluent sampled during different months, where subcluster A1 and B1 grouped the effluent samples taken during March and subcluster A2 and B2 that taken during February. Each of these subclusters were further separated to form grouping associated with pretreatment (untreated and FT). The unweighed pair group method with arithmetic mean (UPGMA) dendrogram for effluent sampled during December and January showed similar groupings (data not shown).

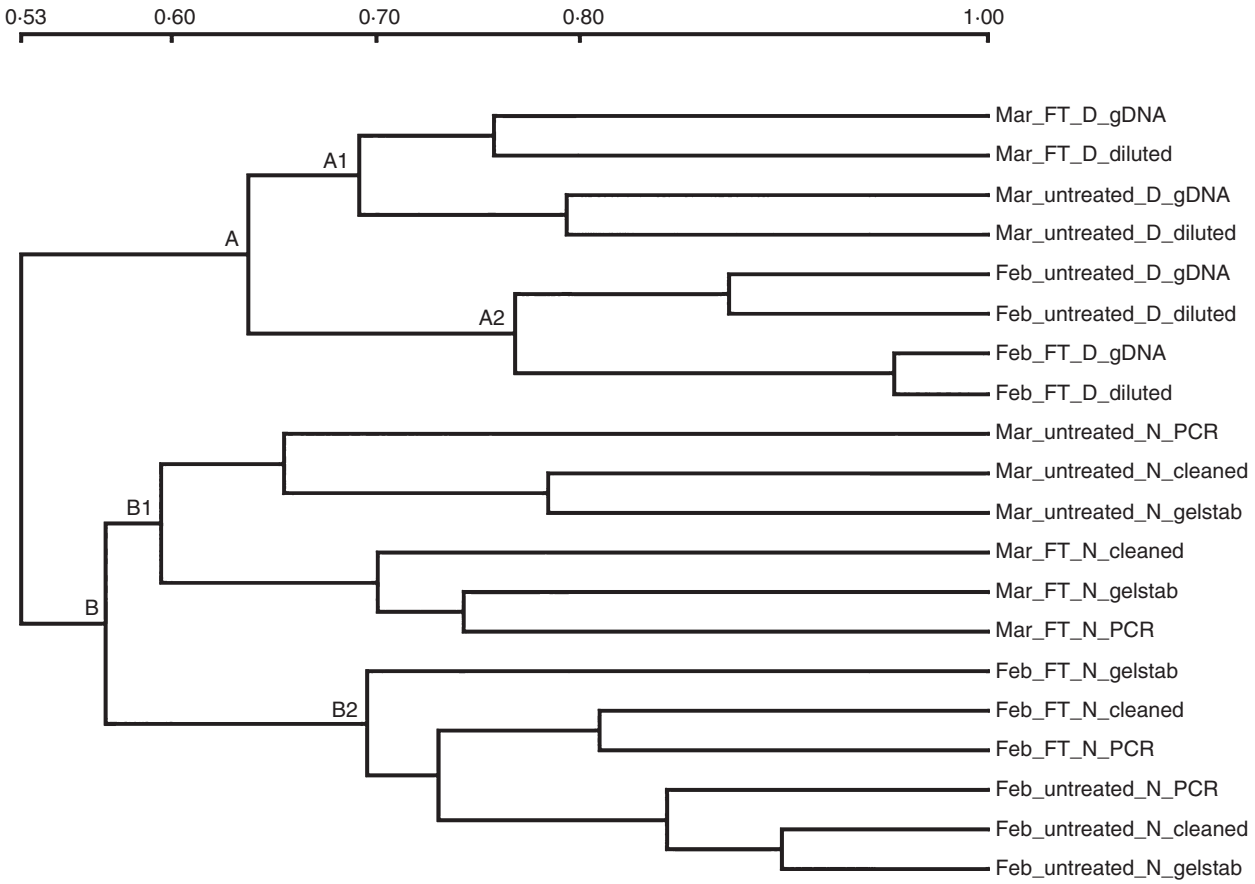

Figure 1 UPGMA dendrogram representing cluster analysis of 16S rDNA banding profile of the diversity similarity for effluent samples taken from a high-throughput poultry abattoir during February and March. 'Untreated' represents samples that were centrifuged only before DNA extraction. FT represents effluent samples that were treated to remove excess fat before DNA extraction. Undiluted (D_gDNA) or 10× diluted genomic DNA (D_diluted) was used as template for direct (D) amplification; or a nested PCR approach (N) using either amplified 16S PCR product (N_PCR), the same PCR product stabbed from an agarose gel (N_gel stab) cleaned from the gel (N_cleaned) as template.

The effects of diluting gDNA, pretreating effluent samples to remove excess fat, as well as the using a nested PCR approach for amplification of DGGE-PCR product on diversity are presented in Table 1 with supporting data presented as in Fig. S1. Change/decrease in inter-sample similarities showed dilution of gDNA template to have very little impact on similarity (7·6%), but can also contribute substantially (20·6%). When considering the influence that pretreatment to remove excess fat has on similarity, a decrease of 24·7–36·6% was evident. A nested PCR approach to amplify PCR-DGGE product resulted in a 31–48·5% decrease in similarity. These results are in accordance with the notion of Park and Crowley (2010) that an indirect PCR approach introduces bias. Finally, the use of pretreatment and a nested PCR approach can decrease similarity up to 51%. Therefore, in this setting using this particular extraction method, the use of raw effluent, undiluted extracted gDNA as template in a direct PCR approach is recommended for maximum diversity detection.

Construction of bacterial diversity fingerprints for each abattoir

Although South African abattoirs are probably of the most water-efficient in the world, 7 million m^3 of effluent is still deposited into municipal sewers per annum (Anon 2004). The Free State province has 21 high-throughput red meat and three high-throughput poultry abattoirs situated near towns and their effluents are all discharged into the local municipal water treatment plants. Much information is available on the COD, BOD, SS, pH, conductivity, OA, nitrogen and phosphorus content of these effluents, as the local municipalities are directed by the Department of Water and Sanitation to monitor and calculate surge charges (DWA 2011). Wastewater treatment

works very often experience overloading with effluent surges and investigations yield poor results since response times are inadequate. This is especially problematic where, for instance, poultry and red meat abattoirs are both connected to the sewage system by the same section of pipeline, resulting in the inability to prove either party responsible. Being able to track the source of the effluent through a fingerprinting technique could contribute towards industries adopting a good basic industrial waste control philosophy, rather than practicing unmonitored dumping to avoid financial implications.

Analysis of the bacterial diversity fingerprints obtained for the different abattoir effluents clearly showed a distinct and consistent fingerprint for the poultry abattoir over a period of 4 months (Cluster B, Fig. 2). Genetic material from bacterial family Porphyromonadaceae and genus Roseobacter (uncultured) were present in all samples (Table 2). As expected from effluent containing bird and mammalian faecal content, genera belonging mainly to three bacterial groups were detected; Furmicutes, Bacteroidetes and Actinobacteria (Balleste and Blanch 2011; Ziganshin et al. 2013). Furthermore, the three largest members of the Bacteroidales order; genera Bacteroides, Porphyromonas and Prevotella, that have been targeted for microbial source tracking associated with faecal contamination, were also present (Dorai-Raj et al. 2011). As were faecal indicators Aeromonas spp and Clostridium spp. (McMahan et al. 2012). Sequencing results obtained from excised bands also hint that specific screening for Anoxybacillus flavithermus (band position 20, Fig. 2) as marker for poultry abattoir effluent seems plausible. However, traceability should first be established in a downstream source, such as the raw wastewater reaching the wastewater treatment plant (WWTP). Other genera unique to poultry abattoir included Patulibacter and Oleispira.

DGGE profiles originating from the red meat abattoir effluent tended to vary considerably with the maximum Dice Coefficient similarity determined merely 33·2% (Cluster A, Fig. 2). This was not entirely unexpected, since this red meat abattoir slaughters different species. Slaughtering on the sample days during December (RM_Dec) and February (RM_Feb) was mainly pigs, January (RM_Jan) cattle and sheep and March (RM_Mar) pigs and cattle. Arguably, the diversity profiles should

Table 1 Inter-sample decreases in similarity introduced by DNA dilution, treatment to remove excess fat and an indirect PCR approach

Sampling months	Dec	Jan	Feb	Mar
Factor	Δ Similarity (%)			
Dilution (n = 1)	9·8	7·6	12·6	20·6
FT (n = 5)	24·7	36·6	25·8	35·8
Nested PCR (n = 12)	31·0	36·8	34·3	48·5
FT and nested PCR (n = 6)	38·8	40·8	39·4	51·0

FT, pretreated to remove excess fat; (n), the number of similarity values included in the calculation, data presented in Fig. S1.

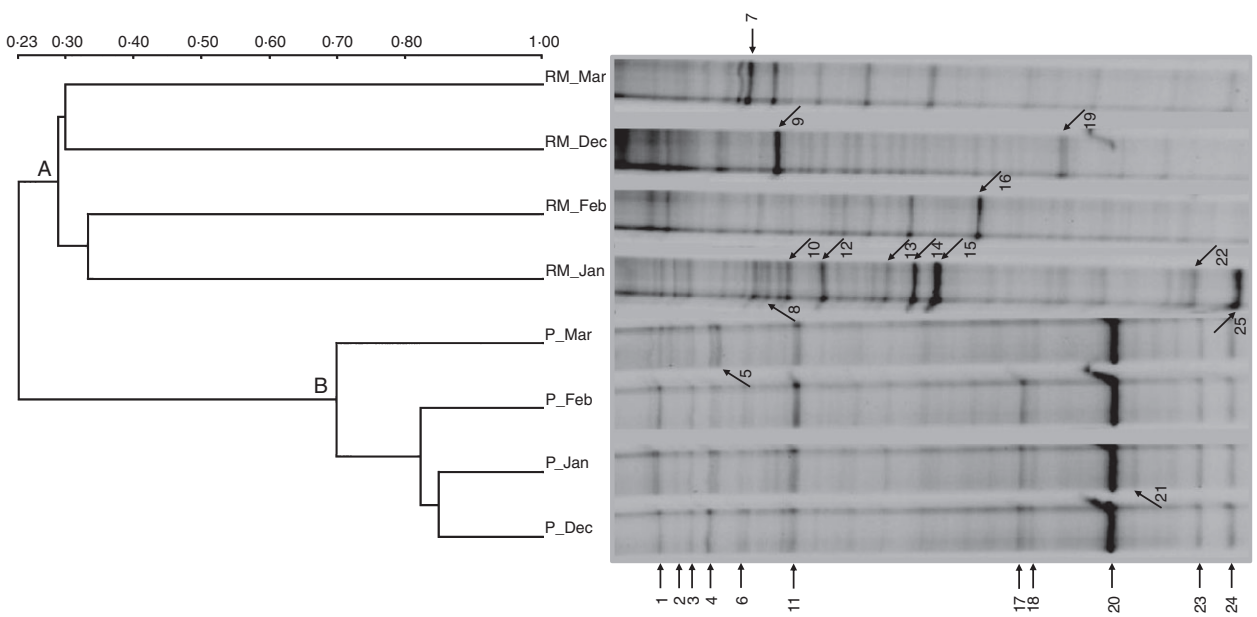

Figure 2 PCR-DGGE and cluster analysis of 16S rDNA banding profiles derived from high-throughput abattoir effluents. RM represents results from red meat abattoir effluent and P that of poultry abattoir effluent sampled over a 4-month period. Numbered arrows represent band positions that were excised and sequenced for identification (Table 2).

Table 2 Closely related sequences to denaturing gradient gel electrophoresis (DGGE) bands based on the NCBI nucleotide sequence database

Band #	Presence*	Database match with accession number in parentheses	*E* value	Identity (%)	Length (bp)	Phylum	Family
1	P/RM	*Bacteroides luti* strain UasXn-3 16S ribosomal RNA gene, partial (NR_125463.1)	6·0E-66	97	164	Bacteroidetes	Bacteroidaceae
2	P/RM	*Prevotella paludivivens* strain JCM 13650 16S ribosomal RNA gene, partial (NR_113122.1)	3·0E-58	98	132	Bacteroidetes	Prevotellaceae
3	All	Uncultured *Roseobacter* sp. isolate DGGE gel band K312-2-7 16S ribosomal RNA gene, partial (GQ351422.1)	2·0E-07	95	144	α-Proteobacteria	Rhodobacteraceae
4	P/RM	*Aeromonas hydrophila* strain ATCC 7966 16S ribosomal RNA gene, complete (NR_074841.1)	6·0E-28	80	160	γ-Proteobacteria	Aeromonadaceae
5	P/RM	*Anoxybacillus kestanbolensis* strain K4 16S ribosomal RNA gene, partial (NR_025733.1)	7·0E-55	93	162	Firmicutes	Bacillaceae
6	P	Uncultured *Patulibacter* sp. clone 6344 16S ribosomal RNA gene, partial (KF506773.1)	3·0E-09	89	141	Actinobacteria	Patulibacteraceae
7	RM	No significant similarity found			159		
8	P/RM	Uncultured Fusobacteria bacterium clone A27 16S ribosomal RNA gene, partial (KF951505.1)	7·0E-46	96	144	Fusobacteria	
9	P/RM	*Moraxella osloensis* strain DSM 6998 16S ribosomal RNA gene, partial (NR_113392.1)	3·0E-68	96	163	γ-Proteobacteria	Moraxellaceae
10	P/RM	No significant similarity found			164		
11	P/RM	Uncultured bacterium clone ADFI7QG3A12HL50Z/1175 16S ribosomal RNA gene, partial (FJ471462.1)	2·0E-42	97	163		
12	All	Uncultured Porphyromonadaceae bacterium clone Gull287-158 16S ribosomal RNA gene, partial (FJ221085.1)	1·0E-38	89	161	Bacteroidetes	Porphyromonadaceae
13	P/RM	*Aeromonas hydrophila* strain ATCC 7966 16S ribosomal RNA gene, complete (NR_074841.1)	2·0E-50	93	145	γ-Proteobacteria	Aeromonadaceae
14	RM	*Porphyromonas* sp. 2070 16S ribosomal RNA gene, partial (FJ848565.1)	1·0E-54	92	158	Bacteroidetes	Porphyromonadaceae
15	P/RM	Uncultured *Clostridium* sp. clone 16504 16S ribosomal RNA gene, partial (KP103995.1)	1·0E-47	96	122	Firmicutes	Clostridiaceae
16	RM	*Peptostreptococcus russellii* strain RT-10B 16S ribosomal RNA gene, partial (NR_115155.1)	5·0E-56	96	161	Firmicutes	Peptostreptococcaceae
17	P	*Aeromonas veronii* strain A112 16S ribosomal RNA gene, partial (KJ561049.1)	3·0E-64	98	146	γ-Proteobacteria	Aeromonadaceae
18	P/RM	*Aeromonas hydrophila* subsp. ranae strain Au-1D12 16S ribosomal RNA gene, partial (NR_042518.1)	2·0E-70	97	164	γ-Proteobacteria	Aeromonadaceae
19	P/RM	*Comamonas denitrificans* strain 3R2-18 16S ribosomal RNA gene, partial (GU195190.1)	5·0E-43	88	159	β-Proteobacteria	Comamonadaceae
20	P	*Anoxybacillus flavithermus* strain 3 from China 16S ribosomal RNA gene, partial (KJ459347.1)	4·0E-69	99	160	Firmicutes	Bacillaceae
21	P	*Oleispira antarctica* strain RB-8 16S ribosomal RNA gene, partial (NR_025522.1)	2·0E-40	93	167	γ-Proteobacteria	Oceanospirillaceae
22	RM	*Peptostreptococcus anaerobius* strain NCTC 11460 16S ribosomal RNA gene, partial (NR_042847.1)	6·0E-50	95	136	Firmicutes	Peptostreptococcaceae
23	P	*Clostridium tertium* strain JCM 6289 16S ribosomal RNA gene, partial (NR_113325.1)	9·0E-39	90	163	Firmicutes	Clostridiaceae
24	P/RM	*Caloramator proteoclasticus* strain Uruguayensis 16S ribosomal RNA gene, partial (NR_026265.1)	3·0E-43	92	138	Firmicutes	Clostridiaceae
25	RM	*Aeromonas* sp. AE7 16S ribosomal RNA gene, partial (EU724048.1)	2·0E-71	99	153	γ-Proteobacteria	Aeromonadaceae

*Band presence: Poultry abattoir effluent only (P), Red meat abattoir effluent only (RM), all samples analysed (All), both P and RM, but not all samples (P/RM).

then group according to species slaughtered, but judging by UPGMA clustering (Fig. 2), this is not the case. Genetic material from Porphyromonas and Peptostreptococcus genera, as well as unidentified bacteria was unique to red meat abattoir effluent.

This research demonstrated that DGGE has the potential to be used as fingerprinting technique for food industry effluents and was able to produce repeatable fingerprints for poultry abattoir effluent over a 4-month period. It also pointed out that a robust approach with no pretreatment to remove PCR inhibitors, such as fat, and direct amplification from genomic DNA yielded optimal/maximal bacterial diversity fingerprints for analysis. The results also hinted that the V3 region of the 16S rRNA gene might not be an appropriate target gene if the aim is to establish consistent fingerprints for different effluent contributors. Furthermore, sequencing results provided valuable information which could assist in the search for other probable, less universal genes to target.

Materials and methods

Sampling and pretreatment

Effluent samples (50 ml) were collected over a period of 4 months (December–March) from a high-throughput poultry- and red meat abattoir in and near Bloemfontein situated in Central South Africa. Samples were taken at 13:00 (poultry abattoir) and 15:00 (red meat abattoir) when the abattoirs were fully operational. Effluent samples were collected from the last drain on the premises and subjected to two different processes; firstly centrifugation at 7000 ***g*** for 7 min after which the supernatants were discarded and the pellets were utilized in further analyses (untreated samples). The second process involved the removal of excess fat using 25% (m/v) ammonium hydroxide (Pal Chemicals, Dorking, Surrey, UK), 99·9% (m/v) ethanol (Merck, Darmstadt, Germany), petroleum ether (Saarchem, Krugersdorp, SA) and 10% (m/v) SDS (Sigma-Aldrich, Johannesburg, RSA) as described by Drake et al. (1996). The resulting pellets were stored at −80°C and used in subsequent analyses. After the 4 months sampling period, all the frozen pellets were processed as one batch.

DNA extraction and 16S rDNA amplification

Genomic DNA was extracted using a glass bead and detergent extraction method described by Labuschagne and Albertyn (2007); very similar to the method described for DNA extraction from activated sludge (Singka et al. 2012). The 16S rRNA gene was targeted for amplification of 1300 bp fragments using primer set 63-F (5′-CAGGCC TAACACATGCAAGTC-3′) and 1387-R (5′-GGGCGGWG TGTACA AGGC-3′) (Marchesi et al. 1998). PCRs were performed in a total volume of 25 μl containing 1 μl of genomic DNA, 2·5 μl reaction buffer, 0·2 mmol l^{-1} dNTPs, 0·5 μmol l^{-1} of each primer and 1 unit of Supertherm Taq polymerase (JMR Holdings, London, UK). Reaction conditions included an initial denaturation step at 94°C for 3 min, 30 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 1·5 min. Final elongation was performed at 72°C for 10 min in the G-Storm GS482 thermal cycler (Gene Technologies, Somerset, UK). PCR products were separated in an agarose gel (1%), stained with 0·05% Goldview (Guangzhou Geneshun Biotech, Guangzhou, China) and visualized under UV light.

Direct and nested PCR approaches

Direct as well as nested PCR approaches were followed to amplify a 233 bp that covers the third Hypervariable (HV) region of the 16S rRNA gene sequence for DGGE analysis. Direct amplification entailed the use of undiluted (1 μl) or diluted (10×) genomic DNA (1μl) as template, while nested PCR required the use of the pre-amplified 1300 bp fragment in a second amplification. The nested approach was further extended by using unprocessed 16S rDNA PCR product (1 μl), an agarose gel stab (Bjourson and Cooper 1992) and PCR product cleaned from the gel with the Illustra GFX PCR DNA and Gel Band Purification Kit (GE Healthcare Life Sciences, Pittsburgh, PA) (1 μl) as template. All templates and primer set 341-F^{GC} (5′-CCTACGG GAGGCAGCAG-3′) with incorporated 40 bp GC-clamp at the 5′-end and 517-R (5′-ATTACCGCGGCTGCTGG-3′) were used to amplify the shorter 233 bp fragment (Muyzer et al. 1993). PCRs were performed in a total volume of 50 μl of the same setup as previously described. Initial denaturation was performed at 95°C for 5 min, 30 cycles of 95°C for 45 s, 55°C for 45 s and 72°C for 1 min. Final elongation was performed at 72°C for 10 min. In order to reduce possible inter-sample PCR variation, two sets of PCRs were performed as independent duplicates and pooled before loading on the DGGE gel. DNA fragments were separated in an agarose gel (2%) and were stained and visualized as previously described.

After influence of pretreatment and PCR approach on diversity was assessed. The subsequent PCR products for DGGE analysis were generated from raw effluent samples (no pretreatment to remove fat) using a direct approach (1 μl undiluted gDNA) in the same reaction setup under the same conditions.

Denaturing gradient gel electrophoresis

DGGE analysis was performed on 30 μl of the 233 bp GC-clamped PCR amplicons using the D-Code Universal

Mutation Detection system (Bio-Rad, Johannesburg, RSA) essentially as described by Muyzer et al. (1993). Sequence-specific separation of the 233 bp fragments was obtained in a 7% (w/v) polyacrylamide (Acrylamid/Bis 37·5 : 1) gel in 1× TAE buffer containing a 40–60% linear denaturant gradient. The 100% denaturant solution contained 40% (v/v) deionized formamide and 7 mol l^{-1} urea. Electrophoresis was performed with a constant voltage of 130 V at 60°C for 5 h. Gels were stained with 0·05% GelStar® (Lonza, Slough, UK) for 15 min, rinsed with ultra-pure water and photographed under UV light. At least two representatives of each band position were excised from the gel on a DarkReader (Clare Chemicals Research, Dolores, CO), each band incubated in 50 μl ultra-pure water at 60°C for at least 5 h and 5 μl used as template for re-amplification. Re-amplified fragments (0·5 μl) were used as template for direct sequencing.

Sequencing analysis

Sequencing was performed on the ABI Prism 3130 XL genetic analyser using the Big Dye® Terminator V3.1 Cycle Sequencing Kit and DNA was precipitated with EDTA and ethanol (Applied Biosystems, Carlsbad, CA). Both strands of amplified DNA were sequenced, using primers 341-F (5′–CCTACGGGAGGCAGCAG-3′) and 517-R (5′-ATTA CCGCGGCTGCTGG-3′) in separate reactions, to eliminate sequencing artefacts and to ensure accuracy of data generated. The sequences obtained were compared to those present in the National Centre for Biotechnology Information (NCBI) database using the BLAST algorithm (MEGABLAST) and identity was determined based on the highest scores. Data were only discussed to genus level, since the short fragment used for sequencing could lead to misinterpretation.

Data processing

DGGE digital images were captured on the Molecular Imager Gel Doc™ XR and analysed with Quantity One® 1-D Analysis imaging software (Bio-Rad). To generate a densitometric profile, a 5% band intensity threshold was set for band selection, individual bands were matched according to their positions in the gel based on a 1·5% position tolerance and peak areas used to determine intensities (Julien et al. 2008). Every band in each sample was included in the comparison and both band position and intensity were considered for similarity comparison computation applying Dice Coefficient. Similarity data sets were tested for outliers and decreases/changes (Δ) in similarity were calculated from average values for each sample (lane) contributing data to a specific parameter (dilution, pretreatment, nested PCR). Cluster analysis of profile similarity was performed using UPGMA also called weighted average linkage. UPGMA gives the most plausible clusters and are affected the least by samples that are outliers.

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Conflict of Interest

The author has no conflict of interest to declare.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1 Dice coefficient matrices derived from DGGE profiles showing inter-sample similarities for each effluent sample.