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Ecological Media Reveal Community Structure Shifts in a Municipal Wastewater Treatment Train

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Abstract

Unique ecological/habitat media derived from four phases of a municipal wastewater treatment plant revealed the highest diversity (2.55-2.86) and evenness (0.79-0.87) for the raw sewage (R) medium. Richness was, however, inoculum- and media-dependent hence inocula R and P recorded the highest counts on media A and F, respectively.

Keywords: Municipal wastewater; Habitat media; Ecological index; Community structure

Introduction

The continuing need for robust, sustainable and reliable (bio) technologies that are also characterized by minimal carbon emissions/footprints must be matched by an equally growing and sound knowledge base of the underpinning microbial communities. Despite their analytical power/capacity, ecogenomic tools do not provide phenotypic or physiological characterization of novel and previously uncultivated microbial strains [1]. A discrepancy that limits a more complete understanding of microbial and process dynamics. Although research continues on culturing strains that were considered unculturable, particular focus is on soil ecosystems [2-4]. A limited understanding exists, in particular, of the phenotypes underpinning a key biotechnology, wastewater treatment.

As a result, directed research must address the well-documented limitations of molecular-based methods [5] so that seemingly important community members or representatives of functionally significant groups can be cultivated for further analyses. Despite the debate for/against cultivation, with/without molecular analyses [6,7], the approach has been exemplified by many researchers [1,4,8-11] who used different approaches, and remains essential to elucidate the physiological responses of process microbial communities for subsequent exploitation.

Material and Methods

Habitat media preparation and inoculation

Samples (1 L) were collected from four stages of a municipal wastewater treatment plant - raw sewage (R), primary settlement tank (P), aeration tank (mixed liquor) (A) and final effluent (pre-UV treatment; F). Following thorough gentle mixing, 50 mL aliquots were centrifuged (15,000 rpm x *g*; 15 minutes, 4°C) and the supernatants series filtered (Whatman: Grade 2; Nalgene: 0.45 µm; 0.2 µm) and autoclaved (121°C; 20 minutes; 15 lb psi). Each sterile filtrate was mixed 1:1 (v/v) with autoclaved 2X technical agar (Oxoid, U.K.) and allowed to set at room temperature for 48 hours prior to duplicate inoculations with 100 µL of 10-fold dilution of the original wastewaters.

Colony DNA extraction and PCR

Following incubation at 25°C for 72 hours, total colony scrapes were re-suspended in 200 µL sterile saline (0.9% w/v NaCl) for DNA extraction (FastDNA™ SPIN Kit, MP Biomedicals, U.K). The 16S rRNA gene (V3 region) was amplified with the F357GC/R518 primer set [12] with 1 µL DNA templates. The PCR was made [13] with 25 µL reaction volumes on a Primus 96 Plus (MWG Biotech, Ebersberg, Germany) and amplicons (5 µL) visualised on 1.5% (w/v) agarose gels stained with SYBR Safe (Molecular Probes, Eugene, U.S.A).

DGGE community profiling

DGGE profiling [12] used an Ingeny Phor U system (Ingeny, Leiden, The Netherlands). Amplicons (20 µL) were loaded onto a 10% (w/v) polyacrylamide gel with a 30-65% denaturing gradient and electrophoresed (18 h; 60°C; 100V) in 0.5× TAE buffer. The gels were stained for 20 minutes with SYBR Gold (Molecular Probes, Eugene, U.S.A.) and digitised (Alpha Imager Gel Documentation System; ProteinSimple, Santa Clara, U.S.A.). Bands per lane were quantified (Phoretix 1D Pro gel analysis software; TotalLab, Newcastle, U.K.) and cluster analysed by the unweighted pair group method with arithmetic averages (UPGMA) [14,15].

Ecological index and statistical analyses

Bacterial community diversity, evenness and similarity were assessed by the Shannon-Wiener diversity (H'), Shannon evenness (E) [16] and Sørensen indices [17], respectively. Inoculum- and media-dependent differences in community structure, taxa richness and community similarity were evaluated by two-way ANOVA tests (Microsoft Office Excel 2007; Microsoft, Redmond, U.S.A)

Results and Discussion

For all inocula, the Shannon-Wiener diversity (2.7 ± 0.06) and evenness (0.8 ± 0.02) were highest after cultivation on the raw sewage medium with statistically significant (p=0.0009) decreases recorded (0.4, H'; 0.12, E) for the other three media (Figure 1). These findings probably related to the high nutrient availability in the raw sewage tank and decreased types/concentrations of the subsequent [18]. Only the community from the primary settlement tank inoculated on its respective medium showed similarly high diversity and evenness values as the raw sewage culture, indicating a marked medium change.

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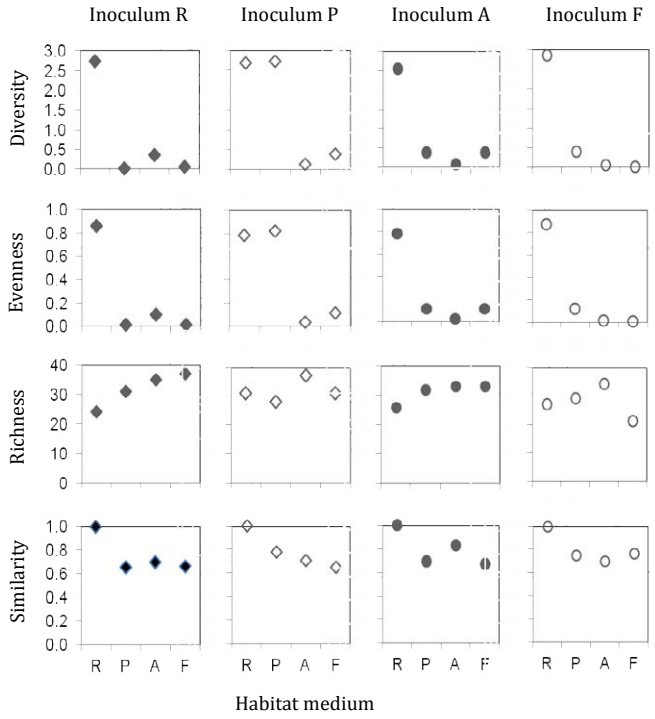

Figure 1: Ecological indices for the raw sewage (R), primary settlement tank (P), aeration tank (A), and final effluent (pre-UV) microbial communities following culture on their respective and three other habitat media.

In general, the average taxa richness of all inocula increased (27 ± 1.5 to 34 ± 1) through the treatment process indicating a change in the selection pressure [18,19]. The one exception was for the P-inoculum cultured on its respective medium, which decreased from 31 to 28. The Sørensen index of similarity indicated comparable community composition throughout the treatment process and suggested that species abundance only varied at different stages.

Emergent research has been made to culture previously uncultured microbial strains from soil ecosystems with only limited investigations for wastewater management. This is in direct contrast to the multiple molecular-based analyses that have been applied, developed and optimised to characterise the complex microbial communities in different wastewater biotechnologies. Matching and complementing genotypic tools with culture-based (phenotypic) analyses will facilitate: (i) identification of novel strains; (ii) quantification of their upper and lower physiological limits and function characterisation; (iii) culture maintenance of important monocultures/ communities [20]; and (iv) more informed exploitation in wastewater treatment plants for increased efficiency/stability/reliability.

Conclusion

This study exemplified the use of wastewater-based media to culture microbial communities that characterized specific phases of a continuous treatment train. DGGE-based analysis then facilitated measurements of diversity, evenness, taxa richness and similarity between treatment stages. Future work should entail detailed physiological/phenotypic studies of the cultivated strains/communities and, subsequently, sequencing to allow genotypic comparisons with these and uncultured wastewater species in existing databases.

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