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Article *in* Journal of Environmental Monitoring · August 2011

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COMMUNICATION

Amplified ribosomal DNA restriction analysis as a routine tool to assess toxicant driven changes in hindgut bacterial populations of *Porcellio dilatatus* (Crustacea: Isopoda)†

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Received 11th February 2011, Accepted 8th June 2011
DOI: 10.1039/c1em10137d

Changes in saprophytic organism's gut microbial communities may present a threat to organic matter breakdown which can ultimately lead to soil function impairment. In this study, Amplified Ribosomal DNA Restriction Analysis (ARDRA) was evaluated as a potential simple molecular tool to assess shifts in bacterial community structure in hindgut populations of *Porcellio dilatatus* exposed to contaminated food. This prospective tool can also be used for a variety of purposes and samples prior to the use of more specific and sophisticated methods.

1. Introduction

Terrestrial isopods are saprophytic organisms that carry a key role in organic matter decomposition, a process of great importance in soil fertility since it promotes carbon and nitrogen recycling of terrestrial environments.[1–3]

The vast diversity of bacteria in isopod hindgut has been confirmed by several authors.[4,5] The presence of contaminants in soils or in the leaf litter, which isopods feed on, ought to influence the bacterial communities present in the hindgut either by affecting the gut of the host or the microorganisms themselves. These microorganisms have been suggested to be involved in the digestive processes of cellulose, lignocellulose and phenolic food compounds, abundant in the isopods' diet.[6]

Simple molecular methods, like Amplified Ribosomal DNA Restriction Analysis (ARDRA), used as a first approach to study the complex population of rRNA PCR products directly amplified from community DNA, sometimes designated "community ARDRA", are helpful to detect changes in bacterial communities. ARDRA profiles reflect, at least the major members of the community which yielded amplicons,[7] and have been successfully used for assessing the effect of contaminants in soil,[8] resulting in distinguishable fingerprints for different bacterial structures.[9]

The aim of this study was to evaluate the potential of ARDRA to assess and monitor shifts in hindgut bacterial community structures of the terrestrial isopod *Porcellio dilatatus* exposed to food contaminated with a heavy metal (zinc) and a pesticide (chlorpyrifos).

2. Materials and methods

The exposure to zinc and chlorpyrifos was conducted over a 14 day period using the terrestrial isopod *Porcellio dilatatus* (Brandt, 1833). The isopods came from a culture established in our laboratory for more than 5 years in conditions described by Lemos *et al.*[2] During the experiment, each animal was placed individually in a 90 mm diameter plastic box, with a layer of plaster of Paris at the bottom, and fed on alder leaves collected from a reference site (Lower Mondego Valley, Coimbra, Portugal). The leaves were oven dried at 60 °C for 2 days, and then remoistened with distilled water to soften the surface. Afterwards they were contaminated with zinc (doses of 1000, 5000 and 10 000 μg of Zn per mg of dry leaf) or chlorpyrifos (doses of 0.01

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† Electronic supplementary information (ESI) available. See DOI: 10.1039/c1em10137d

Environmental impact

Modifications in saprophytic organism's gut microbial community may represent a potential threat to the animal's fitness itself but also to the organic matter breakdown processes, which can ultimately lead to soil function impairment. Therefore, there is a need to develop effective tools that can provide rapid information about these changes. In this communication, Amplified Ribosomal DNA Restriction Analysis (ARDRA) is evaluated as a potential simple molecular tool to assess shifts in bacterial community structure in hindgut populations of *Porcellio dilatatus* exposed to contaminated food.

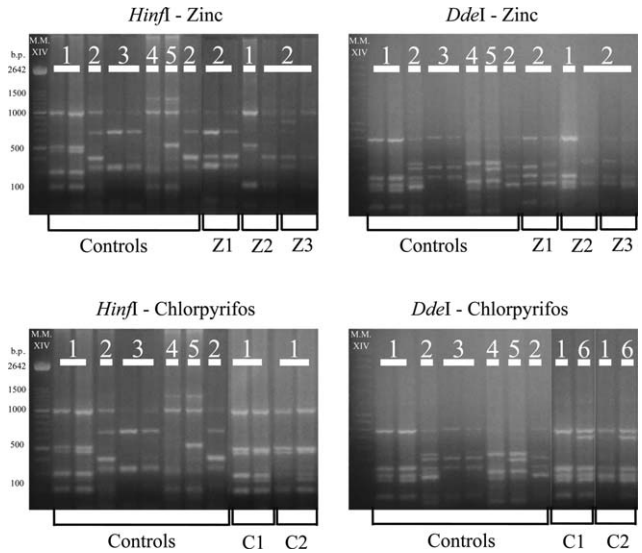

Fig. 1 ARDRA profiles (generated by digestion with restriction enzymes *Hinf*I and *Dde*I), numbered 1 to 6, obtained from hindgut bacterial populations of woodlice control group and exposed to zinc and chlorpyrifos. Zinc doses: Z1 (1000 μg of Zn per mg of dry leaf); Z2 (5000 μg of Zn per mg of dry leaf); Z3 (10 000 μg of Zn per mg of dry leaf). Chlorpyrifos doses: C1 (0.01 μg of chlorpyrifos per mg of dry leaf), C2 (0.1 μg of chlorpyrifos per mg of dry leaf). M.M.-DNA Molecular Weight Marker XIV (100 bp ladder).

and 0.1 μg of chlorpyrifos per mg of dry leaf) and given to the isopods. All experiments were run in the same conditions described by Lemos and co-workers.[2]

From the experiment described above, eight guts were used for controls and two guts were used per each toxicant concentration (each gut is an individual replica and lane in the gel; Fig. 1). The entire guts of adult isopods were dissected, DNA was extracted, PCR amplification of the 16S rRNA gene was carried out. The amplicons were digested with either *Hinf*I or *Dde*I ARDRA routine-use restriction endonucleases[9] (Roche Diagnostic GmbH, Germany), and profiles were then determined by comparison of the presence and absence of bands in different samples—for extensive complete protocols see ESI†.

3. Results

The different ARDRA profiles obtained were used to compare the hindgut bacterial populations of the isopods exposed to contaminated and uncontaminated food. Five different ARDRA profiles were observed in the control group (isopod fed with uncontaminated leaves) numbered from 1 to 5 (Fig. 1). When the animals were exposed to leaves treated with zinc, a decrease in the complexity of the community was detected as only two profiles were obtained.

As for the woodlice exposed to chlorpyrifos contaminated food, a decrease in the number of ARDRA profiles was also observed. In this particular case, when using the enzyme *Hinf*I in the exposed animals, only profile number 1 was observed. However, when the enzyme *Dde*I was used, two different profiles were obtained (1 and 6); profile 6 was not detected in any other condition.

4. Discussion

Although effects of toxicants on isopod gut microbiota have already been studied using conventional plate count methods,[1,10,11] the simple approach here described enables the correlation of significant changes in gut bacterial community structure with the presence of certain dosages of toxicants in the isopods' diet. The presence of the contaminants in isopods diet had a clear effect on the gut bacterial community structures. Indeed, a reduction in the variety of bacterial communities was visible when either zinc or chlorpyrifos were added to the food. This effect may be interpreted as the result of the selection of certain bacterial populations resistant to the stress conditions caused by the presence of the toxic substances. A similar effect was observed in previous studies, where isopods collected from metal-contaminated sites had more tolerant and stable bacterial communities than isopods from reference sites.[11] Thus, it can be expected that the less adapted/tolerant bacterial populations become less numerous or even extinct.

The combined action of digestive enzymes of isopods and microorganisms in the gut has been shown to be fundamental for the rapid and complete degradation of organic materials.[1] Besides the impairment of the isopods' food assimilation process and its probable individual and population consequences, gut microbial community disturbance by environmental contaminants might have severe consequences on decomposition processes, thus having increased ecological relevance. Moreover, the study of the impact of contaminants on isopod gut bacteria has already been proposed as an important complement for the evaluation of the toxicity of these chemicals in the terrestrial environment.[10]

The different ARDRA profiles obtained indicated that differences in bacterial populations could be readily detected. Therefore this culture-independent methodology can be successfully used for rapidly monitoring microbial changes in isopod hindgut, as used before for other bacterial communities. Furthermore, this method represents a quick and inexpensive way of providing a comprehensive framing of the bacterial community structure enabling an easy comparison between different stress conditions that affect organisms, correlating to the presence of contaminants and subsequent changes in the isopod hindgut bacterial communities' composition.

Despite this technique's lack of ability to distinguish taxonomic/functional groups *per se*, it can be complemented with methodologies able to identify the resident and ingested microbiota and the way they relate to exposure to toxicants. This would present an interesting research perspective to provide the ecological relevant effects of these microbiota shifts. Moreover, this approach is most surely not limited to the organ and/or organism here used. It ought to work for other samples and diverse scopes, thus adding a supplementary value to this technique.

5. Conclusion

The present study explores the application of ARDRA as a tool to detect changes in bacterial communities' composition of the gut of terrestrial isopods. Despite this body of work presenting ARDRA profiling as a ecotoxicological tool, it can also be considered as a foundation study for supplementary studies undertaking the identification of isopod gut's key bacteria species and/or functional groups that vary upon exposure. This will enable us to assess the potential threats to the isopod's fitness and to litter degradation

processes, and in the worst case scenario, which would be the potential ecological consequences.

References

1 M. Hassall, J. G. Turner and M. R. W. Rands, Effects of terrestrial isopods on the decomposition of woodland leaf litter, *Oecologia*, 1987, **72**, 597–604.

2 M. F. L. Lemos, C. A. M. van Gestel and A. M. V. M. Soares, Endocrine disruption in a terrestrial isopod under exposure to bisphenol A and vinclozolin, *J. Soils Sediments*, 2009, **9**, 492–500.

3 P. C. J. Van Vliet and P. F. Hendrix, in *Soil Biological Fertility—a Key to Sustainable Land use in Agriculture*, ed. L. K. Abbott and D. V. Murphy, Springer, Dordrecht, The Netherlands, 2004, ch. 4, pp. 61–80.

4 D. Drobne, Bacteria adherent to the hindgut of terrestrial isopods, *Acta Microbiol. Immunol. Hung.*, 1995, **42**, 45–52.

5 R. Kostanjšek, J. Strus and G. Avgustin, Genetic diversity of bacteria associated with the hindgut of the terrestrial crustacean *Porcellio scaber* (Crustacea: Isopoda), *FEMS Microbiol. Ecol.*, 2002, **40**, 171–179.

6 M. Zimmer, The fate and effects of ingested hydrolyzable tannins in *Porcellio scaber*, *J. Chem. Ecol.*, 1999, **25**, 611–628.

7 A. A. Massol-Deya, D. A. Odelson, R. F. Hackey and J. M. Tiedje, in *Molecular Microbial Ecology Manual*, ed. A. D. L. Akkerman, J. D. van Elsas and F. J. de Bruijn, Kluwer Academy Publishers, Dordrecht, 1995, pp. 1–8.

8 E. Smit, P. Leeflang and K. Wernars, Detection of shifts in microbial community structure and diversity in soil caused by copper contamination using amplified ribosomal DNA restriction analysis, *FEMS Microbiol. Ecol.*, 1997, **23**, 249–261.

9 J. W. Bodnar, W. Zempsky, D. Warder, C. Bergson and D. C. Ward, Effect of nucleotide analogs on the cleavage of DNA by the restriction enzymes AluI, DdeI, HinfI, RsaI, and TaqI, *J. Biol. Chem.*, 1983, **258**, 15206–15213.

10 D. Drobne, M. Rupnik, A. Lapanje, J. Strus and M. Janc, Isopod gut microflora parameters as endpoints in toxicity studies, *Environ. Toxicol. Chem.*, 2002, **21**, 604–609.

11 A. Lapanje, D. Drobne, N. Nolde, J. Valant, B. Muscet, V. Leser and M. Rupnik, Long-term Hg pollution induced Hg tolerance in the terrestrial isopod *Porcellio scaber* (Isopoda, Crustacea), *Environ. Pollut.*, 2008, **153**, 537–547.