Actinobacterial resources from termite guts for regional bioindustries

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The State government of Queensland launched a well-funded 10 year strategy for bioindustry development in 1999 with the aim of making Queensland the Asia-Pacific hub of biotechnology. This strategic approach into diversifying the region's economic base through the development of knowledge industries has rapidly transformed traditional methods to cutting edge technologies utilised for bioindustry. Microbially mediated technologies have thus gained importance for the production of biofuels and bioproducts, bioenvironmental management of natural resources, bioremediation, waste and wastewater management [1].

Bioconversion of lignocellulosic material has been receiving significant attention thanks to its practical applications in various agro-industrial processes, such as efficient conversion of plant biomass to fuels and chemicals, delignification of paper pulp and digestibility enhancement of animal feedstock. Enzymes that degrade, or help degrade lignocellulose, are of great interest to pulp and paper industry due to their bleach-boosting properties, which reduce environmentally unfriendly chlorine consumption [2]. Microbes active in composting and wood-decay produce a series of non-specific extracellular peroxidases that are involved in ligninolysis, but can also attack a broad range of aromatic and phenolic compounds such as lignin which is polyphenolic by nature [3]. In this context, termite gut-associated microorganisms involved in the degradation of aromatic compounds, hemicellulose and lignin [4] can be a valuable source for the needs of regional bioindustries in Australia.

Presently, there are some 2000 termite species described in the world. Australian range of termite fauna is diverse and represented by the families Mastotermitidae, Termopsidae, Kalotermitidae, Rhinotermitidae and Termitidae. These five families include about 30 genera with 258 described species and at least 90 undescribed species [5]. Actinobacteria have been frequently described in termite guts since the detection of *Micromonospora* species by Hungate (1946) [6] and Sebald and Prévot (1962) [7]. Since the late 1970s large numbers of streptomycetes were isolated by Bignell *et al.* (1979) [8], followed by *Arthrobacter, Aureobacterium, Cellulosimicrobium, Cellulomonas, Kocuria, Microbacterium, Micrococcus, Rhodococcus* and *Nocardia* species [4]. However, culture independent molecular studies overseas have revealed that the majority of the gut actinobacterial symbionts have not yet been cultured [9]. Also, very few published papers exist in the literature on the diversity of termite gut associated microorganisms in Australia [10] and their potential applications in bioindustry. Accordingly, it is important that continuous efforts are made towards the design of improved isolation techniques in order to: culture representatives of termite gut associated actinobacterial taxa; document their diversity; and detect their potential for use in large scale bioindustrial applications.

Guts of the subterranean termite species *Coptotermes lacteus* (Froggatt) were studied at the University of the Sunshine Coast (USC) using a selective isolation technique recently described by Kurtböke and French [5]. Termites were collected from local termite mounds (Figure 1) using a baiting technique described by French [11]. Guts of the termites were removed aseptically and were exposed to genus and species specific phages for removal of background bacteria, which impede the growth of actinobacteria on isolation plates. Once these unwanted microbial taxa were removed on the isolation plates, previously undetected and novel actinomycetes were successfully cultured from the gut samples [5]. Molecular sequencing results confirmed that the isolates belonged to the actinobacterial genera *Steptomyces Micromonospora, Nocardia, Rhodococcus, Actinomadura, Saccharopolyspora, Geodermatophilus, Microbispora* and *Pseudonocardia* [5].

Members of the genus *Streptomyces* have been studied more commonly since the 1950s and their potential in the degradation of

naturally occurring biopolymers in soil has been well documented over the past 2 decades. As a result, 50 selected streptomycete species (Figure 2) isolated using the bacteriophage technique (five different mounds were studied; for each mound 20 termite guts were extracted and pooled together to obtain a composite sample for the site) were tested for their hydrolytic abilities [3, 12] on agar plates incorporated with the test substrates. Isolates from the termite guts were found to produce amylase, cellulose (Figure 3), xylanase (Figure 4), ligninase and keratinase in greater numbers when compared to streptomycete species cultured from adjacent soils (Table 1).

Decolourisation of Poly-R is an indication of the reduction of the quinone groups present in this substrate catalysed by lignin

Figure 1. Baiting for termites.

Figure 2. Streptomycete species isolated from the termite guts.

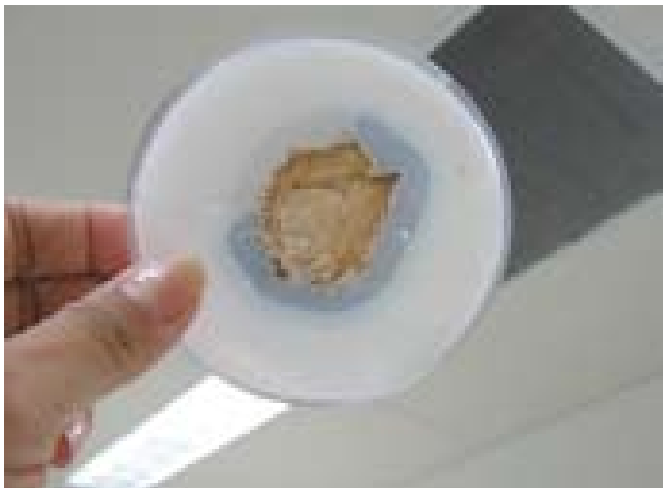

Figure 3. Cellulase production by the streptomycete colony indicated by the clear zones around the colony.

Figure 4. Xylanase production by the streptomycete colony indicated by the clear zones around the colony.

Table 1. Hydrolytic abilities of actinobacteria.

Isolate numbers with hydrolytic ability*	From termite gut	From mound surrounding soil
Amylase	48	38
Cellulase	25	13
Xylanase	30	11
Poly-R decolourisation indicating the presence of lignin peroxidase, Mn-dependent peroxidase, laccase	18	3
Keratinase	10	2

* Total number of isolates tested: 50 from each side. Numbers indicate the number of isolates showed clearance of the substrate incorporated into the agar plates (see Figures 3-5).

peroxidase, Mn-dependent peroxidase and laccase [3] (Figure 5). Although similar species were detected both in the gut samples and surrounding soils, findings related to the increased numbers of hydrolytic species from the gut samples might indicate that, following ingestion, actinobacteria might play a significant role in the termite guts. These bacteria might be adapting to the gut environment to produce hydrolytic enzymes that break down lignocellulosic material ingested by the insect. In addition, tests using these isolates as baits to attract termites indicated that termites were able to recognise hydrolytic species and get attracted towards them outside of the gut environment (Figure 6).

Termites, together with their microbial symbionts, have a highly significant impact on biodegradation and biorecycling as well as shaping soil functions and properties in the tropics and sub-tropics [13]. A sound understanding of these efficient biorecycling systems, such as that for lignocellulose and the symbioses providing this efficiency, will greatly benefit applied microbiology and biotechnology and contribute to the needs of regional bioindustries (e.g. agro-food, oil, animal feed, detergent, pulp and paper, textile, leather, petroleum, and specialty chemical and biochemical industries).

Figure 5. Halo of decolourisation surrounding streptomycete colony on Poly R-478 plates.

Figure 6. Actinobacteria recognition test by *Coptotermes lacteus* (Froggatt) species (termites were monitored for their move towards the bottles containing hydrolytic enzyme producing and non-producing actinobacteria isolates).

The most important step towards microbially mediated bioindustrial applications is, however, to culture these termite gut symbionts in order to understand their diversity, distribution and functions in biorecycling systems [14]. The use of phage battery reported here will be one of the important selective isolation tools for the detection of bioindustrially important actinofloral taxa from termite guts. Further spectral analysis of the enzymes and their application for regional bioindustries are underway as well as a study of the attraction of termites to their symbionts leading to nest mate recognition in natural environments.

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