

Bioprospecting of Termite and Termite mound soil for enzymatic potential: A review

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Abstract

Termites, as ecologists, have a central function in lignocellulosic biomass breakdown, nutrient recycling, and soil consolidation. Their guts, symbiotic microflora, and their surrounding mound soils offer a rich source of enzymes, specifically cellulase, ligninase and xylanase having extensive biotechnological potential. This article brings to light new developments in the bioprospecting of Termites and mound soils for enzymic potential, investigating their microbial diversity, ability to produce enzymes, as well as their industrial application in biofuel, waste treatment, agriculture, and pharmaceuticals.

Keywords: Termite, enzyme, Termite mound soil, symbiotic microbiota

Introduction

The caste system in Termite colonies is a complex system of labour division under three main roles: i) Reproductives (winged, unmated alates), ii) Workers (false workers or pseudergates), and iii) Soldiers, which is largely involved in defence of the colony (Snyder, 1926; Krishna, 1970) [13, 31]. Each of these castes is morphologically specialized to perform a particular function to ensure the smooth operation of the colony. Ecologically, Termites are divided according to their nesting and feeding habits into four broad groups: i) Wood-dwellers, ii) Ground-dwellers, iii) Arboreal carton nest builders, and iv) Earthen mound builders. Evolutionally and functionally, Termites are usually classified as lower and higher Termites. Lower Termites are characterized by a symbiotic relationship with a dense population of gut-dwelling prokaryotes and protists. The protists ingest wood cellulose particles through endocytosis and metabolize them to acetate, which serves as the main energy and carbon source for the Termite host, a paradigm example of mutualistic symbiosis (Ohkuma, 2003) [21]. On the other hand, higher Termites only possess a single family 'Termitidae' but this comprises over 75% of all the Termite species. Though their gut also harbours an enrichment of microbial population, they do not possess any protists such as lower Termites. Instead, their cellulose digestion solely relies on a diverse population of bacteria, exhibiting a more evolved symbiotic digestive strategy (Ohkuma, 2003) [21].



Fig 1: Termite



Fig 2: Termite Mound

Literature review

Very comprehensive literature survey of about 150 research articles, the activities of Termite, Termite gut microbiota and Termite mound soil derived enzymes highlight their tremendous biocatalytic potential fascinates to work on its applications in biotechnology and allied sciences.

Termites: Longevity, Microbiome, and Ecological Significance

Mechanisms of anti-ageing and longevity in Termites have intrigued scientists for a few decades. Termite royals are primarily the queen and king, have demonstrated exceptional longevity and extended reproductive capacity and are thus very valuable study subjects in ageing (Tasaki *et al.*, 2018) [33]. Ecologically, Termites are key decomposers in a large number of ecosystems, and they play a significant role in improving soil structure and fertility through bioturbation and organic material processing (Bignell and Eggleton, 2000; Holt and Lepage, 2000) [4, 11]. They play a vital role in the nutrient cycling process, most significantly through carbon turnover, and their potential as models for biofuel research. Recent research on the Termite microbiome has provided new insights into Termite symbioses. Termites are among the 3 only groups of

animals capable of digesting lignin, an extremely recalcitrant, very highly complex plant cell wall polymer. This is attributed to separate mechanisms in lower and higher Termites.

In lower Termites, symbiotic protists in their gut that are cellulolytic flagellates capable of breaking down lignin are responsible for the digestion of lignin. In higher Termites, this role is achieved through a mixture of endogenous Termite enzymes and enzymes from their multifarious gut bacterial symbionts (Tokuda and Watanabe, 2007) ^[34]. These microorganisms are so specialized that some are found nowhere else in nature (Brune, 2006) ^[5]. Termites are also one of the only animal groups with biological nitrogen fixation, adding still more value to their ecological worth. Their voracious appetites allow some Termite species to consume quantities of food several times their body weight on a daily basis. By sheer numbers and persistence, they are able to break down recalcitrant hard organic materials such as coconut shells and municipal solid waste materials such as thermocol materials that are otherwise refractory to composting or vermicomposting. Along the route, Termite zoome is a high-quality protein feedstuff, particularly for inclusion in poultry feed (Ravindran and Blair, 1993; Sankar G, 2007) ^[26, 27]. Termites also yield valuable biochemicals and bioactive chemicals, which have prospective biotechnological, biofuel, and industrial enzymology applications (Warnecke *et al.*, 2007; Weng *et al.*, 2008) ^[35, 36].

Enzymatic Activity of Termite:

Termites have been used for centuries by different cultures for both food and medicinal uses. Termites of 45 species, representing four families, have been documented as part of human utilization worldwide. 43 species as food items in humans or animals, and nine species are used in traditional medicine (Figueiredo *et al.*, 2015) ^[7]. Termites like *Macrotermes falciger* are highly nutritious, containing around 44.3% fat and 41.8% protein in terms of dry weight (Phelps, 1975) ^[24]. These medical applications address a variety of conditions, including especially respiratory diseases like asthma, bronchitis, sinusitis, and hoarseness, wounds, and conditions of pregnancy. Termites are occasionally used in spiritual purposes as well. One of the species most frequently mentioned for medicinal application is *Nasutitermes macrocephalus*, broadly applied in Brazil against respiratory afflictions. In Nigeria, *Macrotermes nigeriensis* is utilized not just for wound healing and maternity care but also as a protective charm (Figueiredo *et al.*, 2015) ^[7]. Such uses underscore the cultural and pharmacological importance of termites in traditional knowledge systems. Termites' protein is fairly digestible by both humans and rats, but their enzymatic activity is impressive especially against cellulose. Termites possess remarkable lignocellulose-degrading capacity, with endogenous enzymes and the metabolic processes of symbiotic gut bacteria. Scharf (2015) ^[29] categorizes Termite biotechnology into two types, Termite-directed biotechnology with a focus on pest management and Termite-inspired biotechnology with a focus on industrial uses of enzymes from Termites. Termite digestomes is a blend of host and microbial enzymes are high in carbohydrate-active enzymes (CAZymes), such as cellulases, xylanases, and lignin-degrading enzymes. They possess broad applicability in biomass conversion for

biofuels, paper, feed processing, and waste treatment. Purwadaria *et al.* (2016) in their study identified high endo- β -D-1,4-glucanase (CMCase) activity in Termite extracts, which may contribute to digestion of poultry feeds containing lignocellulose. Fagbohunka *et al.* (2021) illustrated that Termites' cellulase enzymes are more active in natural substrates (leaves, wood, food waste, paper) compared to synthetic substrates (nylons, plastics). The percentage of activity varied up to: Leaves: 62–229% Papers: 28–194% Food wastes: 17–113% Wood: 9–61% The high enzymatic efficiency highlights the contribution of Termites toward the degradation of organic wastes and possible bioremediation. Franco Cairo *et al.* (2016) Applied metaproteomic analysis to contrast enzymatic activity among Termite castes (*Coptotermes gestroi*). Extracts from worker caste exhibited greater activity than soldiers against different natural polysaccharides. Current omics-based research (Scharf, 2015; Chakraborty *et al.*, 2023) ^[29] has established the consistency of CAZyme repertoires in Termites, particularly the ones utilized in plant cell wall deconstruction. Recombinant Termite enzymes hold promise in Lignin degradation, Solubilization of cellulose and hemicellulose, Feedstock bioconversion an interesting enzyme cocktail, the HEC-H cocktail from Termites, has been found to be effective in hydrolysing agricultural residues containing hemicellulose and amorphous cellulose central to biorefinery industries (Mafa *et al.*, 2020) ^[17]. In a novel application of Termite enzymology, Bagampadde *et al.* (2022) ^[2] explored Termite saliva-derived binding agents in laterite gravel stabilization. The incorporation of mucopolysaccharides and cellulases resulted in dramatic decrease in plasticity and shrinkage of gravel. The research suggests future sustainable construction applications of Termite biomaterials. Studies by Mogilicherla *et al.* (2022) ^[18] indicate that higher Termites are heavily dependent on symbionts to breakdown lignin. Further functional genomic studies will identify species-targeted gene candidates for RNAi based pest management. Moreover, Haiyang Wu *et al.* (2020) ^[9] described a new structural feature insertion of CBM domains in Termite Xylan degrading enzymes, placing them nearby such enzymes in ruminants and humans. The enzymatic toolbox of Termites, refined through millions of years of symbiosis and coevolution, is a goldmine for biotechnology, sustainable agriculture, waste management, bioenergy, and green architecture. More metagenomic and proteomic findings will unlock even more potential from these tiny but powerful bioengineers.

Enzymatic Activity of Termite Mound Soil

Termites employ their faeces and saliva in building nests. In nest building, they blend the soil from the top layer, usually 25 to 35 cm deep. Consequently, termite-infested soil is very distinct from the surrounding ground. Physically, termite mounds add a lot of environmental heterogeneity, particularly when the mound soil is very different from nearby topsoil (Lind *et al.*, 2023) ^[16]. Soil from Termite mounds has high cellulase, saccharase, and β -glucosidase activity, irrespective of climate, altitude, or Termite type (Santhoshkumar *et al.*, 2020) ^[28]. Soil binding protein were extracted from termite mound soil (Pariyath, 2014) ^[23]. Mound soil glycoproteins increase compaction, water retention, and compressive strength with potential as a bio-binder in rural road construction equal to commercial terrazyme. Water, ethyl acetate and petroleum ether extract

of termitarium exhibit broad-spectrum antibacterial activity (Subramanian *et al.*, 2022) ^[32], including activity against *Pseudomonas putida*, *Streptococcus mutans*, *Lactobacillus acidophilus*, *E. coli*, etc. Phenylacetic acid, produced by *Reticulitermes speratus*, acts to suppress fungal pathogens such as *Metarhizium anisopliae* and *Athelia termitophila* (Nakashima *et al.*, 2024) ^[19]. Termite guts and mound soils are populated with enzyme-producing bacteria which includes Cellulolytic (e.g., *Mesobacillus jeotgali*, *Bacillus fusiformis*) (Korsa *et al.*, 2023) ^[12], Lignolytic (e.g., *Pseudomonas alcaligenes*, *Brevibacillus sp.*) (Latifah *et al.*, 2021) ^[15], Chitinolytic/lipolytic/proteolytic isolates with possible Termite biocontrol potential (Sindhu *et al.*, 2016) ^[30]. Pullulanase of *Bacillus safensis* demonstrates industrial resilience (Olaniyi *et al.*, 2022) ^[22]. Metagenomic studies have identified new and industrially significant genes for cellulases and xylanases of Termite (Nimchua *et al.*, 2012; Kumar *et al.*, 2019) ^[14, 20]. Microbial diversity within Termite mounds enhances landscape-scale soil health (Baker, 2020) ^[3]. Sudden lysis of Termite gut microbes in foreign systems such as rumen (Azizi *et al.*, 2019) ^[1] could restrict direct transfer. Encouraging results need to be scaled up for rural infrastructure, bioremediation, and green industrial applications. The enzymatic and microbial diversity of Termite systems, both mound and gut, is a biotechnological treasure chest of many functions. Termites have the secrets to sustainable applications in agriculture, industry, and the environment. Interdisciplinary research integrating microbiology, soil science, engineering, and molecular biology can realize the full potential of these "soil engineers." While considerable research has been conducted on Termite biology and behaviour, there remains a notable gap in understanding the functional applications of Termite mound soil. Specifically, enzymatic and medicinal activities of Termite mound soil are unexplored. The distinctive biochemical microenvironment of Termites may result in novel, sustainable solutions, and hence this is a promising and fascinating research field. Thus, there is significant scope for emerging researchers to explore the multifaceted potential of Termite soils in building a more sustainable future.

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