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Isolation and identification of termite gut symbiotic bacteria with lignocellulose-degrading potential, and their effects on the nutritive value for ruminants of some by-products

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ABSTRACT

The termite gut contains different kinds of lignin and lignocellulose degrading microbes. This study was conducted to isolate and identify termite gut symbiotic bacteria with lignocellulose-degrading potential, and evaluate their effects on the chemical composition and *in vitro* digestibility of wheat straw and date leaves. Termite gut contents were extracted and cultured in 9 different culture media containing lignin and lignocellulosic materials that had been prepared from water-extracted sawdust and wheat straw. Three superior bacteria capable of growing on all media, and with higher lignin peroxidase activity, were selected and subjected to molecular identification. Following this, wheat straw and date leaves were incubated with the isolated bacteria in liquid medium for 6 weeks. 16S rRNA sequence analysis showed that these isolates possessed 97, 99 and 97% similarity with *Bacillus licheniformis*, *Ochrobactrum intermedium* and *Microbacterium paludicola*, respectively. The highest ($P < 0.05$) dry matter (DM) loss in wheat straw and date leaves was observed following treatment with *B. licheniformis*. In the case of wheat straw, the organic matter (OM) and neutral detergent fiber (NDF), and for date leaves OM, NDF and acid detergent lignin (ADL) contents were not influenced by the treatments ($P > 0.05$). The greatest and lowest ($P < 0.05$) ADF content of wheat straw was observed as a result of treatment with *B. licheniformis* and *O. intermedium*, respectively. However, bacterial treatments decreased ($P < 0.05$) ADF content of date leaves when compared to the control. Acid detergent lignin content of wheat straw was decreased ($P < 0.05$) by bacterial treatments in comparisons to the control. For wheat straw, the highest and lowest ($P < 0.05$) value of crude protein (CP) was observed in the case of *M. paludicola* and *O. intermedium* treatments, respectively. For date leaves, the CP content of the control treatment was highest ($P < 0.05$) among treatments. For wheat straw, bacterial treatments enhanced ($P < 0.05$) DM, OM and ADF digestibility when compared to the control. However, highest and lowest ($P < 0.05$) CP digestibility was observed using *O. intermedium* and *M. paludicola*, respectively. For date leaves, treatment with *B. licheniformis* significantly increased ($P < 0.05$) digestibility of DM, OM and NDF when compared to the others. However, CP and ADF digestibility was not different ($P > 0.05$) between experimental groups. Overall, the results of this study showed that

Abbreviations: ADF, acid detergent fibre; ADL, acid detergent lignin; CP, crude protein; DM, dry matter; Lignin(sa), lignin measured by solubilisation of cellulose with sulfuric acid; M9, media 9; NCBI, National Center for Biotechnology Information; NDF, neutral detergent fibre; OM, organic matter; PCR, polymerase chain reaction; SBM, Sterile basal media; TB, Terrific buffer.

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the isolated bacteria partially changed the chemical composition of wheat straw and date leaves while, they improved digestibility of nutrients. These bacteria are suitable candidates for increasing nutritive value of by-products for ruminants.

1. Introduction

Numerous agro industrial by-products are produced annually in all countries around the world. Large proportions of these by-products are important feedstuffs for ruminant animals and can be used as a potentially significant source of energy. However, the use of these materials as ruminant feed is limited because of their complex structure, low protein and high lignin content (Nasehi et al., 2014). The complex network formed between cellulose, hemicellulose and lignin reduces their digestibility in ruminants, due to lack of microbial ligninolytic activity in the rumen (Falcon et al., 1995).

Different physical and chemical methods have been used to increase the nutritive value of such by-products (Fazaeli et al., 2004; Nasehi et al., 2014). Although these methods have advantages, they are costly, relatively ineffective and environmentally unfriendly and require the application of technology (Sharma et al., 1993). Recently, biological delignification of lignocellulosic material has been considered as an alternative approach. Biological pre-treatment typically uses microbes such as brown, white and soft-rot fungi to improve the suitability of by-products for enzymatic hydrolysis (Galbe and Zacchi, 2007).

Three groups of organisms are able to biodegrade lignin namely, white rot fungi, some soil microbes and termites. In recent years, increased attention has been given to the role of bacteria in lignin degradation in agricultural by-products. Insects that utilize wood as a food source are beetles, cockroaches and termites. Termites are especially well known for their ability to break down the lignin barrier and digest carbohydrate polymers (Hyodo et al., 1999; Watanabe and Tokuda, 2010). Early studies on lignin degradation by termite guts have been elegantly described by Breznak and Brune (1994), in which ^{14}C-[lignin]-lignocellulose was administrated to the termite *Coptotermes acinaciformis,* but no significant release of respired $^{14}CO_2$ was detected. Butler and Buckerfield (1979) demonstrated that lignin was digested by collecting $^{14}CO_2$ respired by termites fed with natural and synthetic lignins and related compounds. Kato et al. (1998) incubated bacteria from the gut of the termite *Nasutitermes takasagoensis* in medium containing a lignin compound, and reported that 28% of dealkalized lignin and 60–95% of lignin dimer compounds were degraded. Borji (2003) isolated 3 bacterial species from the *Anacanthotermes vagans* termite gut, including *Bacillus* sp., *Enterobacter* sp., and *Ochrobacterium* sp. These bacteria could grow on different media containing lignin and lignocellulosic materials prepared from water extracted wheat straw and sawdust as a sole source of carbon and energy. There are 2700 termite species with different types of gut microbes and little work has been done on the processing of lignocellulosic materials by isolated ligninolytic bacteria from this ecosystem.

Therefore, the aim of the present study was to isolate and identify symbiotic lignocellulosic degrading bacteria from the termite gut, and to investigate their effects on the nutritive value of wheat straw and date leaves.

2. Materials and methods

2.1. Insects

Higher termites *Microcerotermes diversus* (Silvestri) (Isoptera: Termitidae) were collected from nests on the orange trees located at Khuzestan Ramin Agricultural and Natural Resources University farm, and maintained under darkness at 30 °C with 60 percent humidity in the laboratory. Only worker termites were used. *Microcerotermes diversus* is the most economically destructive termite in structures in southwest Iran (Habibpour, 2010).

2.2. Lignin preparations

Samples of sawdust and wheat straw were milled through a 0.5 mm screen (Wiley mill, Swedesboro, USA). Before use as a growth substrate, sawdust and wheat straw were extracted with boiling water for 1 h (4 changes of water during extraction) and dried for 48 h at 60 °C. Lignin was isolated from both substrates using 3 different methods.

2.2.1. Dioxane lignin

Ground sawdust and wheat straw (0.5 mm) were extracted with ethanol-benzene (1:1) in a Soxhlet extractor for 50 h and dried in a vacuum desiccator. Extractive-free residues were then extracted for 12 h at 90 °C with dioxane-water (9:1) containing the equivalent of 0.2 N HCl. The extract was concentrated under a vacuum and the lignin was precipitated in deionized distilled water. The precipitated lignin was washed with water, dried, and washed with petroleum ether (Browning, 1967).