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Short communication

Effect of roughage fibre content on fibrolytic activities and volatile fatty acid profiles of *Neocallimastix* sp. YAK11 isolated from rumen fluids of yak (*Bos grunniens*)

Y.C. Cao, H.J. Yang*

State Key Laboratory of Animal Nutrition, College of Animal Science and Technology, China Agricultural University (CAU), Beijing 100193, PR China

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ABSTRACT

A 10-day pure culture of *Neocallimastix* sp. YAK11, isolated from rumen fluids of yak (*Bos grunniens*) was evaluated for fibrolytic activity, apparent dry matter disappearance (ADMD), ferulic acid, reducing sugars and volatile fatty acids (VFA). The fungus was grown on oat straw (OS), corn stalks (CS), rice straw (RS) and wheat straw (WS). Recovered activity of ferulic acid esterase peaked at day 5 in all roughages, and the lowest activity was found with low fibre OS throughout the incubation ($P<0.0001$). The recovered activity of acetyl esterase peaked at day 6 with WS and RS and at day 7 with CS and OS ($P<0.0001$). The highest acetyl esterase activity was found with high fibre WS throughout the incubation ($P<0.0001$). Xylanase activity increased with all roughages and was maintained at a high level throughout the incubation ($P=0.0102$). The lowest xylanase activity was found in WS ($P<0.0001$), but cultures grown on OS, CS and RS showed no differences. The highest ADMD and reducing sugar values occurred in OS and the lowest in WS ($P<0.0001$). Ferulic acid release did not differ between four roughages. ADMD was positively correlated with xylanase activity ($r=0.51$, $P<0.01$). Total VFA concentration was greatest in OS ($P<0.0001$) and highly correlated with fibrolytic enzyme activities ($r>0.42$, $P<0.01$). Molar proportions of acetate and valerate did not differ in the roughages. The lowest propionate and branch chained VFA levels occurred in WS ($P=0.0115$), and the highest butyrate levels occurred in OS throughout the 10-d incubation ($P=0.0464$). The highest ratio of non-glucogenic to glucogenic acids (NGR) occurred in WS ($P=0.0481$). NGR peaked for all roughages around day 3 and then slightly declined for the rest of the experiment. In general, the isolate was capable of a pronounced degradation that would depend on the fibre content of the roughages. Screening of fungi from rumen fluids of yak would appear to be a useful strategy to obtain highly active esterases and polysaccharide hydrolases.

1. Introduction

Rumen fungi are able to colonize fibres by anchoring to plant materials by the rhizoid and producing a wide range of fibrolytic enzymes, including xylanase, cellulases, ferulic acid esterase (FAE) and acetyl esterase (AE), which act synergistically on fibrous feeds (Yue et al., 2009). Role of polysaccharide hydrolases in fibre degradation have been well documented

Abbreviations: ADF, acid detergent fibre; ADMD, apparent dry matter disappearance; AE, acetyl esterase; CMCase, carboxymethyl cellulase; CP, crude protein; CS, corn stalks; DM, dry matter; FA, ferulic acid; FAE, ferulic acid esterase; NDF, neutral detergent fibre; NGR, ratio of non-glucogenic to glucogenic fatty acids; OS, oat straw; RS, rice straw; VFA, volatile fatty acids; WS, wheat straw.

* Corresponding author. Tel.: +86 10 6273 3124; fax: +86 10 6273 4859.
E-mail address: yang_hongjian@sina.com (H.J. Yang).

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in the literature (Sijtsma and Tan, 1993). In last decades, FAE was identified as responsible for cleaving the ester linkage between the polysaccharide main chain of xylans or pectins and monomeric or dimeric ferulic acid (Borneman et al., 1990), and AE liberate acetyl groups from acetylated polysaccharides such as pectins and xylans (Timell, 1967). Substantial variation has been noted among fungal isolates from domestic or wild animals with respect to their ability to degrade fibrous feeds, their polysaccharide hydrolase profiles and their ability to function in different ecosystems (Paul et al., 2010). The yak (*Bos grunniens*), grazed in Qinghai Tibetan Plateau with an elevation of above 3000 m, is an important animal for local people as a source of transportation, milk and meat (An et al., 2005). One strain of *Neocallimastix*, isolated from the yak faeces at a zoo, was found to have ability to yield exoglucanase, endoglucanase, beta-glucosidase, xylanase and beta-xylosidase (Sijtsma and Tan, 1993). However, no literature on esterase activity is yet available for the fungi isolated from rumen fluid in yak. In this study, we hypothesized that the yak rumen would be populated by specific fungi with high esterase activity, and the objective of this study was to determine the capacity of *N.* sp. YAK11 isolated from rumen fluid of yak to degrade agricultural crop stalks or straw.

2. Material and methods

2.1. Microorganism

The strain YAK11 was isolated from the rumen fluids of Datong yaks in Qinghai with a roll-tube technique (Hungate, 1969). Sequences of the strain have been deposited in GenBank with accession numbers GQ403047 for a partial first internal transcribed spacer and 5.8S rDNA, GQ403048 for a partial first internal transcribed spacer and GQ403049 for a partial 18S rDNA. The isolation, identification and incubation were done completely as described by Yue et al. (2009). The fungus was incubated anaerobically at 39 °C with a substrate of chopped wheat straw (2.0 mm) and was transferred every 4 days.

2.2. Roughages

Oat straw, corn stalks, rice straw and wheat straw were used in the present experiment. Baiyan No. 2 oat (*Avena sativa* L.) straw was harvested at Qumei town, Shigatse area of Tibet, China. Zhengdan 958 corn (*Zea mays* L.) was harvested after maturity at 130 days (dough stage) from the crop fields of Baicheng area of Jinlin province of China, and the stalk was kept after removal of ears. Wuyungeng No. 7 rice (*Oryza sativa* L.) and Yannong 19 wheat (*Triticum aestivum* L.) were harvested from the crop fields of Lingbi county of Suzhou area of Anhui province, China. All stalks and straws were sun-dried, chopped and ground to pass a 2 mm screen (AK-400B, Aoli Ltd., Wenling, China).

2.3. Experimental design and sampling

The experimental design consisted of four roughage treatments (OS, oat straw; CS, corn stalks; RS, rice straw; WS, wheat straw). Each roughage (80 mg) was added in four Hungate's tubes containing 9.0 ml basal medium (Hungate, 1969) and autoclaved at 121 °C for 20 min. At the end of transferring microorganism every 4 days (Section 2.1), 1 ml inoculum was taken and added to the tubes containing 1600 IU/ml penicillin and 2000 IU/ml streptomycin and incubated at 39 °C for 1–10 days. The incubation was repeated in three runs, and substrate free tubes, served as blanks, were incubated in each run of the incubation.

For the analysis of fibrolytic activity and VFA, *etc.*, cultures (0.7 ml) were sampled every 24 h from each tube with a 1 ml syringe (a needle inner diameter of 0.4 mm) after gaseous end-products in the headspace of tubes were vented. Afterwards, fresh medium (0.7 ml) was immediately compensated for the withdrawn sample. At the end of the incubation, the biomass cultures in each tube were centrifuged at 1000 × *g* for 10 min at 4 °C and the pellets were dried at 65 °C for 48 h to determine the residual dry matter (DM). Apparent DM disappearance (ADMD) was calculated as the DM loss, represented as the difference between initially incubated DM and residual DM, corrected by blanks.

2.4. Chemical analyses, enzyme assays and VFA analyses

The methods of AOAC (1999) were used for measurements of DM (ID 930.5) and crude protein (N×6.25; ID 984.13). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined and expressed inclusive of residual ash whereas alpha amylase was not used, but sodium sulphite was used for NDF determination (Van Soest et al., 1991). The concentration of alkali-extractable ferulic acid (FA) in the roughages was determined by a high-performance liquid chromatographic method (Yang et al., 2009). The samples of OS contained (per kg DM) 83.1 g CP, 585.4 g NDF, 319.5 g ADF, 4.6 g FA; CS contained 69.4 g CP, 698.7 g NDF, 402.1 g ADF, 15.6 g FA; RS contained 74.2 g CP, 705.6 g NDF, 403.1 g ADF, 6.8 g FA; WS contained 26.0 g CP, 815.0 g NDF, 591.0 g ADF, 7.6 g FA.

Following the method of Yue et al. (2009), FAE, AE, xylanase and carboxymethyl cellulase (CMCase) activities were determined with standard substrate solutions of 100 μM methyl ferulate, 1.0 mM *p*-nitrophenyl acetate, 10 g/l birchwood xylan and 10 g/l carboxymethyl cellulose, respectively. One unit of enzyme activity was defined as the amount of enzyme releasing 1.0 μmol of FA, *p*-nitrophenol or reducing sugar (xylan or glucose) per minute per millilitre culture.