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Identification of novel enzymes to enhance the ruminal digestion of barley straw

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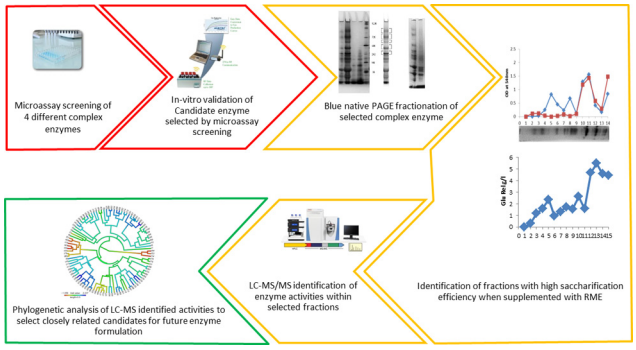
GRAPHICAL ABSTRACT

Graphical representation of experimental work flow adopted to identify enzyme activities with potential to enhance ruminal digestion of barley straw. Different stages of experimental work flow i.e., screening, enzyme discovery and selection of candidate enzymes for future formulations are shown in Red, yellow and green boxes respectively.



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ABSTRACT

Crude enzyme extracts typically contain a broad spectrum of enzyme activities, most of which are redundant to those naturally produced by the rumen microbiome. Identification of enzyme activities that are synergistic to those produced by the rumen microbiome could enable formulation of enzyme cocktails that improve fiber digestion in ruminants. Compared to untreated barley straw, Viscozyme® increased gas production, dry matter digestion ($P < 0.01$) and volatile fatty acid production ($P < 0.001$) in ruminal batch cultures. Fractionation of Viscozyme® by Blue Native PAGE and analyses using a microassay and mass-spectrometry revealed a GH74 endoglucanase, GH71 α-1,3-glucanase, GH5 mannanase, GH7 cellobiohydrolase, GH28 pectinase, and esterases from Viscozyme® contributed to enhanced saccharification of barley straw by rumen mix enzymes. Grouping of these identified activities with their carbohydrate active enzymes (CAZy) counterparts enabled selection of similar CAZymes for downstream production and screening. Mining of these specific activities from other biological systems could lead to high value enzyme formulations for ruminants.

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1. Introduction

Sustainable agriculture and animal production represent grand challenges for humanity in the coming decades (Alexandratos and Bruinsma, 2012). With an ever growing human population and more affluent societies, the global demand for food and meat and milk in particular, is projected to increase substantially (Elam, 2010). Ruminant livestock are in a unique position to satisfy the growing demand for high quality protein as they can produce it from crop residues and food by-products. However, often less than 50% of the energy in low quality forages is digestible by cattle (Hatfield et al., 1999). Consequently, large amounts of cereal grains are fed to finishing cattle to enhance the efficiency of growth. Increased global appetite for meat and milk will further heighten demand for feed grains and could threaten food security in some regions of the world (Tenenbaum, 2008). Consequently, alternative cost-effective feed ingredients that promote the sustainable intensification of beef and dairy production must be identified to meet future global demands. Forages such as alfalfa hay or cereal straws (e.g. barley, wheat) could fulfill this need if technologies to increase the conversion of these substrates into energy in the rumen could be developed.

Globally, ≈73.9 Mt of crop residues are produced annually, with most of them left to decay in the field or burned (Kim and Dale, 2004). These residues can be used as feed for cattle (Sokhansanj et al., 2006), but due to their low digestibility they are often abandoned. If the digestibility of this material could be increased it would represent a global feed source for ruminants that could be used to offset the use of grain in ruminant production systems. The digestibility of plant cell wall is highly correlated with the structural complexity of plant cell walls (McCann and Carpita, 2008; Badhan and McAllister, 2016). The composition and layered architecture of the plant cell wall, extent of cross-linkages among polysaccharides, degree of lignification, crystallinity, size of micro-fibrils and protein cross-linkages all can contribute to the recalcitrance of plant cell walls (Himmel et al., 2007; McCann and Carpita, 2008). Furthermore, inhibitors that are natural components of the plant cell wall or are generated during hydrolysis can also adversely affect its enzymatic saccharification (McCann and Carpita, 2008).

The rumen harbors a vast array of cellulolytic microorganisms including bacteria, protozoa and fungi that works synergistically to digest plant fiber (Ribeiro et al., 2016). However, the complex plant cell wall is rarely completely digested by ruminal microflora. Limited penetration of cellulolytic microbes into the interior of the plant cell, insufficient retention time of feed within rumen and rate-limiting enzyme activities, have all been reported to act as constraints to ruminal cellulose digestion (Weimer, 1996; Ribeiro et al., 2016). Characterization of total tract indigestible fiber residues (TTIR) could help identify those undigested plant cell wall moieties that escape ruminal digestion and provide insight into factors that limit plant cell wall digestion (Badhan et al., 2015). Metagenomics and metatranscriptomic studies have indicated an absence or scarcity of GH7 (endoglucanase and cellobiohydrolase), GH44 (endoglucanase and xyloglucanase), GH12 (xyloglucanase and endoglucanase), GH52 (β-xylosidase) and GH62 (arabinofuranosidase) activity within the rumen (Dai et al., 2015). It has also been shown that supplementing mixed rumen enzymes with endoglucanase (GH7), arabinofuranosidase or acetyl xylan esterase activity enhances the cellulosic saccharification of mixed rumen enzymes (Badhan et al., 2014, 2015).

Previous crude commercial enzymes assessed in ruminants were formulated primarily for industrial applications and not to confer synergism to the natural enzyme profile of the rumen microbiome (Bhat and Hazlewood, 2001). In fact, commercial enzyme mixtures often contain redundant enzyme activities that are already produced in abundance by the rumen microflora (Meale et al., 2014). Hence an informed and specialized approach towards development of enzyme formulations targeted at enhancing ruminal fiber digestion is needed. The aim of this study was to fractionate a candidate commercial enzyme preparation and through proteomic analysis identify those CAZymes that interact with mixed rumen enzymes in a manner that enhances plant cell wall digestibility.

2. Materials and methods

Graphical representation as shown in Fig. 1 describe the workflow, sequence and interaction of experiments adopted in this study to

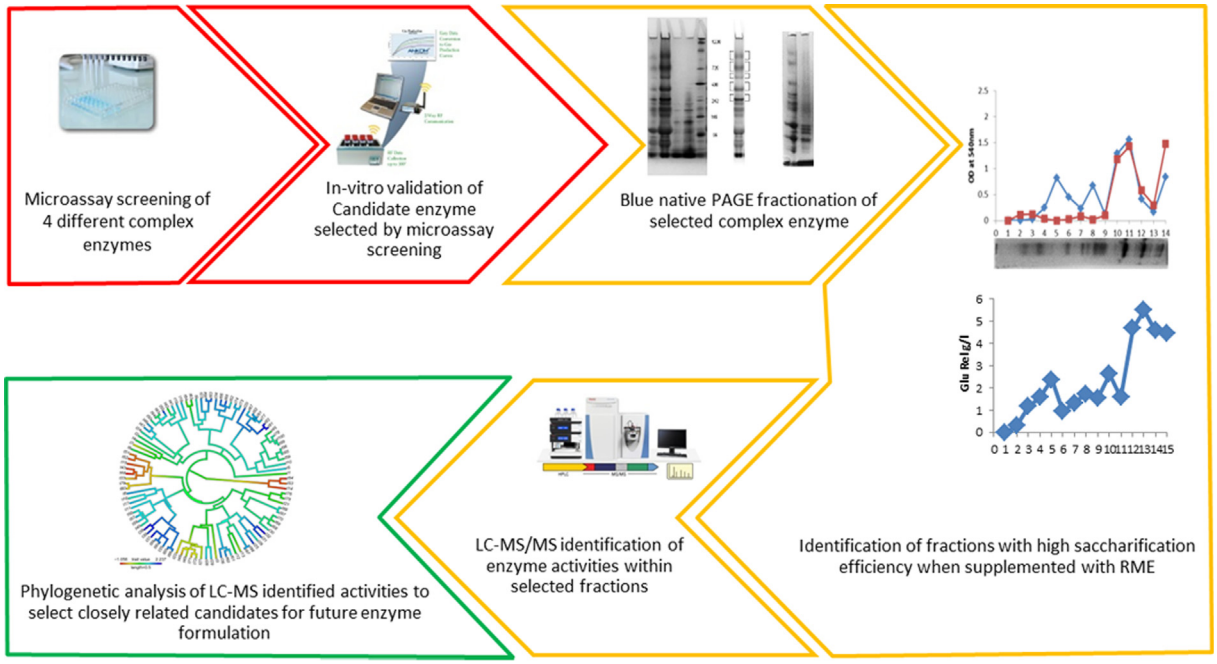

Fig. 1. Graphical representation of experimental work flow adopted to identify enzyme activities with potential to enhance ruminal digestion of barley straw. Different stages of experimental work flow included screening, enzyme discovery and selection of candidate enzymes for future formulations are shown in Red, yellow and green boxes, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)