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Isolation and characterization of yeasts from fermented apple bagasse as additives for ruminant feeding

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ABSTRACT

Solid-state fermentation can be used to produce feeds for ruminants, which can provide an enriched population of yeasts to improve ruminal fermentation. Fermentation of apple bagasse was performed to obtain a yeast-rich product, with the objective of isolating, identifying, and characterizing yeast strains and testing their capability to enhance *in vitro* ruminal fermentation of fibrous feeds. Yeasts were isolated from apple bagasse fermented under *in vitro* conditions, using rumen liquor obtained from cannulated cows and alfalfa as a fibrous substrate. A total of 16 new yeast strains were isolated and identified by biochemical and molecular methods. The strains were designated Levazot, followed by the isolate number. Their fermentative capacity was assessed using an *in vitro* gas production method. Strain Levazot 15 (*Candida norvegensis*) showed the greatest increase in gas production ($p < 0.05$) compared with the yeast-free control and positively affected *in vitro* ruminal fermentation parameters of alfalfa and oat straw. Based on these results, it was concluded that the Levazot 15 yeast strain could be potentially used as an additive for ruminants consuming high-fiber diets. However, further studies of effects of these additives on rumen digestion, metabolism, and productive performance of ruminants are required.

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Introduction

A search for alternative, low-cost feed sources that can replace higher-cost conventional feeds is one of the main goals of animal nutritionists.[1,2] The solid-state fermentation (SSF) process involves fermentation of solid substrates in the absence (or near absence) of free water and is frequently used for substrates containing enough moisture to support the development of fermenting microorganisms.[3] Because SSF stimulates the growth of microorganisms, this process offers numerous opportunities for processing of agro-industrial residues, such as protein enrichment of sugarcane.[4] Apple bagasse is a residue produced during extraction of apple juice. As an agricultural byproduct, apple bagasse is rich in soluble carbohydrates and pectins and thus can be utilized, after an SSF process, as a feed for ruminants. The fermentative process enhances the nutritive value of apple bagasse by promoting the growth of microorganisms, which increase its protein content and improve its digestibility.[5] The use of yeasts such as *Saccharomyces cerevisiae* as feed additives has been shown to improve the health and productivity of ruminants[6,7] and to offer a natural way to manipulate animal productivity by favorably modifying microbial fermentation and improving dry matter and neutral detergent fiber (NDF) digestibility,[8] feed consumption,[9] milk production, and live weight gain.[10] A previous *in vitro* study has demonstrated that the addition of fermented apple bagasse (FAB) to ruminal fermentation of alfalfa increased the viable yeast counts and concentration of lactic acid in the ruminal medium within 24 h.[11] Other studies have shown that when certain yeast strains are provided, rumen conditions are improved.[12,13] Similarly, significant increases were found in the *in vitro* forage degradability when rice bran was treated with *Candida utilis*, and the author attributed this effect to the stimulation of rumen microbes by the yeast.[14] Marrero et al.[15] demonstrated that supplemented yeasts were able to survive for 24 h under the rumen conditions. These results confirm that certain microorganisms, when placed in a new habitat, are able to exploit the resources of the environment in which they are inoculated, as, for example, yeasts utilize the scarce oxygen present in the rumen, thus favoring anaerobic conditions.[16] Based on the assumption that the addition of FAB would improve the ruminal fermentation, specifically due to the yeasts present in FAB, the objective of this study was to isolate, identify, and characterize yeast strains from FAB for their potential use as microbial additives in ruminant production systems.

Materials and methods

Fermented apple bagasse

Apple bagasse was obtained from Confrutta, S.A. (Chihuahua, Mexico) and fermented as described by Castillo-Castillo et al.[5] Briefly, apple bagasse was ground and mixed with urea, ammonium sulfate, and a mineral salt mixture containing macro- and microelements, which were added to final concentrations of 1.5%, 0.2%, and 0.5%, respectively.[17] Then, 342 g of the sample was placed into a sterile 500-mL Erlenmeyer flask for SSF. The flasks were plugged with cotton and incubated under static conditions at 32 °C for 48 h.

In vitro ruminal fermentation of apple bagasse

Non-lactating, rumen-cannulated Holstein dairy cows (average body weight: 550 ± 25.5 kg, $n = 3$) were used as donors of ruminal liquor. The cows were fed twice daily with 4.0 kg of a concentrate (51.0% corn, 23.5% wheat bran, 10% cottonseed meal, 8.49% corn gluten meal, 2.0% sugarcane molasses, 1.5% soybean meal, 1.0% bypass fat, 0.8% $CaCO_3$, 0.5% urea, 0.5% animal fat, 0.2% NaCl, and 0.5% trace mineral and vitamin premix) and 4 kg of corn silage (on a dry matter basis). The rumen fluid was sampled at 6:00 a.m., before the morning feeding, and transferred to the laboratory in hermetically sealed sterile bottles. The ruminal liquor was filtered through six layers of cheesecloth under complete CO_2 atmosphere to provide anaerobic conditions. Fermentation was performed in 20 serum flasks (250 mL) at 39 °C with mechanical agitation. The filtered rumen fluid was mixed with a buffer solution at a ratio of 1:2 (50:100 mL) and added to a mixed substrate of FAB and alfalfa hay (50:50 ratio), which were previously milled and sieved through a 2-mm sieve before their transfer to the flasks for fermentation. After 24 h, the flasks were withdrawn from the incubator, and their entire contents were collected, homogenized, and filtered through six layers of cheesecloth. The filtrates were used for inoculation of a medium in roll tubes, as described below. The chemical composition of the alfalfa (% of dry basis) was as follows: organic matter (OM), 89.07; ash, 10.93; crude protein (CP), 18.02; NDF, 65.6; acid detergent fiber (ADF), 39.0; and ether extract (EE), 2.02. The chemical composition of the fermented apple bagasse (% of dry basis) was as follows: OM, 89.94; ash 10.05; CP, 35.05; NDF, 48.31; ADF, 37.54; and EE, 5.2.

Culture of microorganisms

Yeasts were cultivated under strict anaerobic conditions according to the method described by Hungate.[18] Roll tubes were inoculated in triplicate with three dilutions (10^4, 10^5, and 10^6) of the filtered contents of the ruminal fermentation flasks, according to Caldwell and Bryant,[19] and incubated at 39 °C for 24 h. The isolation medium included malt extract agar (Difco™, Sparks, MD, USA) supplemented with 0.01 g/L of chloramphenicol. The yeast isolates present in the ruminal ecosystem were obtained from colonies that grew in the roll tubes with the highest dilution, following the method of Marrero et al.[20] Cultures with different macroscopic characteristics were inoculated and incubated following the method described by Marrero et al.[21]

Biochemical and microscopic characterization of Levazot strains

To ensure that the isolated yeasts do not belong to the genus *Saccharomyces*, the isolates (designated Levazot, followed by the isolate number) were grown at 30 °C for 72 h on the following specific medium for non-*Saccharomyces* yeasts: malt agar extract (3.2 g/L), peptone (1.8 g/L), dextrose (10 g/L), K_2HPO_4