
Neocastilligmycota SP in Cowdung Helping in Biomethanation- Review

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Abstract

Mammalian herbivores do not produce the cellulolytic or hemi-cellulolytic enzymes to degrade ingested plant material; instead they mainly rely on symbiotic associations with microorganism (i.e. anaerobic fungi, bacteria, the methanogenicarchaea and protozoa) that reside within their gut. Within this microbial consortium, the anaerobic fungi are known to be key players in the degradation of the lignocellulosic plant fibre in the rumen.

Keywords: Anaerobic, fungi, microorganisms, rumen, degrade

INTRODUCTION

Zoospores of anaerobic fungi in the rumen were originally classified under the category as protozoa, until it was recognized that these ‘flagellates’ represented mainly the dispersal phase of a zoosporic fungus. The initial acceptance of these novel rumen fungi was quiet complicated; however, due to the long held belief that all the fungi were obligate aerobes. However, Researchers showed that the cell walls of these organisms contained chitin, confirming their correct placement in the kingdom fungi. Since this time, research has started to uncover the basis for the novel mechanisms that mainly enable these fungi to live in the absence of oxygen (Sawyer et al., 2019).

Taxonomy Anaerobic fungi belong to the phylum the Neocallimastigomycota, the earliest diverging lineage unequivocally assigned to kingdom Fungi and are closely related to the chytrids (phylum Chytridiomycota). While they share pivotal morphological features with their chytrid relatives, they possess the distinctive anaerobic physiology and flagellar

apparatus. Furthermore, the genetic analyses have consistently shown that the Neocallimastigomycota form a distinct, the well-supported clade basal to the chytrids. The phylum Neocallimastigomycota currently comprises the six genera, each distinguishable by morphological features: thallus morphology (the rhizoidal vs. bulbous) and zoospore flagellation (monoflagellate vs. polyflagellate) (André et al., 2016). Although the conclusive genus identification of anaerobic fungi availing microscopic approaches can be challenging, assignment of isolates into the bulbous (*Caecomycetes*, *Cyllamyces*), hyphaelmonocentric (*Neocallimastix* and *Piromyces*) and thehyphael polycentric (*Orpinomyces* and *Anaeromyces*) by direct examination of colonies/cultures is reasonably quiet straight forward. However, difficulties in observing zoospore release, the pleomorphic growth form and also variable sporangial morphology of some isolates can mainly make further identification challenging (Zupančič et al., 2011). This has led to disagreement as to the validity and the distinctiveness of some taxa. Prior to the advent of DNA barcoding, the difficulty of transporting the viable cultures and the absence of any established repository for these fungi also made the inter lab comparisons very difficult (Lever, et al., 2012).

ROLE OF FUNGI

Advanced proventricular microbial ecosystem is the main reason why ruminants represent the most evolved cooperative animal-microorganism relationship. Symbiotic microorganisms in the rumen hydrolyze plant fibers with the production and further generation of end fermentation products (the volatile fatty acids) that can be utilized by host animal as nutrients. In the rumen, the cellulose, hemicellulose and other compounds of plant diet are hydrolyzed under the anaerobic condition by the complex microbial community consisting of bacteria, protozoa and fungi (Bader, et al., 1992). While the symbiotic relationship with bacteria and protozoa is common natural phenomenon, the beneficial cohabitation with filamentous fungi is less common, and symbiosis with the anaerobic fungi is unique feature of ruminant and some non-ruminant herbivores. Rumen anaerobic fungi produce bioactive plant biomass hydrolyzing enzymes and some authors suggested that the contributions of the rumen fungi to cell wall degradation may greatly exceed the fermentation part of the bacteria. Improvement of biomass hydrolysis by bioaugmentation availing anaerobic ruminal microbes is one of the ways to enhance decomposition of the lignocellulosic plant material and consequently improve biogas production. Instigated by this idea the presented projects has attempted to apply rumen anaerobic fungi into the biogas producing microbial consortium (Tokushige et

al., 1972). The ruminal fermentation is a very highly developed natural cellulose-degrading system, and also it has been already proposed that anaerobic system of ruminant animal can serve as a specific model for improvement of mainly converting cellulosic biomass into transportation fuels, particularly ethanol. The more so the inspiration for the biogas production enhancement can be sought in the rumen, the most elegant and the highly evolved cellulose digesting system in nature (Ostrem et al., 2004).

ANAEROBIC FUNGI

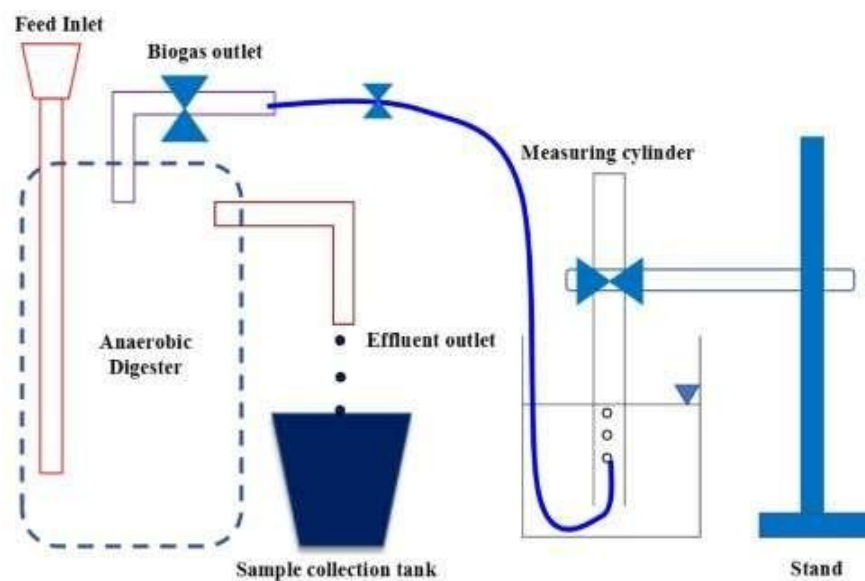
Rumen or the gut fungi are the only anaerobes in kingdom fungi classified recently in separate phylum Neocallimastigomycota mainly encompassing class Neocallimastigomycetes, order Neocallimastigales, and the family Neocallimastigaceae. Inside this family six genera of the anaerobic fungi including Neocallimastix, Piromyces, Ceacomycetes, Orpinomyces, Anaeromyces and the Cyllamyces have been described up to now, even if the latest research and findings indicate that this number is not terminal. Anaerobic fungi synthesize a variety of specific hydrolytic enzymes necessary for plant cell-wall degradation encompassing cellulases, Xylanases, mannanases, glucosidases, the glucanases, the pectinases, and phenolic acid esterases (Bilitewski et al., 1997). Enzymes of anaerobic fungi involved in the biomass degradation pertain to the several different GH families based on the amino acid sequence similarities, hydrophobic cluster analysis, and the stereochemistry of cleavage of the β -1-4 bonds. β -glucosidases (cellobiases) represents usually family GH1 or sometimes GH3, exoglucanases (cellobiohydrolases) are registered in the GH6 and endoglucanases (cellulases) are generally of family GH5, but some are classified in the family 6, 9, 45 and 48. Known enzymes of anaerobic fungi involved in xylanase degradation are the xylanases of GH family 10 and 11, licheninases of family GH16, mannanases of the family GH26, and esterases of CE family 1, 2, 3 and 6 (Atelge et al., 2020). This broad spectrum of hydrolases and the mycelial growth mainly enable fungi to penetrate plant cell walls, access the fermentable substrates not available to surface-acting bacteria, colonize the more recalcitrant components of the forage, weaken and degrade plant tissue and reduce the size of plant particles. The ability of the anaerobic fungi to specifically penetrate deeply into plant tissues that are not accessible to the rumen bacteria hints that they have a special role in fiber digestion. Some of these bioenzymes act individually and are free in medium, whereas others are the constituents of large (hemi) cellulase multi-enzyme complexes remarkably similar to the cellulosomes of the anaerobic bacteria (Deublein et al., 2011). Cellulosomal enzyme complexes are extremely active and can

mainly degrade both amorphous and crystalline cellulose. Anaerobic fungi are the only known members of the kingdom Fungi possessing cellulose and this can mainly explain their cellulolytic superiority over aerobic cellulolytic fungi. Anaerobic fungi grown on the soluble carbon source produce acetate as the major end-product, followed by formate, ethanol, variable amounts of lactate and malate and also small amount of succinate. Relative ratio of the short chain fatty acids however mainly differs between genera. All isolates also produce CO₂ and H₂. Final products and the fermentation efficiency are dependent on hydrogen concentration. Cultivation of anaerobic fungi (Bucker et al., 2020). Biogas is a potential source of energy, because year by year the demand for energy is increasing alarmingly (Sreeremya, 2016a). Isolation of potential microorganisms from cow and goat ruminal fluid acts as a stimulating factor in energy production (S.Sreeremya, 2016b). Even microorganism collected from direct cow dung or biogasifiers where cow dung used as raw material also serve as energy stimulating factor for biomethanation process (Sreeremya et al., 2017a). It is considered as a biopotential source for macro- and micronutrients. The assessment of microorganisms isolated from different biological sources can be further assessed for the presence of cellulose (S. Sreeremya, 2017b). Specific indicator dye is used named Congo red (S.Sreeremya, 2016c). If ruminal fluid is collected and to understand the biomethanation process, especially the presence of anaerobic bacteria-Balloon method is implemented in a pilot scale (S.Sreeremya 2016d). The biomethanation process is an inevitable phenomena in biofuel production (Dr.S.Sreeremya 2020). There can be both plant based as well as animal based biomethanation process (Dr.S.Sreeremya 2019). Biofuel and biogas being the sustainable energy resources (S. Sreeremya, 2018), it's imperative to promote the synthesis of sustainable energy (S.Sreeremya 2017c).

The techniques for the cultivation of anaerobic bacteria described by few researchers in the year (1969) and the roll-tube technique described by researcher named Joblin (1981) are still the most common methods available in maintenance and isolation of AF, respectively. Both are batch culture techniques, requiring routine feeding or the sub-culturing every 4 to 14 days (depending on the culture volume) and thereby making the culturing process much rather tedious routine. Descriptions of the cultivation techniques and examples of widely available (Wang et al., 2018).

ANAEROBIC FUNGI IN ANAEROBIC DIGESTION

Co-digestion of the lignocellulosic biomass with animal (e.g. cattle) manure is already a frequently implemented technique to boost the biogas production in full-scale digesters. Hence, in order to assess the full extent of the AF bioaugmentation effects on AD, it is prudent to investigate and verify a possible bioactive presence of AF within running full-scale reactors fed with the cattle manure or cattle slurry (Rouhollahi et al., 2020).



Figuer no. 1 Pilot Scale Anaerobic Assessment

Cooperation: Methanogens, Anaerobic Fungi, and Bacteria

Because AF are known to produce H₂ and the formate and methanogens are known to convert those products into methane, the relationship between AF and the methanogens is of great interest in biogas production. The first natural co-cultures of the AF and methanogens were isolated in the 1980es and 1990es (Sun et al., 2015).

CONCLUSION

Biomethanation is a process by which sustainable energy is synthesized. It is a process in which microbial consortium is involved. The biofilm of cowdung along with Neocatilligmycota species help in potential biomethanation process.

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