CHARACTERIZATION OF AMAFERM® EFFECTS ON THE RUMEN FUNGUS *NEOCALLIMASTIX FRONTALIS* EB 188

PART 1. ZOOSPORE DEVELOPMENT AND PHYSIOLOGY

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AMAFERM stimulated the secretion of protein, and cellulolytic and amylolytic enzymes.

SUMMARY

DOSE OF AMAFERM USED
AMAFERM powder < 1 part per 20,000; AMAFERM liquid < 1 part per 555,000

AMAFERM accelerated the growth of motile zoospores of the rumen fungus *Neocallimastix frontalis* EB188, with a resulting increase in production of both cellulolytic and amylolytic enzymes. This stimulation effect did not come from live *Aspergillus oryzae* spores contained within AMAFERM.

VALUE

By stimulating the secretion of protein, and cellulolytic and amylolytic enzymes by rumen fungus, AMAFERM resulted in greater fermentation of cellulose, and thus increased the release of soluble sugars.

PROTOCOL

Type of Animals/Experimental Units

- Rumen fungus *Neocallimastix frontalis* EB188 culture obtained from the ATCC (Rockville, MD) as acquisition number 76100

PROTOCOL (CONTINUED)

Number of Animals/Experimental Units

- At least triplicates of 5 cultures for each treatment

Trial Design

- Complete randomized design

Treatments

- Control, AMAFERM, wheat bran – carbon source was either cellulose or as indicated

Diet Information

- N/A

Data Collection

- Extracellular and intracellular protein and enzymes, gas production, zoospore production and maturation, cellulose degradation

DISCUSSION OF RESULTS

- In cultures of growing fungi, the presence of AMAFERM resulted in a higher level of cellulase than wheat bran-treated or control cultures, as early as 40-hour incubation, with the peak of cellulase secretion reaching levels of up to 150% of control
- There were more zoospores produced at earlier time points in the AMAFERM-treated culture
- Reducing sugars for the AMAFERM culture were 0.1-0.2 µmol/ml higher after 20 hours, compared with the control – indicating a greater fermentation and release of soluble sugars from the degradation of cellulose
- Protein and µ-glucosidase secretion from the AMAFERM-treated culture occurred earlier, and the total enzyme production was higher throughout the growth period
- Within stationary cultures, AMAFERM stimulated earlier zoospore production and maturation into germination entities, when cellulose was the sole carbon source
- The relative total numbers of motile, non-motile and germinated zoospores in AMAFERM cultures vs. the control cultures were 120%, 151.1% and 197%, respectively
- Measurements of intracellular components from zoospores showed that AMAFERM extract increased the amount of protein and the numbers of total zoospores by as much as twofold. All enzymes measured, including malate dehydrogenase, lactate dehydrogenase, amylase and CMCase were in greater amounts in the AMAFERM cultures (Table 1)

DISCUSSION OF RESULTS (CONTINUED)

- Similar stimulating effects on fungal growth and physiology were found in this study when un-autoclaved AMAFERM was added to the culture
  - Adding AMAFERM resulted in the increased production of protein, gas, acetate and major enzymes, including cellulase and µ-glucosidase
  - The numbers of motile zoospores and their maturation into germination entities were more rapid in AMAFERM-treated culture
- *Aspergillus oryzae* spores (approximately 3,300/g) contained within the product are not viable (do not germinate) under anaerobic conditions, indicating factors other than the live spores are responsible for the stimulation effect (unpublished results)

Table 1
Measurements of intracellular components from zoospores after 50 hours AMAFERM culture

Protein	[µg/ml (as BSA)]	AMAFERM	-	136
			+	274
Zoospores	10^6	AMAFERM	-	2.55
			+	3.44
CMCase	(mIU/ml)	AMAFERM	-	0.215
			+	0.295
ß-glucosidase	(mIU/ml)	AMAFERM	-	0.761
			+	1.992
Amylase	(mIU/ml)	AMAFERM	-	4.19
			+	17
MDH	(mU/mg)	AMAFERM	-	4.36
			+	27.76
LDH	(U/l)	AMAFERM	-	0.354
			+	0.962
Reducing sugar	[µmol/ml (glucose)]	AMAFERM	-	0.284
			+	0.439

CMCase: Carboxymethyl cellulase; MDH: Malate dehydrogenase;
LDH: Lactate dehydrogenase; BSA: Bovine serum albumin

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