Indian Journal of Animal Sciences **82** (9): 1022–1025, September 2012

Potential of anaerobic rumen fungi *Orpinomyces joyonii* to degrade lignified feedstuffs

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Received: 1 October 2011; Accepted:6 January 2012

Key words: Digestibility, Feruloyl esterase, *Orpinomyces joyonii,* P-coumaroyl esterase

There are evidences of definite positive relationships between anaerobic fungi in the rumen and the increased voluntary intake of low digestible fibrous feeds, growth and milk production (Dey *et al.* 2004, Sehgal *et al.* 2008 and Saxena *et al.* 2010). Manipulation of anaerobic fungal numbers and their activity in the rumen has the potential to benefit the domesticated ruminants by better utilization of nutrients from these roughage based diets (Sehgal *et al.* 2002, 2008, Jha 2009, Sanjay 2009). Rumen anaerobic fungi account for up to 8–12% of the microbial biomass in rumen (Rezaeian *et al.* 2004). Administration of elite fiber-degrading anaerobic fungus isolated from goat to sheep increased intake and digestibility of lignocellulosics feeds (Lee *et al.* 2001). Present work was carried out to study the enzymes p-coumaroyl esterase and feruloyl esterase activity and *in vitro* dry matter digestibility (IVDMD), *in vitro* neutral detergent fiber digestibility (IVNDFD) and *in vitro* acid detergent fiber digestibility (IVADFD) of lignified wheat straw, paddy straw and paddy and wheat straws based total mixed ration incorporated with the zoospores of anaerobic rumen fungi *Orpinomyces joyonii.*

Rumen fungi *Orpinomyces* joyonii were procured from Fungal Biotechnology Laboratory, Dairy Microbiology Division, NDRI, Karnal, and tested to check the purity of the cultures before being used as inoculums (Thareja *et al.* 2006, Tripathi *et al.* 2007a). The fungal cultures were grown and maintained as per the modified Hungate's roll-tube technique (Joblin 1981). The roll-tubes were kept in CO_2 incubator at 39±1°C and the colonies were sub-cultured routinely in Joblin's broth supplemented with anti-bacterial antibiotics (penicillin and streptomycin).

Enzymes p-coumaroyl esterase and feruloyl esterase activity of *Orpinomyces joyonii* were estimated as per O'Neil *et al.* (1996). For enzyme assays, zoosporess were grown for 6 days in paddy straw, wheat straw and TMR medium, containing 0.5% paddy straw, 0.5% wheat straw and 0.5% TMR, respectively, as the sole carbon source. Zoospores supernatant (1 ml) was mixed with 1 ml MOPS buffer (pH 6.8) containing 100 mg of starch free wheat bran. Reaction mixtures were incubated at 39°C/ 30 min. Reaction was stopped by keeping in boiling water for 5 min. Samples were centrifuged and the supernatant was filtered through 0.45 micron filter before injecting into HPLC for analysis. One unit of activity was defined as the amount of enzyme that catalyses the release of µmol product (ferulic or p-coumaric acid)/ min.

In vitro dry matter digestibility (IVDMD) trials were carried in 100 ml serum bottles having butyl rubber stopper and incubated at 39±1°C for 48h. *In vitro* digestibility (IVDMD, NDF and ADF) of paddy straw, wheat straw and paddy and wheat straws based TMR (PS: WS: Conc. Mixture, 30: 30: 40) were carried out as per Tilley and Terry (1963), in triplicates using following treatments.

Control (C_1) – 0.5g paddy straw +40 ml buffer +10 ml SRL +5 ml broth without fungal zoospores

Treatment (T_1) –0.5g paddy straw +40 ml buffer + 10 ml SRL + 5 ml broth with fungal zoospores

Control (C_2) –0.5g Wheat straw + 40 ml buffer + 10 ml SRL + 5 ml broth without fungal zoospores

Treatment (T_2) –0.5g Wheat straw + 40 ml buffer + 10 ml SRL + 5 ml broth with fungal zoospores

Control (C_3) – 0.5g TMR +40 ml buffer + 10 ml SRL +5 ml broth without fungal zoospores

Treatment (T_3) –0.5g TMR +40 ml buffer + 10 ml SRL +5 ml broth with fungal zoospores

For this, fresh rumen liquor was collected before feeding from the rumen of permanently fistulated bull fed on a standard diet containing wheat straw, green fodder maize and concentrate mixture to meet their nutritional requirements as per NRC (2001). Rumen liquor was strained through four layers of muslin cloth. All the flasks were flushed thoroughly with CO_2 and then placed in incubator at 39±1°C for 48h.

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Table 1. Morphological characteristics of rumen fungi *Orpinomyces joyonii* isolated from rumen liquor of cattle

Fungal isolate	Thallus morphology	Nature of growth	Shape of sporangium	Rhizoid type	Zoospore flagellation
Orpinomyces joyonii	Polycentric	Exogenous	Many globose sporangia	Highly branched	Polyflagellated

After incubation, samples from each trial were centrifuged at 2800 g for 10 min. The pellets were dried at 100°C for 24h and analyzed for percent IVDMD (Tilley and Terry 1963), neutral detergent fiber (NDF) and acid detergent fiber (ADF) acid contents (Van Soest *et al.* 1991). Data were recorded as mean±SD of 3 independent replicates and were statistically analyzed using ANOVA after suitable transformation, and standard deviation was calculated (Snedecor and Cochran 1980).

Rumen fungal isolate, *Orpinomyces joyonii* maintained through routine sub-culturing was selected for this study owing to their maximum hydrolytic activity when subjected to *in vitro* fiber degradation and cultured using modified Hungate's technique (Joblin 1981). Revival of *Orpinomyces joyonii* was carried out using modified Roll tubes technique and were inspected routinely for the appearance of colonies and confirmed by phase contrast microscopy on the basis of morphological features. Morphological characteristics of rumen fungi *Orpinomyces joyonii* are shown in Table 1. These findings were compared to their parent culture morphologically. It can be concluded that the revived fungal culture showed identical morphology to the parent cultural characteristics.

Enzyme p-coumaroyl and p-ferulyl esterase activity of Orpinomyces joyonii: Activity of p-coumaroyl esterase and p-feruloyl esterase increased significantly ($P \leq 0.01$) as compared to control groups (Table 2). Little information regarding the activity of enzyme p-coumaroyl esterase and p-feruloyl esterase on paddy straw, wheat straw and TMR could be found in the literature. Anaerobic rumen fungi *Orpinomyces joyonii* produced higher p-coumaroyl esterase and p-feruloyl esterase enzymatic activities, which can degrade lignin and thus has a potential to release more energy from lignified roughages. To increase the release of nutrients from highly lignified feedstuffs, *Orpinomyces joyonii* should be exploited under field conditions as direct fed microbial. Yang *et al.* (2009) showed that 138 and 71 g/kg of alkali-extractable ferulic acid were released from Chinese wild rye grass hay by crude enzyme solution of YQ1 and YQ3, respectively, while 92 and 94 g/kg of alkali-extractable p-coumaric acid were released by crude enzyme solution of YQ1 and YQ3, respectively. These different hydrolysis results might be due to synergism occurred between ferulic acid esterase and other fibrolytic enzymes in the degradation of large feruloyl-polysaccharides (Borneman *et al.* 1992).

Table 2. Enzyme p-coumaryl and p-ferulyl esterase activity (micro mol/ml/h) of *Orpinomyces joyonii* grown on paddy straw, wheat straw and TMR (mean± SE)

Treatment•	p-coumaryl esterase	p-ferulyl esterase
C_1	24.09±2.73	78.93±3.92
T_1	39.98±1.93**	178.93±4.91**
C_2	29.94±4.72	84.92±5.11
T_2	89.14±5.61**	205.09±10.23**
C_3	45.82±3.71	99.34±9.01
T_3	109.13±2.99**	295.83±5.88**

•Samples were analyzed in triplicates, **significant (P<0.01).

In vitro *fiber digestibility (IVDMD) using fungal zoospores of* Orpinomyces joyonii: *In vitro* dry matter digestibility (IVDMD) increased significantly (P<0.05) by 5.04, 6.17 and 9.69% points as compared to their respective controls (Fig. 1). The efficiency of degradation of DM was quite comparable with the earlier observations (ManiKumar *et al.* 2004) where the fungal cultures were administered. Results were also compared with finding of Shelke *et al.* (2009), in which *in-vitro* DM digestibility was observed to be similar for normal zoospores of *Neocallimastix* sp.GR-1 and *Piromyces* sp.WNG-12. Our results are also quite comparable with Dey *et al.* (2004) and Thareja *et al.* (2006), observed with normal fungal culture. *In vitro* neutral detergent fiber digestibility (IVNDFD) and *in vitro* acid detergent fiber digestibility (IVADFD) increased significantly (P<0.05) in fungal zoospore administered group as compared to their respective control groups without administration of fungal zoospores. There was 2.96, 2.29 and 5.02% increase in IVNDFD and 2.7, 2.38 and 3.7% increase in IVADFD of paddy straw, wheat straw and total mixed ration, respectively. Earlier studies showed that administration of elite fungal cultures led to a significant increase in the per cent disappearance of NDF contained in WS and TMR based on WS (ManiKumar *et al.* 2004, Thareja *et al.* 2006, Dayanand 2007). Dey *et al.* (2004) and Shelke *et al.* (2009) observed that IVNDFD for different incubation periods under different conditions differed significantly ($P \leq 0.05$). The present study indicated that fungal zoospores were quite efficient in reducing the NDF content in PS, WS and TMR under *in-vitro* system. The disappearance of ADF correlates positively to the degradation and utilization of highly complex polysaccharides present in dry roughages this might be due to high production of p-coumaroyl and feruloyl esterase enzymes. These enzymes are able to breakdown the side chain ester linkages of lignin and hemicelluloses, thus makes more cellulose available for other rumen microbes. Our study

showed that with incorporation of fungal zoospores, complex polysaccharides present in PS, WS and TMR could be utilized to release energy which can be further used by the animals for increasing their productive performance. Our results are in agreement with Dayanand *et al.* (2007), Thareja *et al.* (2006) and Jha (2009). The ADL degradation was in concurrence with Lee *et al.* (2000), Manikumar *et al.* (2002, 2004) and Shelke *et al.* (2009).

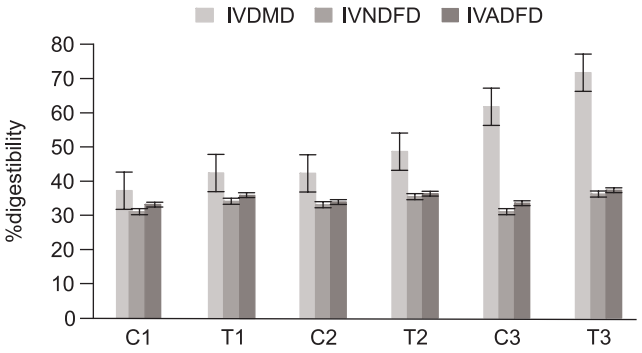

Fig. 1. Per cent *in vitro* fiber digestibility of paddy straw, wheat straw and total mixed ration incorporated with fungal zoospores of *Orpinomyces joyonii.*

In the present study, fungal zoospores of *Orpinomyces joyonii* significantly increased *in vitro* fibrolytic activities, clearly suggesting that the introduction of such fibrolytic fungal strain into the rumen could possibly improve nutrient utilization in ruminants from lignified feedstuffs. Based on the results obtained, the fibrolytic potential of *Orpinomyces joyonii* could be exploited through their administration into ruminants for improved nutrient utilization.

SUMMARY

The work was carried out to study the enzymes p-coumaroyl esterase, p-feruloyl esterase activity and *in vitro* fiber digestibility of wheat straw, paddy straw and total mixed ration (paddy straw: wheat straws: concentrate mixture in the ratio of 30:30:40) incorporated with the zoospores of anaerobic rumen fungi *Orpinomyces joyonii.* Significant increase in p-coumaroyl and p-feruloyl esterase activity was observed when *Orpinomyces joyonii* were incubated with paddy straw, wheat straw and TMR based medium as compared to control (without *Orpinomyces joyonii*). *In vitro* fiber digestibility of paddy straw, wheat straw and TMR increased significantly as compared to their respective control. It might be concluded that there were 5.0–9.7, 2.3–5.0 and 2.4–3.7% increase in IVDM, IVNDF and IVADF digestibility of paddy straw, wheat straw and total mixed ration incorporated with the zoospores of anaerobic rumen fungi *Orpinomyces joyonii*.

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