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Short Communication

Fatty Acid Analysis of Anaerobic Ruminal Fungi *Neocallimastix*, *Caecomyces* and *Orpinomyces*

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ABSTRACT

This study aimed to explore the fatty acid composition of the isolated ruminal fungi *Neocallimastix*, *Orpinomyces* and *Caecomyces*. Fatty acids of carbon chain lengths ranging from 12 to 24 were analyzed as methyl esters. *Neocallimastix* and *Caecomyces* had oleic acid ($C_{18:1}$) as the major fatty acid, while *Orpinomyces* had equal concentrations of oleic ($C_{18:1}$) and palmitic acid ($C_{16:0}$). All fungal isolates had similar amounts of myristic ($C_{14:0}$) and stearic acid ($C_{18:0}$), although they showed various profiles for lauric ($C_{12:0}$) and nervonic ($C_{24:1}$) acids. © 2010 Friends Science Publishers

Key Words: Fatty acid; Rumen fungi; *Neocallimastix*; *Caecomyces*; *Orpinomyces*

INTRODUCTION

The chemical composition of rumen microorganisms have been investigated mainly, because of their remarkable nutritive value to ruminants (Storm & Orskov, 1983; Kemp *et al*., 1984). Cellular lipids of rumen bacteria received great impetus by researchers, because of the fact that they could be used in identification of the microorganisms (Ifkovits & Ragheb, 1968) and reflect the symbiotic relationship between the ruminal bacteria and their host (Kunsman, 1970). It is, now, well known that lipid composition of the rumen microbes is an important component of animal nutrition (Or-Rashid *et al*., 2007). The fatty acids of *P. communis* and *N. frontalis* have been analyzed and high levels of monoenoic acids with chain lengths of up to C: 24 were found, whereas no polyenoic acids were reported (Kemp *et al*., 1984; Body & Bauchop, 1985). However polyenoic acids from rumen fungi were reported by the recent study of Koppova *et al*. (2008). This study was focused to analyze some fatty acids of anaerobic rumen fungi *Neocallimastix*, *Caecomyces* and *Orpinomyces* and the differences about the fatty acids compositions of three genera were examined.

MATERİALS AND METHODS

Anaerobic techniques used in this study were based on Hungate (1969). Anaerobic medium preparation and rumen fungi isolation were accomplished as described earlier by Comlekcioglu *et al*. (2008). Purification of the fungal isolate was carried out in agar containing roll tubes as described by Joblin (1981) and partial characterization (at genus level) was performed according to morphological data (Ho & Barr, 1995) as seen under light microscope.

For lipid extraction anaerobic fungi were grown on glucose (0.5% w/v) containing medium for 3 days and the fungal biomass was harvested by centrifugation at 1250 g for 10 min. Fungal biomass were washed thrice with sterile ddH_2O and freeze dried by the aid of freeze dryer. Lipid extraction was performed according to Folch (1957) and methyl esters of fatty acids were prepared according to Ozogul and Ozogul (2007). The fatty acid composition was analyzed by a GC Clarous 500 with autosampler (Perkin–Elmer, USA) equipped with a flame ionization detector and a fused silica capillary SGE column (30 m x 0.32 mm ID x 0.25 lm BP20 0.25 UM, USA). The oven temperature was 140°C, held 5 min, raised to 200°C at the rate 4°C/min and held at 220°C at 1°C/min, while the injector and the detector temperatures were set at 220 and 280°C, respectively.

Fatty acids of fungal isolates were compared by analysis of variance using the SPSS V12 statistical package program and a significance level of 0.05 was used.

RESULTS

Isolated rumen fungus, which had spherical rhizoids and monocentric life cycle, were readily determined as *Caecomyces* (Fig. 1a). *Neocallimastix* isolates were distinguished with their extensive rhizoidal system, monocentric life cycle and polyflagellated zoospores (Fig. 1b). Polycentric fungi with polyflagellated zoospores were identified as *Orpinomyces* (Fig. 1c). Identification of all isolates was corrected by using ITS sequences of these isolates (Data not shown).

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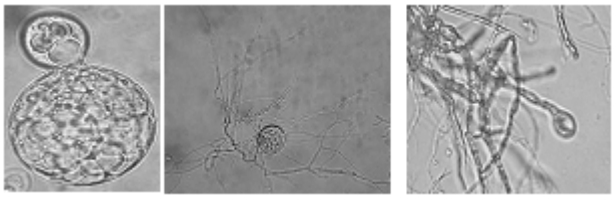
Fig. 1: Micrographs of exemplified fungal isolates used in present study. Bulbous body of *Caecomyces sp* (a), thallus structure of *Neocallimastix sp* (b) and fine rhizoidal network of *Orpinomyces sp* (c)



a **b** **c**

Table I: Relative amounts (% of total fatty acid) of analyzed fatty acids in *Neocallimastix* sp., *Orpinomyces* sp. and *Caecomyces* sp

Fatty Acid	Fatty acid composition*		
	Neocallimastix	*Orpinomyces*	*Caecomyces*
C12:0	8.00 ± 0.43^{b}	12.4 ± 0.43^{a}	4.15 ± 0.32^{c}
C14:0	6.27 ± 0.05^{a}	6.03 ± 0.35^{a}	6.74 ± 0.57^{a}
C15:0	1.00 ± 0.05^{a}	0.68 ± 0.02^{b}	1.18 ± 0.07^{a}
C16:0	21.54 ± 0.82^{b}	27.97 ± 0.30^{a}	27.04 ± 1.40^{a}
C16:1	1.13 ± 0.07^{a}	1.75 ± 0.32^{a}	0.56 ± 0.01^{b}
C18:0	17.61 ± 2.27^{a}	17.73 ± 4.84^{a}	21.59 ± 2.69^{a}
C18:1	32.56 ± 0.2^{a}	27.48 ± 2.20^{b}	33.25 ± 2.84^{a}
C18:2 n6	1.26 ± 0.18^{b}	1.38 ± 0.18^{b}	3.18 ± 0.29^{a}
C20:0	ND	ND	ND
C20:1	1.22 ± 0.1^{a}	0.59 ± 0.01^{b}	0.81 ± 0.01^{b}
C24:1	4.90 ± 0.52^{a}	3.15 ± 0.03^{b}	ND

*Values represent the means of two samples produced from independent cultures, Fatty acid determination was carried out in triplicates ($n = 3$), a, b, c superscripts in the same row means that different superscripts are statistically significant ($P<0.05$), ND: Not detected

Fatty acid composition of isolated anaerobic rumen fungi are shown in Table I. All fungi showed similar distribution for analyzed fatty acids. According to its percentage, pentadecanoic ($C_{15:0}$) for *Orpinomyces*, palmitoleic ($C_{16:1}$) for *Caecomyces*, cis-11 eicosenoic acid ($C_{20:1}$) for both fungi were remained <1.0. Palmitic acid ($C_{16:0}$), stearic acid ($C_{18:0}$) and oleic acid ($C_{18:1}$) were significantly higher than lauric ($C_{12:0}$), myristic ($C_{14:0}$), linoleic ($C_{18:2}$) and nervonic acid ($C_{24:1}$) ($p<0.05$). Oleic acid ($C_{18:1}$) had been found highest for monocentric fungi *Neocallimastix* and *Caecomyces* (32.56 & 33.25%, respectively), however polycentric fungi *Orpinomyces* sp. had equal concentrations of palmitic ($C_{16:0}$) and oleic acids (27.97 & 27.48%, respectively). Lauric acid ($C_{12:0}$) levels were found to be different regardless of reproduction origin (monocentric/polycentric) of the fungal isolates ($P<0.05$). Palmitic ($C_{16:0}$) and cis-11 eicosenoic ($C_{20:1}$) acid concentrations were similar for the isolates of *Orpinomyces* and *Caecomyces*, which were different from *Neocallimastix* sp. Furthermore pentadecanoic acid ($C_{15:0}$) concentrations of *Neocallimastix* sp. and *Caecomyces* sp. were tend to be similar, although *Orpinomyces* sp. showed significantly different level for the same fatty acid ($P<0.05$). Linoleic acid ($C_{18:2}$) was detected in *Neocallimastix*, *Caecomyces* and *Orpinomyces* but *Caecomyc*es sp. had more linoleic acid than the other isolates ($P<0.05$). Arachidic acid ($C_{20:0}$) was not detected for all studied fungi and nervonic acid ($C_{24:1}$) was not found in *Caecomyces*.

DISCUSSION

Neocallimastix, *Caecomyces* and *Orpinomyces* isolates had $C_{18:1}$ and $C_{16:0}$ as the most abundant fatty acids as reported in earlier study of Kemp *et al*. (1984) and Body and Bauchop (1985) for *Piromyces communis* and *N. frontalis*, respectively. $C_{18:0}$ was the third major fatty acid in our isolates like *N. frontalis* (Body & Bauchop, 1985) but $C_{12:0}$ was more abundant than $C_{18:0}$ in *P. communis* (Kemp *et al*., 1984). Whether $C_{24:1}$ was common in *Neocallimastix* sp., *Orpinomyces* sp. and previously reported fungi with relative differences in concentrations (Kemp *et al*., 1984; Body & Bauchop, 1985), this fatty acid was not determined in *Caecomyces* isolate of present study. Koppova *et al*. (2008) found that the unknown strain KM2CC1 was the only strain that contained the highest amount of oleic acid among the analysed six anaerobic fungi. Even this study seems to have results more likely with Kemp *et al*. (1984) and Body and Bauchop (1985). Linoleic acid ($C_{18:2n6}$) was detected in all tested rumen fungal isolates in this study and also reported by Koppova *et al*. (2008).

In the view of taxonomical approach rumen fungi, which belongs to Neocallimastigomycota, was differed from other studied fungal species by the absence or minor concentrations of polyenoic acids. This difference could be related to the obligately anaerobic growth conditions of rumen fungi (Body & Bauchop, 1985). However, the use of fatty acids as an additional tool on the beside of morphological and phylogenetical classification of rumen fungi needs more comprehensive studies that should includes more strains of all rumen fungal genera to clarify the differences between the genera of rumen fungi and also identify the relationship between Neocallimastigomycota with other fungal phyla.

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