Diversity of filamentous fungi associated with anaerobic and aerobic phase of sugar cane silage treated with biological additives

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Introduction The ensiling of sugarcane in the farms has operational advantages in relation to daily cutting. However, the fermentation process of sugarcane silage is difficult to control, and the microorganisms are still poorly studied, which can have a significant interference in forage quality. The presence of undesirable microorganisms such as filamentous fungi can significantly affect the nutritional value of silage, their appearance and acceptance by the animals, besides being a risk to people handling these silages. The growth of filamentous fungi can be accompanied by the mycotoxins production. The main genera producers of mycotoxins are *Aspergillus*, *Fusarium* and *Penicillium*. The species belonging to these genera are able to synthesize a number of enzymes that degrade the silage. The objective of this work was to enumerate and identify filamentous fungi that occur in sugarcane silages treated with different microbial inoculants.

Materials and Methods There were used silos of PVC tubes with 10 cm diameter and 60 cm length, which were capped and sealed with special rubber caps for PVC tubes adapted with Bunsen valves. The treatments consisted of 14 microbial inoculants containing strains isolated from sugarcane silage (Avila et al., 2009) and pre-selected in the laboratory. After 126 days storage, the silos were opened and 2 kg of silage were placed in plastic buckets, which were maintained for 10 days at 24°C (± 1.5°C). Samples were collected at the silos opening and after 5 and 10 days of exposure to air for pH measurement and counting of filamentous fungi. Twenty five g of sample were placed in 225 mL of sterile peptone water (0.1%) and stirred for 20 minutes and serial decimal dilutions were inoculated in DRBC media (Dichloran Rose Bengal Chloramphenicol) and DG 18 media (Dichloran Glycerol Medium Base). The plates were incubated for 7 days at 25°C. The purification of the fungi was done by successive streaking in the media MEA (Malt Extract Agar). Identification of filamentous fungi was done by macroscopic and microscopic analysis according to Barnett & Hunter (1987), Samson (2004) and Samson & Houbraken (2009).

Results and Discussion Most of the fungi were observed 10 days after the silo opening time, showing high population and a larger number of morphotypes in DRBC media. The media comparison showed that DRBC was the most suitable for fungi isolation from sugarcane silage. The incidence of fungi was lower in silage treated with bacteria strains UFLA SIL 34 and UFLA SIL 46. The species identified in the silages were *Aspergillus foetidus, A. fumigatus, A. ustus, Gymnascella aurantiaca, Paecilomyces variotii, Penicillium carneum, P. citrinum, P. paxilli, P. roqueforti* and *P. solitum*. The species *Penicillium roqueforti* is quoted silage spoiler and producer of the mycotoxin roquefortine and *P. citrinum* producer of citrinin. The species *P. solitum* and *P. carneum* were detected all sampling. The first is cited as silage spoiler and the second can produce peritrema A, mycophenolic acid and patulin that controls bacterial growth and may affect the development of inocula when the silage is exposed to air. *Aspergillus fumigatus* and *Paecilomyces variotii* are pathogens of humans and animals. Positive correlation

was found between the value of silage pH and the total number of isolated fungi and also between the time of exposure to air and the number of isolates (0.84 and 0.83, respectively). These results allowed us to infer that the fungi growth in silage is directly related to the concentration of acids in silage and also with exposure to air. In practical terms, the silo will be rarely exposed to air for 10 days, but the occurrence of poorly compacted silages, the breaking of the seals and even the opening of the silos for animal feeding allows the entry of oxygen in the silo, which might favor the development of filamentous fungi. It is necessary to protect against air infiltration during storage and feedout process avoiding the aerobic deterioration and, also inhibiting the spreading of microorganisms that produce mycotoxins.

References

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Table 1. pH values, population and number of morphotypes of filamentous fungi isolated from sugarcane silages treated with 14 novel strains of lactic acid bacteria after 126 days of fermentation

Silages	Population (cfu/g silage)[1] / Number of morphotypes[2] of filamentous fungi								
	Time of exposure to air (days)								
	0[3]			5			10		
	pH	DRBC	DG 18	pH	DRBC	DG 18	pH	DRBC	DG 18
Control	3.51	0 / 1	0 / 0	5.31	0 / 1	0 / 1	5.2	5.72 / 1	6.00 / 2
Strain 17	3.54	0 / 2	0 / 0	4.79	0 / 1	0 / 2	4.81	6.30 / 2	6.00 / 2
Strain 19	3.61	0 / 1	0 / 0	3.63	0 / 2	0 / 1	4.92	5.40 / 3	5.79 / 1
Strain 24	3.51	0 / 1	0 / 1	4.68	0 / 1	0 / 1	4.99	6.00 / 2	7.46 / 1
Strain 25	3.51	0 / 0	0 / 0	5.12	0 / 1	0 / 1	4.81	4.95 / 3	0 / 1
Strain 27	3.52	0 / 0	0 / 2	5.30	0 / 1	0 / 1	4.94	6.00 / 2	6.77 / 1
Strain 32	3.61	0 / 0	0 / 1	4.54	0 / 1	0 / 0	5.26	6.30 / 3	0 / 1
Strain 33	3.55	0 / 0	0 / 0	4.74	0 / 1	0 / 1	4.98	5.07 / 3	0 / 1
Strain 34	3.60	0 / 0	0 / 0	3.59	0 / 0	0 / 0	5.17	0 / 1	0 / 1
Strain 35	3.68	0 / 1	0 / 0	3.88	0 / 0	0 / 1	5.13	5.23 / 3	6.00 / 2
Strain 41	3.55	0 / 0	0 / 0	4.10	0 / 0	0 / 0	5.38	8.19 / 3	6.69 / 3
Strain 42	3.61	0 / 1	0 / 0	4.26	0 / 0	0 / 1	5.19	8.00 / 3	6.60 / 2
Strain 46	3.66	0 / 0	0 / 0	3.35	0 / 0	0 / 0	4.11	0 / 1	0 / 1
Strain 51	3.67	0 / 1	0 / 1	4.49	0 / 1	0 / 1	4.83	6.00 / 2	4.69 / 1
Strain 55	3.80	0 / 1	0 / 1	4.53	0 / 0	0 / 1	4.97	4.47 / 3	4.00 / 1

[1]Population quantified by plating surface, [2]number of morphotypes isolated from the plating surface and direct, [3]after 126 days of fermentation