Evaluation of king oyster mushrooms strains (*Pleurotus eryngii*) on selective lignin degradation in wheat straw: **An update**

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WageningenUR, Plant Breeding

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1 Introduction

The king oyster mushroom (*Pleurotus eryngii*) is a delicious mushroom with a large market potential (personnel communication Banken Champignons B.V.). Most fresh products in Europe are imported from the Republic of Korea and an improved production system will help to increase the local production. This also includes the use of varieties with an improved yield and quality. Next to the production of edible mushrooms, this species can also be used to upgrade lignocellulose materials. The king oyster mushroom is a white rot fungus that can degrade lignin selectively in lignocellulosic materials (degradation of lignin while hardly any cellulose is degraded;(Tuyen et al. 2013). This will facilitate the access to the polysaccharide cellulose, a source for the generation of bioenergy and bioplastics. In addition, the selective removal of lignin with a white rot fungus increases the digestibility for ruminants ((Kuijk 2016)) and when for this purpose white rot fungi are used that produce edible mushrooms, the treated substrate is generally considered as safe (GRAS) and thus suitable as an animal feedstock. These arguments also accounts for the shiitake mushroom (*Lentinula edodes*). Shiitake is less produced in the Netherlands but has proven to be an interesting species for selective removal of lignin in lignocellulose biomass ((Kuijk 2016).
Most research done on selective degradation of lignin by white rot fungi has been directed to screening of fungal species and substrate combinations. Hardly any research has been done to evaluate the performance of strains within one species. *Pleurotus eryngii* forms a species-complex of fungi growing on roots and lower stem residues of Apiaceae (umbellifers) plants (Zervakis et al. 2014) and found in many part is the world. The fungal collection of Wageningen UR Plant Breeding (WUR-PB) contains a large number (156) of strains assigned to this complex and a proper genotyping of these strains have not been done so far. The collection also contains a large number of shiitake strains (99) which have been collected in the last 40 years by exchanging strains with other collections, sampling from commercial spawn or gifts from third persons. The genetic variation within this species is also not known well. The project has thus first evaluated the genetic diversity within these two species in order to make a selection that represent the genetic variation for each species. The selection of *P. eryngii* has subsequently been evaluated for the selective degradation of lignin in Miscanthus and in wheat straw. The fibre content of the treated wheat straw has been analysed and the effect of this treatment on the digestibility by ruminants by using a in vitro gas production model (IVGP).
A small sample of 3 *L. edodes* strains have been pre-tested on 2 Miscanthus varieties that differ in lignin content. This is done before we will evaluate a large set of strains on lignin degradation in Miscanthus. A good performance (high lignin degradation in combination with a low cellulose degradation) makes a strain useful for upgrading lignocellulose but might also benefit the production of fruiting bodies. Degradation of lignin enhances the access to polysaccharides and can thus potentially improve also mushroom yield.

2 Materials & Methods

All strains from the *Pleurotus eryngii* (156 strains) and *Lentinula edodes* have been grown on MMP medium as described before (Sonnenberg et al. 1996)and freeze dried mycelium was used to extract DNA as described in Sonnenberg et al, 2016 (submitted). (Sonnenberg et al. 2016). The DNA samples of the *P. eryngii* were subsequently used a low complexity sequencing method. The SNP detected were combined in sequence strings and aligned with CLC Main Workbench version 7.6.2. The alignments were exported as a NWK files and used to generate dendrograms (Archeopteryx version 0.972;(Han and Zmasek 2009)). The *L. edodes* strains were genotypes with AFLP as described in Terashima et al.(Terashima et al. 2002).

Each strain and time point for *P. eryngii* on wheat straw was done in duplicate using plastic trays (http://www.microbox-container.com/) filled with ca. 250 gr wheat straw.

Three different Miscanthus varieties varying in lignin content and one wheat straw variety were used. The fungal treatment of these materials was done as described in Kuijk et al. (2016). Fibre analysis was performed according to the method of Van Soest et al. (1991) (Van Soest et al. 1991)and described in more details in Kuijk et al. (Kuijk 2016).

The effect of fungal pretreatment on the digestibility of wheat straw was tested as described by Kuijk et al 2016 (Kuijk 2016). In short, samples were freeze dried after fungal pretreatment and used in a glass vial filled with a sample of ruminant fluid from a cow and flushed with N2 gas to generate an anaerobic environment. The vials were incubated at 37 oC and gas productions were measured during 72 hours and used to calculate the increase in gas productions by the fungal treatment as a measure for the increase in ruminant digestibility.

3 Results

3.1 Genotyping the WUR-PB collection of *Pleurotus eryngii* species-complex.

Of the 156 strains, 140 generated DNA of sufficient quality to be used for genotyping by a low comoplexity sequence mthodology. This method generated sequences of sufficient quality for 378 SNP (≤ 20% missing data per locus). The collection contains a large set of strains isolated from the wild in Iran (Behnamian et al., 2010) (Behnamian et al. 2010). The genotyping methodology could discriminate strains sampled from different geographic locations within the country. Three groups are seen within the Chamahal Bakhtiari region and two groups within the Kourdistan Province region. One of each of these groups and the commercially cultivated varieties show a close relationship (figure 1). Two wild strains collected in the Chamahal Bakhtiari region fall within the group of commercially grown varieties. This might indicate that the commercial varieties originate from this region or represent escapes from cultivated varieties. One of the Kourdistan group is only found on *Ferula ovina* or *F. haussknechtii* host plant whereas strains from other groups are found on different Umbellifer species (in addition on *Smyrniopsis aucheri*, *Kellusia oderatissima, Ferula assa-foetida, Prangos* ferulacea). A dendrogram of the complete collection of Plant Breeding showed a clear grouping of strains more or less according to what was expected based on data available for these strains. The strains of *P. eryngii* that are used to generate edible mushrooms in Europe and the USA

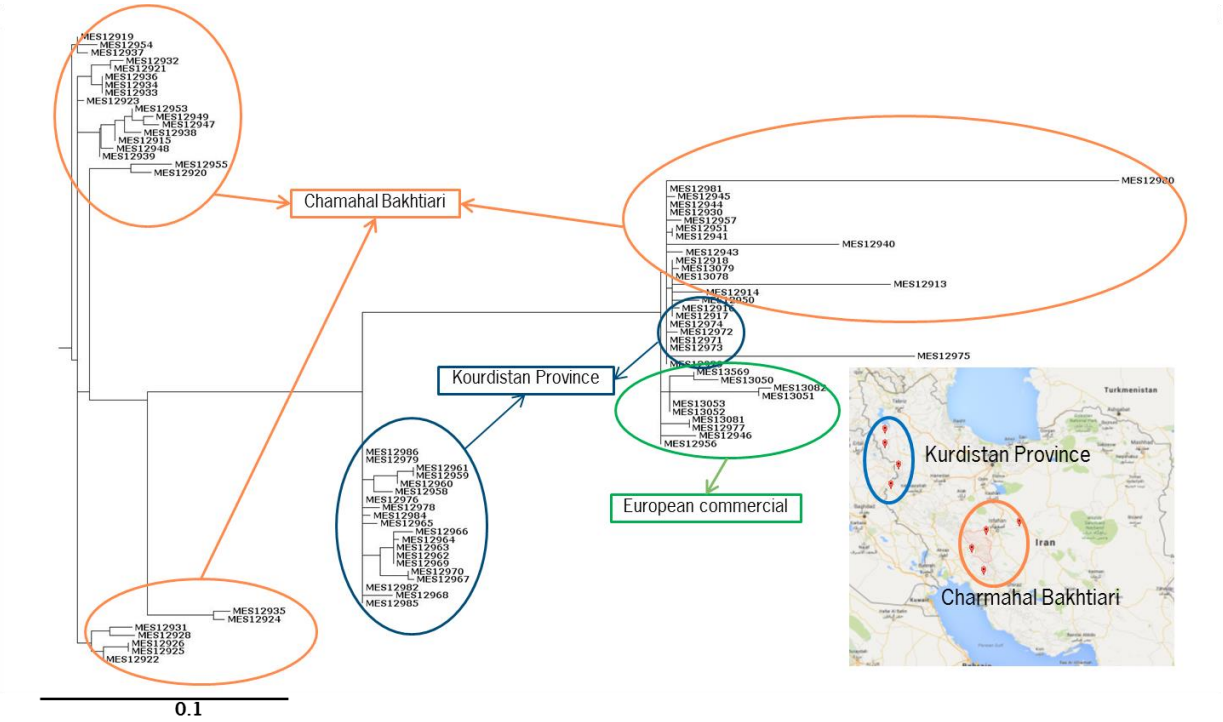

Figure 1. Dedrogram showing the genetic relationship of strains within the species-complex Pleurotus eryngii. Strains were collected in two regions of Iran (Behnamian et al., 2010). Genotyping was done with GBS.

cluster in two groups with a low genetic variation within each group (Figure 2). Although no details are available of their origin, such a clustering was expected since not much breeding is done within the mushroom industry and new varieties are generally not generated by outcrossing but by the screening of multispore cultures. Growers also tend to use the same (and best) variety. The GBS also identified two groups originating from China. Unfortunately, no data on the location of collection are available. One set of these Chinese strains group with a cluster isolated in the Kourdistan region in Iran but are distinct from this group. The other group is representing likely the Chinese *P. eryngii* subsp. *tuoliensis* (Wei Gao, personal communication and Kawai et al, 2008 (Kawai et al. 2008)), a subspecies reported

to have evolved independently in China. Finally, a group was identified collected in Sicily (Italy) and reported to represent possibly *P. eryngii* var. *elaeoselini* (Venturella et al 2000) (Venturella et al. 2000). The genetic variation in the collection shows clearly the potentials for breeding, although an evaluation of variation in useful phenotypes have to be done.

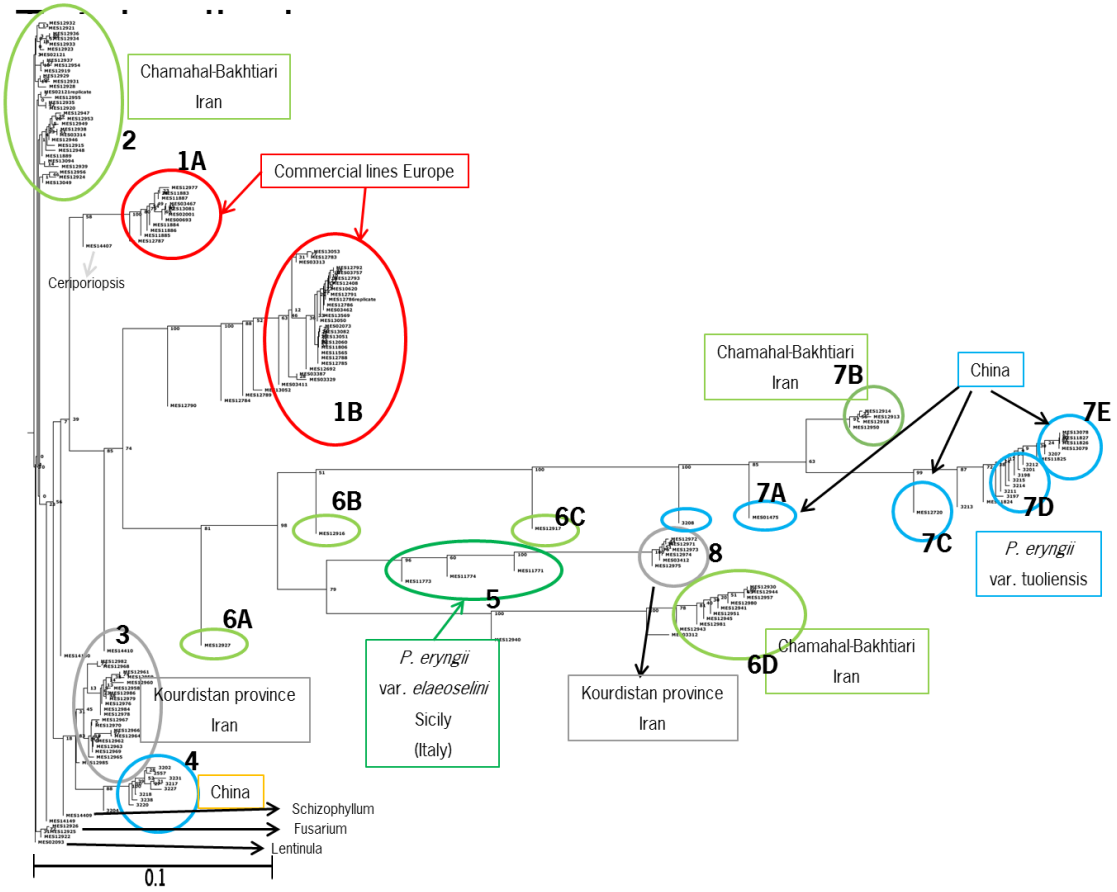

Figure 2. Dedrogram showing the genetic relationship of all strains of the Plant Breeding collection within the species-complex Pleurotus eryngii. Genotyping was done with GBS. Strains collected in the same country or region are encircled in the same colour. The different phylogenetic groups are numbered and from each group 2 strains were selected for selective degradation lignin in wheat straw.

3.2 Genotyping the *L. edodes* collection

Of the 99 strains of *L. edodes* present in the collection, 87 were genotyped. The missing strains did not grow very well and were omitted from genotyping due to the limited time for genotyping. The AFLP analysis revealed a large genetic variation between strains of this species. In most cultivars of edible mushrooms such as *Pleurotus ostreatus (*grey oyster mushroom)*, Pleurotus eryngii* and *Agaricus bisporus* (button mushrooms), commercial lines usually cluster in groups with a low genetic variation. The commercial varieties of shiitake, however, are scattered over all groups indicating a large genetic variation in cultivars. One group stands a bit out of the dendrogram and a ITS sequencing was done. This group also contained one strain as an outgroup (*Pleurotus eryngii*). To verify that these strains were indeed *L. edodes*, an ITS sequencing was done on these strains. Only one strain (MES 02017) appeared to be a different species: *Bjerkandera adusta*. All others are indeed *L. edodes*. This emphasis the large genetic variation of strains within this species.

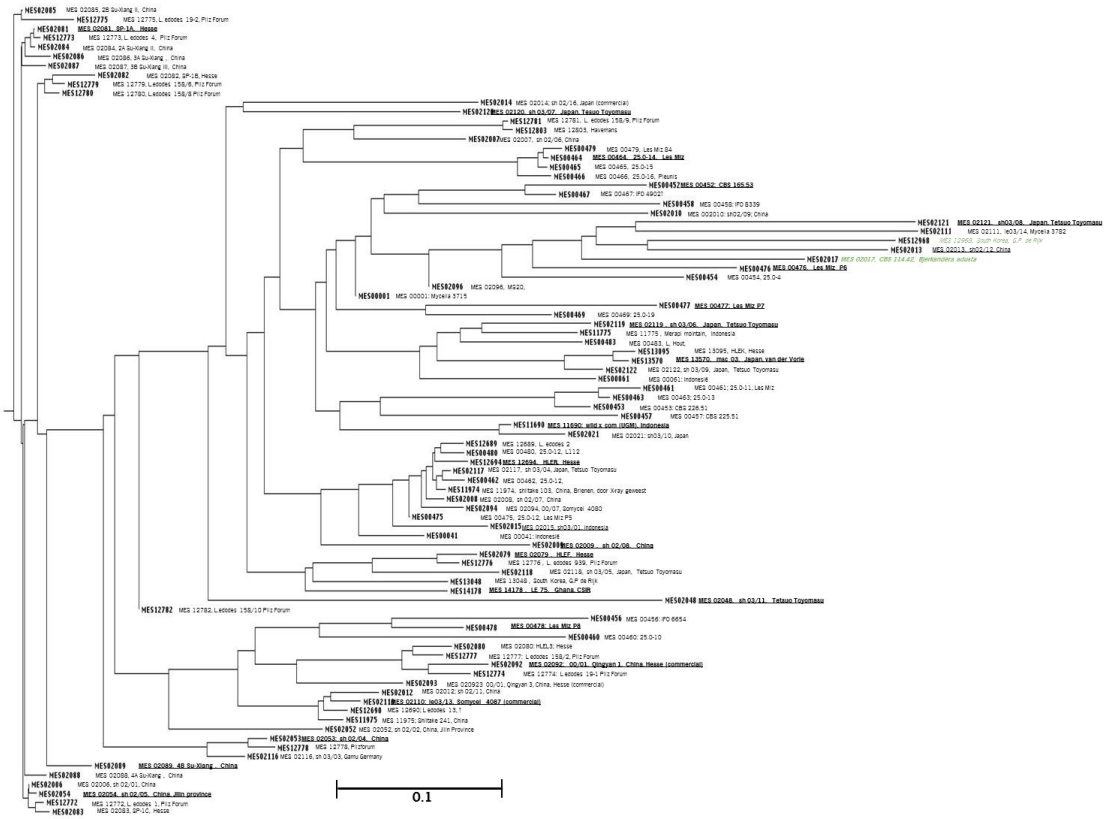

Figure 3. Dendrogram of 87 *Lentinula edodes* strains from the Plant Breeding collection using AFLP. The 2 green strains represent other species (*Pleurotus eryngii* and *Bjerkandera adjusta*).

3.3 Degradation of Miscanthus by two *P. eryngii* strains

In the initial proposal we intended to test a selection of strains of *P. eryngii* on 2 different varieties of Miscanthus with low and high lignin content. In another project, however, it appeared that the strain of *P. eryngii* tested did not grow well on Miscanthus and showed hardly degradation of lignin. As a preliminary test we, therefore, selected 2 strains and analysed the degradation of Miscanthus at two time points, after 4 and 7 weeks. Both strains growth very thin on the substrate and the fibre analysis showed now significant degradation of the different fibres (figure 3). For the subsequent experiments we switch therefore to wheat straw since previous experiments have shown that *P. eryngii* can grow well on this substrate.

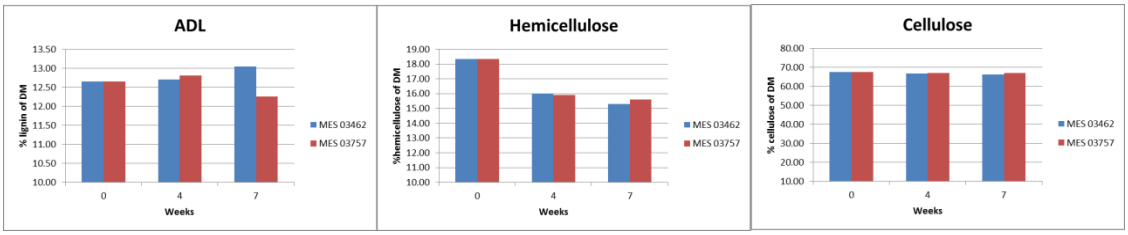

Figure 4. Degradation of fibre components by two *P. eryngii* strains in wheat straw. The fractions of ADL (lignin), hemicellulose (NDF-ADF) and cellulose (ADF-ADL) of the total dry matter are plotted.

3.4 Degradation of wheat straw by a selection of 20 *P. eryngii* strains

After the assessment of the genetic variation of the *P. eryngii* strains within the Plant Breeding collection, a selection was made representing each of the phylogenetic groups (table 1). For each of these 20 strain, six containers (each with ca 250 gram of sterilized wheat straw) was inoculated with spawn. At each time point after inoculation (3, 6 and 9 weeks), 2 containers were freeze dried and used to analyse fibre content (ANKOM based on van Soest et al., 1991). The dry weight of each container was also measured for all samples. These data, however, varied considerably and could not be used to make a good mass balance. The data are thus represented as fractions of dry matter. There are significant differences in fibre content of wheat straw treated with different *P. eryngii* strain. These are particularly evident after 9 weeks of incubation (figure 5). A positive correlation was seen between lignin and hemicellulose content whereas a negative correlation was found between lignin and cellulose content (figure 6). This indicates a selective lignin removal with respect to cellulose and thus an increase in cellulose content after 9 weeks of incubation. This usually gives rise to an crease in digestibility for ruminants (Tuyen et al. 2012)and an improvement in the extend of saccharification with enzymes (Kuijk et al., in preparation). The analysis for digestibility and saccharification are in progress. The best 6 strains (causing the lowest lignin content in wheat straw after 9 weeks) are wild isolates derived from Iran from groups that show a large genetic variation (appendix figure A2). The strains with the lowest performance (high lignin content after 9 weeks incubation) are isolates from the wild from Sicily (Italy) and China, the latter putative var. *tuoliensis*. This latter group of fungi usually need a long time for a complete colonization of substrate and a cold period (14 days at 5-7 °C) before fructification is induced at 15-17 °C (Kawai et al., 2008). The strains with an intermediate performance in lignin degradation are mostly represented by the commercial lines. The differences between the best and worst performing strains are evident (table 2). Statistical analysis are given in the Appendix (Table A1).

Table 1. Selection of strains of the *P. eryngii* collection used for the selective degradation of lignin in wheat straw. The selection was made in such a way that each group (figure 1) is represented.

Strain	Phylogenetic Group
MES 12948	2
MES 12937	2
MES 12962	3
MES 12961	3
MES 11773	5
MES 11774	5
MES 12975	8
MES 12972	8
MES 12787	1A
MES 03467	1A
MES 03757	1B
MES 11565	1B
MES 12927	6A
MES 12917	6C
MES 12980	6D
MES 12943	6D
MES 12913	7B
MES 12720	7C
MES 13078	7E
MES 11825	7E

Strain	ADL	Hemicel.	Cellulose
MES12975	42%	-38%	5%
MES11774	18%	-29%	1%

Table 2. Change in fibre content between the two extremes strains in lignin degradation.

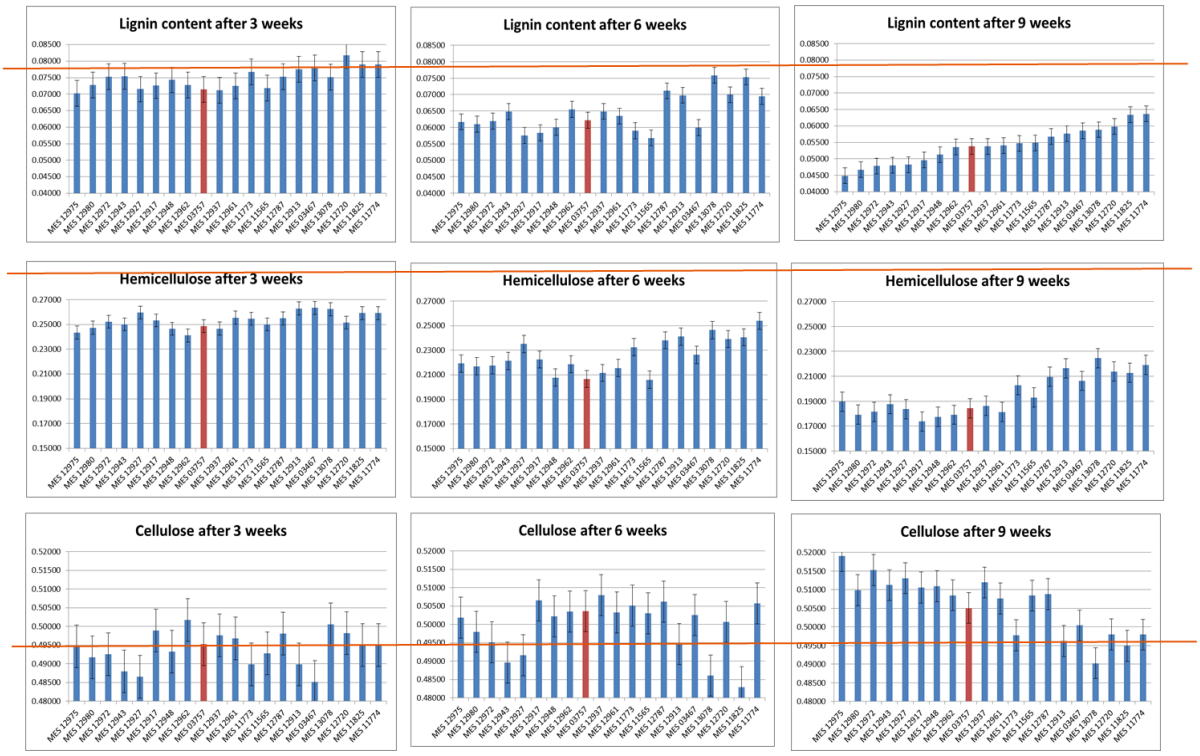

Figure 5. The fibre constitution (fraction of dry weight) of wheat straw incubated for 3, 6 or 9 weeks with 20 different strains of *P. eryngii*. Lignin is expressed as ADL, hemicellulose as NDF-ADF and cellulose as ADF-ADL. The strains are sorted on lignin content after 9 weeks of incubation. The orange line in each graph indicates the content of each fibre in non-inoculated wheat straw. The strain with the red bar represents a strain that is used before in previous work (Tuyen et al., 2012 and 2013).

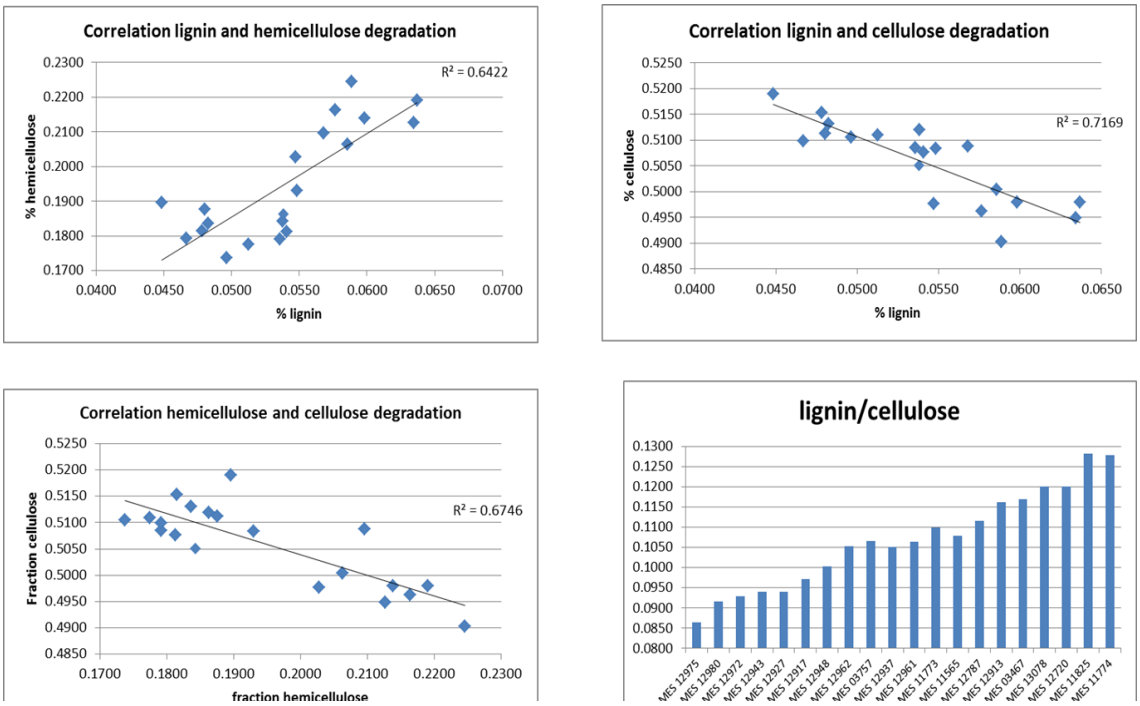

Figure 6. Correlations between changes in content of different fibre fraction after treatment of 9 weeks with 20 different strains of *P. eryngii*. A positive correlation between lignin and hemicellulose content, a negative correlation between lignin and cellulose content and as a result also a negative correlation between hemicellulose and cellulose content. In the last figure (bottom right), strains were sorted on the lignin content of wheat straw after 9 weeks of incubation (as in figure 4). The ratio between lignin and cellulose correlates then almost perfectly

3.5 Influence of fungal pretreatment (*P. eryngii)* on digestibility of wheat straw

To test the influence of the fungal treatment on the digestibility of wheat straw by ruminant, the *in vitro* gas production (IVGP) system was used as described in M&M. A large variation was seen in the

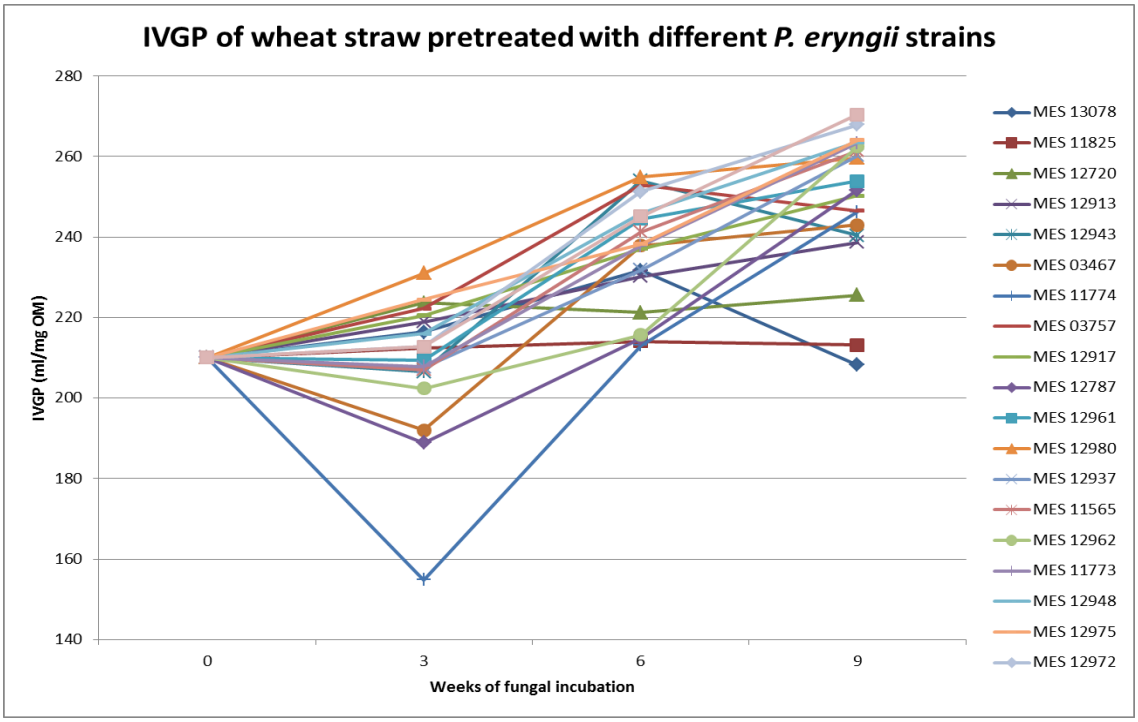

Figure 7. In vitro gas production (accumulation after 72 incubation with ruminant fluid) of fungal treated wheat straw. Samples were incubated for 3, 6 or 9 weeks with 20 different strains of *P. eryngii*.

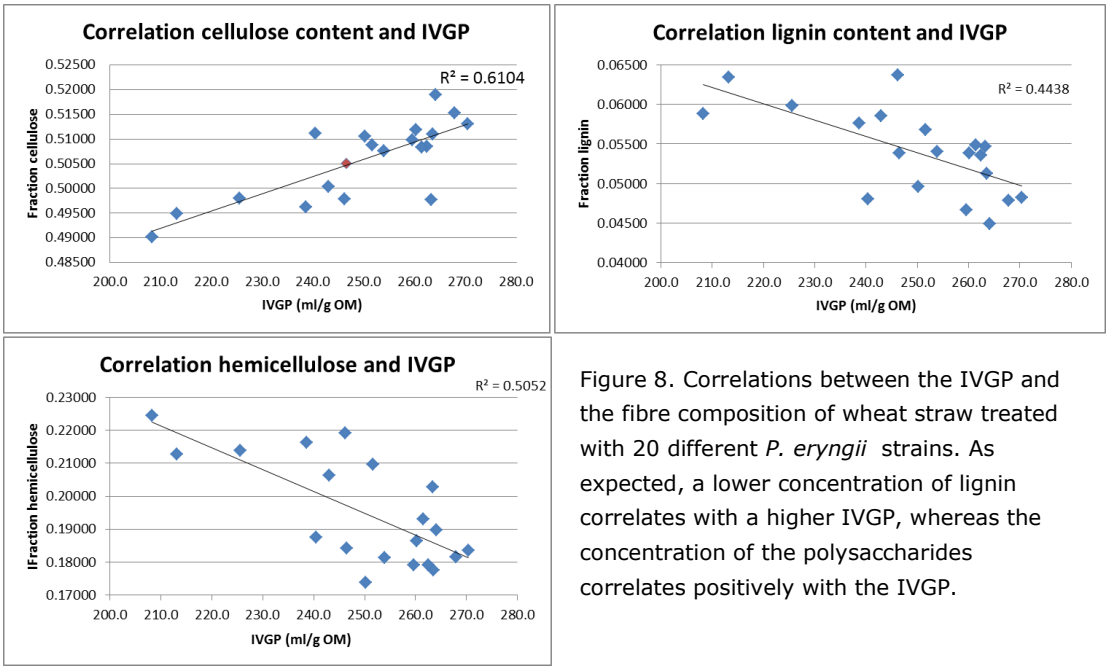

Figure 8. Correlations between the IVGP and the fibre composition of wheat straw treated with 20 different *P. eryngii* strains. As expected, a lower concentration of lignin correlates with a higher IVGP, whereas the concentration of the polysaccharides correlates positively with the IVGP.

gas production after 3, 6 and 9 weeks of fungal treatment. After 9 weeks of pretreatment some strain did not improve gas production at all whereas some increased gas production with almost 30%. The variation in duplicates were large resulting in many treatments not differing significantly (Table 2A). As expected, the differences in gas production correlates with the fibre content of the treated wheat straw. The lignin content of the wheat straw is negatively correlated with the IVGP. That means that removal of lignin enhances the accessibility of the polysaccharides. As expected, we found a positive correlation between the cellulose content and IVGP. The higher the cellulose content the higher is the IVGP. That accounts also for the content of hemicellulose. The pretreatment might have a multiple

effect: removing lignin and making polysaccharides more available, increasing the concentration of polysaccharides and enhancing the degradability of crystalline cellulose. The latter might be the result of the unspecific action of radical molecules generated by the fungus resulting not only in a degradation of lignin but also etching crystalline cellulose enabling endocellulase to attack crystalline cellulose.

3.6 Three Lentinula edodes strains on two Miscanthus varieties

As a preparation on an extended test of shiitake strains on Miscanthus, two Miscanthus varieties were incubated with 3 different *L. edodes* strains. The first variety (E192) has a lignin content of approximately 9.3% and the variety E199 has a lignin content of ca. 7.4%. Incubation periods were 4 and 7 weeks and each treatment carried out in duplicate. As seen with *P. eryngii* on wheat straw, here also a decrease in lignin content accompanies a decrease in hemicellulose and an increase in cellulose content (figure 7). There is a significant decrease in lignin content with all shiitake strains with both Miscanthus varieties after 7 weeks of incubation (Table 3). Although statistical analysis is difficult to perform on samples changing in time, it seems that strain MES 02079 performs less than the other two strains indicating that there is an effect of the genotype of the shiitake strain. The effect of the lignin content in the extend of lignin degradation and enrichment in cellulose seems negligible. It is expected that changes will be larges when the incubation period is extended to 9 weeks (Kuijk 2016).

	Change after 7 weeks incubation		
	ADL	Hemicellulose	Cellulose
Miscanthus E192			
MES 02079	**-21.5%**	**-43.1%**	**0.9%**
MES 02121	**-25.5%**	**-47.4%**	**6.1%**
MES 11910	**-26.1%**	**-43.4%**	**4.2%**
Miscanthus E199			
MES 02079	**-20.1%**	**-44.3%**	**0.9%**
MES 02121	**-31.5%**	**-45.4%**	**5.8%**
MES 11910	**-29.4%**	**-48.0%**	**4.3%**

Table 3. Percentage change in fibre content by 3 different shiitake strains on 2 varieties of Miscanthus.

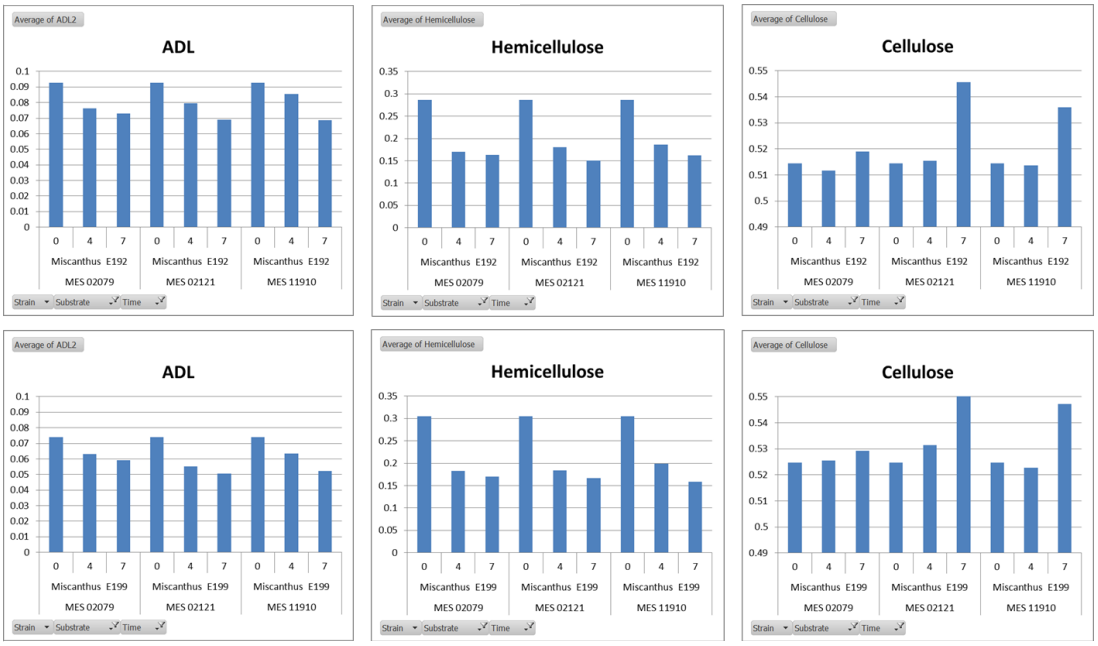

Figure 7. Change in fibre content of two Miscanthus varieties after treatment with three different shiitake strains for 0, 4 and 7 weeks.

4 Conclusion

The strains of *Pleurotus eryngii* and *Lentinula edodes* of the plant breeding collection represent a large genetic variation. The strains of *P. eryngii* cluster in groups that show within each group a limited variation. That is partly due to the sampling in small areas in Iran and due to the fact that the commercial varieties are very similar. The latter is common practise in the mushroom industry where breeding is hardly done and when applied, usually selections are made from multispore cultures that obviously will lead to similar genotypes. In contrast, the genetic variation within the *Lentinula edodes* collection is large. Also the commercial lines used are scattered over the whole dendrogram. This is consistent with the finding that yield, mushroom shape and qualities are also very different between cultivars.

The king oyster mushroom is performing less in selective lignin degradation than other white rot fungi but the genetic variation and the variation in lignin degradation indicates that this species can be improved by breeding. Such breeding program might also improve the utilisation and thus yields in mushroom production. It might also be interesting to grow this species on other lignocellulose materials and see if it performs better on these sources.

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6 Appendix

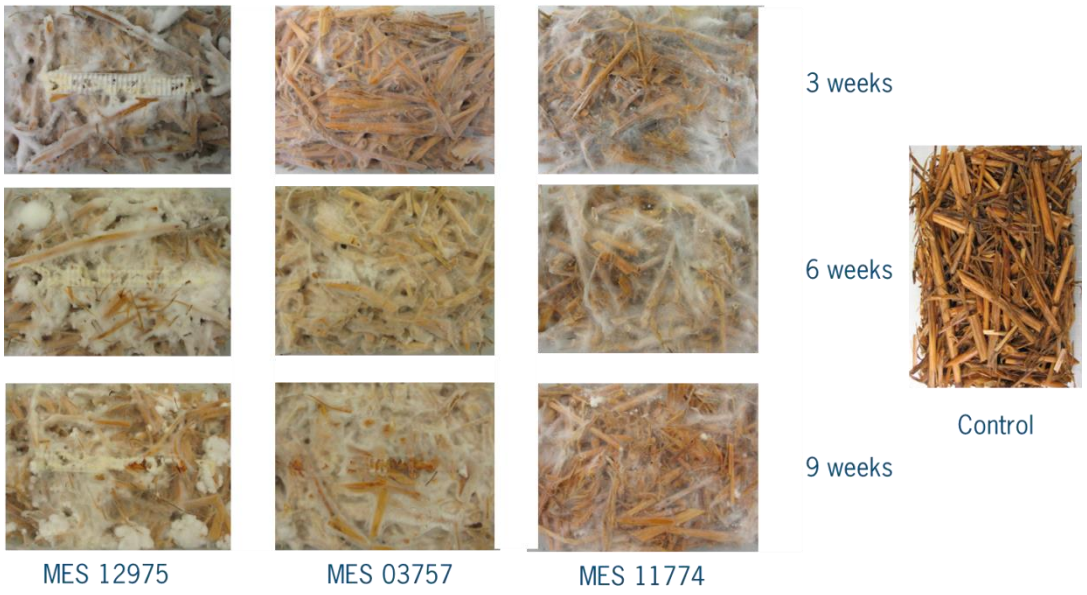

Figure A1. Colonization of wheat straw by 3 different strains of *P. eryngii* on wheats straw. After 9 weeks of incubation strain MES12975 resulted in wheat straw with the lowest lignin content, MES 11774 the highest lignin content and MES 03757 showed an intermediate lignin content in wheat straw.

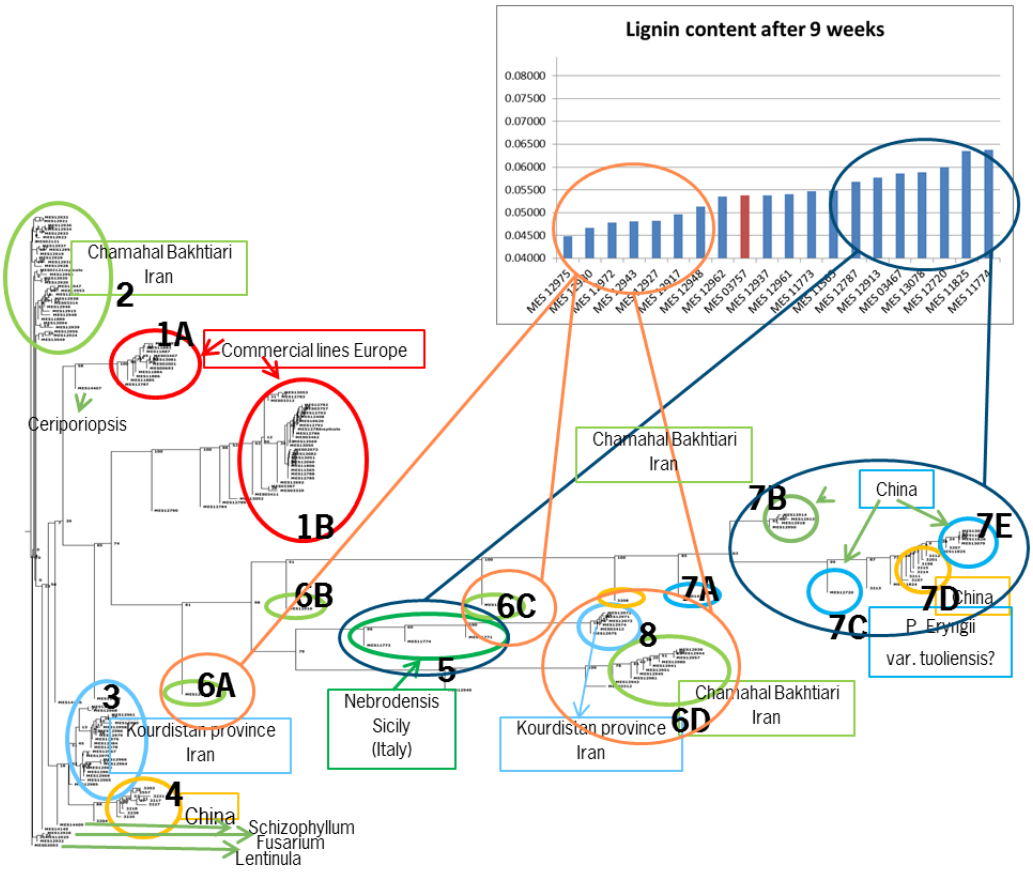

Figure 2A. The 6 strains in reducing lignin content in wheat straw after 9 weeks represent isolates from Iran and show a large genetic variation (orange circles). Strains with a low lignin reduction are nebrodensis and tuolienis varieties. These are strains that take long incubation time for colonisation of substrate and a cold shock before producing fruiting bodies.

Strain	ADL		Strain	Hemicel.		Strain	Cellulose	
MES12975	0.04486	a	MES12917	0.1737	a	MES13078	0.4903	a
MES12980	0.0467	ab	MES12948	0.1775	ab	MES11825	0.4949	ab
MES12972	0.04784	abc	MES12962	0.1791	abc	MES12913	0.4962	abc
MES12943	0.04805	abcd	MES12980	0.1792	abc	MES11773	0.4977	abcd
MES12927	0.04826	abcd	MES12961	0.1813	abc	MES11774	0.4979	abcd
MES12917	0.04963	abcde	MES12972	0.1815	abc	MES12720	0.498	abcd
MES12948	0.05128	abcdef	MES12927	0.1836	abcd	MES03467	0.5004	abcde
MES12962	0.05358	abcdef	MES03757	0.1843	abcd	MES03757	0.5051	abcdef
MES03757	0.0538	abcdef	MES12937	0.1863	abcde	MES12961	0.5076	bcdef
MES12937	0.05382	abcdef	MES12943	0.1876	abcde	MES11565	0.5084	bcdef
MES12961	0.05407	abcdefg	MES12975	0.1896	abcdef	MES12962	0.5085	bcdef
MES11773	0.05472	bcdefgh	MES11565	0.193	abcdef	MES12787	0.5088	bcdef
MES11565	0.05484	bcdefgh	MES11773	0.2028	abcdefg	MES12980	0.5099	bcdef
MES12787	0.0568	cdefgh	MES03467	0.2063	bcdefg	MES12917	0.5105	bcdef
MES12913	0.05764	defgh	MES12787	0.2096	cdefg	MES12948	0.5109	bcdef
MES03467	0.05857	efgh	MES11825	0.2127	defg	MES12943	0.5112	bcdef
MES13078	0.05887	efgh	MES12720	0.2139	defg	MES12937	0.5119	cdef
MES12720	0.05984	fgh	MES12913	0.2164	efg	MES12927	0.5131	def
MES11825	0.06343	gh	MES11774	0.2191	fg	MES12972	0.5153	ef
MES11774	0.0637	h	MES13078	0.2245	g	MES12975	0.519	f

Table A1. Statistical analysis of fibre content. Each strain was incubated in duplicates. Significance of differences are indicated for each fibre fraction in the third column: strains sharing same letters do not differ significantly.

		ADL				Hemicellulose				Cellulose			
Denrogram group	Strain	T=0	T=3	T=6	T=9	T=0	T=3	T=6	T=9	T=0	T=3	T=6	T=9
8	MES 12975	0.07720	0.07026	0.06169	0.04486	0.30653	0.24351	0.21932	0.18963	0.49587	0.49469	0.50182	0.51897
6D	MES 12980	0.07720	0.07272	0.06100	0.04670	0.30653	0.24745	0.21698	0.17919	0.49587	0.49178	0.49799	0.50986
8	MES 12972	0.07720	0.07534	0.06189	0.04784	0.30653	0.25221	0.21770	0.18148	0.49587	0.49260	0.49513	0.51529
6D	MES 12943	0.07720	0.07541	0.06483	0.04805	0.30653	0.25013	0.22141	0.18759	0.49587	0.48797	0.48963	0.51121
6A	MES 12927	0.07720	0.07152	0.05754	0.04826	0.30653	0.25965	0.23513	0.18361	0.49587	0.48653	0.49159	0.51308
6C	MES 12917	0.07720	0.07258	0.05835	0.04963	0.30653	0.25325	0.22259	0.17373	0.49587	0.49891	0.50658	0.51053
2	MES 12948	0.07720	0.07430	0.06007	0.05128	0.30653	0.24652	0.20785	0.17749	0.49587	0.49329	0.50220	0.51094
3	MES 12962	0.07720	0.07272	0.06552	0.05358	0.30653	0.24119	0.21868	0.17915	0.49587	0.50169	0.50352	0.50849
1B	MES 03757	0.07720	0.07147	0.06218	0.05380	0.30653	0.24864	0.20668	0.18428	0.49587	0.49528	0.50361	0.50508
2	MES 12937	0.07720	0.07113	0.06488	0.05382	0.30653	0.24668	0.21165	0.18634	0.49587	0.49757	0.50801	0.51191
3	MES 12961	0.07720	0.07251	0.06345	0.05407	0.30653	0.25552	0.21565	0.18132	0.49587	0.49683	0.50326	0.50762
5	MES 11773	0.07720	0.07678	0.05901	0.05472	0.30653	0.25475	0.23255	0.20276	0.49587	0.48983	0.50509	0.49769
1B	MES 11565	0.07720	0.07184	0.05679	0.05484	0.30653	0.25012	0.20610	0.19299	0.49587	0.49275	0.50303	0.50838
1A	MES 12787	0.07720	0.07533	0.07114	0.05680	0.30653	0.25496	0.23814	0.20964	0.49587	0.49810	0.50618	0.50881
7B	MES 12913	0.07720	0.07756	0.06977	0.05764	0.30653	0.26299	0.24114	0.21639	0.49587	0.48990	0.49470	0.49619
1A	MES 03467	0.07720	0.07794	0.05991	0.05857	0.30653	0.26341	0.22638	0.20634	0.49587	0.48520	0.50258	0.50038
7E	MES 13078	0.07720	0.07514	0.07588	0.05887	0.30653	0.26236	0.24641	0.22455	0.49587	0.50059	0.48613	0.49025
7C	MES 12720	0.07720	0.08172	0.06995	0.05984	0.30653	0.25150	0.23918	0.21386	0.49587	0.49815	0.50072	0.49796
7E	MES 11825	0.07720	0.07899	0.07535	0.06343	0.30653	0.25917	0.24054	0.21273	0.49587	0.49505	0.48292	0.49488
5	MES 11774	0.07720	0.07899	0.06944	0.06370	0.30653	0.25917	0.25403	0.21909	0.49587	0.49505	0.50570	0.49791

Table A1. Fibre analysis of wheat straw samples after treatment with different strains of *P. eryngii*. Data represent percentage of each fraction of the dry weight of wheat straw. Hemicellulose is expressed as NDF-ADF and cellulose as ADF-ADL.

untreated	210	
	Mean	
MES 13078	208.2	a
MES 11825	213.2	ab
MES 12720	225.5	abc
MES 12913	238.6	abcd
MES 12943	240.4	abcd
MES 03467	243	bcd
MES 11774	246.2	bcd
MES 03757	246.5	cd
MES 12917	250.2	cd
MES 12787	251.6	cd
MES 12961	253.9	cd
MES 12980	259.6	cd
MES 12937	260.2	cd
MES 11565	261.5	cd
MES 12962	262.5	cd
MES 11773	263.3	cd
MES 12948	263.6	cd
MES 12975	264.1	cd
MES 12972	267.9	d
MES 12927	270.4	d

Table A2. Statistical analysis of IVGP ("Mean" after 9 weeks of incubation) in the model by different *P. eryngii* strains (MES numbers). Due to a large variation in duplicates, most treatments do not vary significantly.

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