Fungal Occurrence on Sugarcane Filter Cake and Bagasse Isolated From Sugar Refineries in Thailand

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Abstract

This study assessed the distribution and species occurrence of microscopic fungi isolated from two types of self-heating lignocellulosic materials: (1) sugarcane filter cakes (SFC) and (2) sugarcane bagasse (SBG). Three sugar mill refineries in Thailand were chosen: the Eastern Sugar & Cane Co., Ltd. (Site I), the T. N. Sugar Industry Co., Ltd. (Site II), and the Buri Ram Sugar Factory Co., Ltd. (Site III). We isolated 79 strains, which represented 23 species in 11 genera. Eight *Aspergillus* species (37.8% of all species) were identified and within the mycoflora this genus was predominant. The most frequently encountered taxa, which represented either the most dominant fungi or the overlapping species on the two types of lignocellulosic materials (>10%), were *A. fumigatus* (35.4%), *Paecilomyces variotii* (13.9%), and *A. terreus* (12.6%). These taxa correlated with the temperature of the collected samples. Fungal abundance was highest on SBG from Site II (10 species), and the species diversity (1.90) and evenness (0.60) were the greatest on both SFC and SBG from Site II with the highest moisture content. Hence, the fungal diversity correlated with the humidity of the collected lignocellulosic samples. The lowest diversity was observed on SFC from Site III (1 species). A comparison of the fungal communities on the two lignocellulosic materials and at the three selected sites revealed few differences in species composition, and the fungi found in this study are discussed in relation to published data.

Keywords: sugarcane press mud, sugarcane bagasse, sugar mill, fungal diversity

Introduction

Although several studies have been conducted on sugarcane filter cake (SFC) and bagasse (SBG) as lignocellulosic materials, these studies investigated these materials largely for biotechnological and agricultural purposes (Senthil and Das, 2004; Kumar et al., 2010; Sørensen et al., 2011). The fungal biodegradation of lignocellulosic materials has been studied extensively in several countries (Abdel-Sater and El-Said, 2001; Li et al., 2002; Chauhan et al., 2007; Dong et al., 2013; Boonyuen et al., 2014). However, our understanding of the distribution and diversity of the fungi that originate from piles of self-heating lignocellulosic material (i.e. from SFC and SBG) after the sugar production

process in Thailand is limited. In contrast, several studies of SFC and SBG have been performed in Mexico, Brazil, and India (Sandhu el al., 1980; Cortés-Espinosa et al., 2006; Basso et al., 2010; Naik et al., 2012; Pandey et al., 2013).

Therefore, the general purpose of this study was to assess the occurrence and distribution of fungi on both lignocellulosic materials to better understand the fungal diversity in selected sugar mill refineries in Thailand. The specific objectives of this study were: (i) to isolate fungi from SFC and SBG at three selected sugar mill refineries in Thailand; (ii) to compare the species (i.e., dominant, common, and overlapping species) isolated from these two lignocellulosic materials at the three sites; and (iii) to assess fungal abundance and fungal diversity.

Materials and Methods

Collection Sites

SFC and SBG were randomly collected from piles at three selected sugar factories in Thailand. These sites were designated as Sites I, II and III, as follows: (I) the Eastern Sugar & Cane Co., Ltd. (ESC) in Sa Kaeo Province in eastern Thailand (N 13°77', E 102°19', 7 m in elevation); (II) T. N. Sugar Industry Co., Ltd. in Lop Buri Province in central Thailand (N 15°22', E 100°27', 89 m in elevation); and (III) Buri Ram Sugar Factory Co., Ltd. in Buri Ram Province in northeastern Thailand (N 15°22', E 103°23', 174 m in elevation).

Sample Collection

At each collection site, 500 g of approximately 2- to 12-month-old SFC and SBG that remained after the sugar mill's refining process (Figure 1) were collected in sterilized polyethylene bags during March 19-23, 2012. The samples were returned to the laboratory at the National Center for Genetic Engineering and Biotechnology (BIOTEC) for isolation of fungi. Temperatures, pH, and moisture levels in the SFC and SBG are presented and compared with the values reported in previous publications in Table 1. SFC is produced by drum filters and represent the residue of sugarcane juice after filtration. SFC is high in organic matter, phosphorus, and moisture. During the clarification process, the juice is separated at the top and then removed. The SFC is then filtered to separate the suspended matter, which includes insoluble salts and fine bagasse (SBG). SFC is commonly used as a soil conditioner, soil fertilizer, compacting agent, foaming agent, and as animal feed (Van der Poel et al., 1998), and it is also used as a fuel at some sugar factories (George et al., 2010). In contrast, SBG is sugarcane fiber, which accounts for 9-18% of the stalk weight in commercial varieties. The SBG is widely used in compost, for manufacturing paperboard, and as a fuel source to supply electricity and steam for mill boilers and consumers (Meunchang et al., 2005; Dos Santos et al., 2014; Lopes Silva et al., 2014). The chemical composition of both SFC and SBG varies depending on the

Table 1 The temperature, pH, and moisture content of sugarcane filter cake and sugarcane bagasse collected from the three sites in Thailand.

Parameter	SFC[1/]				SBG[1/]				[3/]Prado et al. (2013)[4/]	[3/]Meunchang et al. (2005)[5/]	[3/]Horecký and Saska (2010)[6/]	[3/]Patil et al. (2013)[7/]
	Site I	Site II	Site III	Average[2/]	Site I	Site II	Site III	Average[2/]				
Temperature (°C)	42.34	48.65	47.21	46.06	34.00	36.02	38.07	36.03	—	—	—	—
pH	5.26	7.12	6.29	6.22	4.21	6.85	5.54	5.53	8.2 in SFC	7.7 in SFC; 4.1 in SB	—	4-5 in SFC
Moisture (%)	42.07	62.47	54.85	53.13	22.17	21.05	25.27	22.83	—	—	64 in SFC	50-65 in SFC

[1/] Site I = (1) the Eastern Sugar & Cane Co., Ltd; Site II = (2) T. N. Sugar Industry Co., Ltd; Site III = (3) Buri Ram Sugar Factory Co., Ltd.;
[2/] Data from the present study. Each value represents the average of three replicate samples.
[3/] Data are compared to previous reports from different authors, — = Not determined.
[4/] Report on agricultural use of SFC, no fungi isolated.
[5/] Report on co-composting between SFC and SBG, no fungi isolated.
[6/] Report on thermophilic anaerobic digestion of SFC, no fungi isolated.
[7/] Report on for thermophilic anaerobic digestion of SFC with fungal isolation.

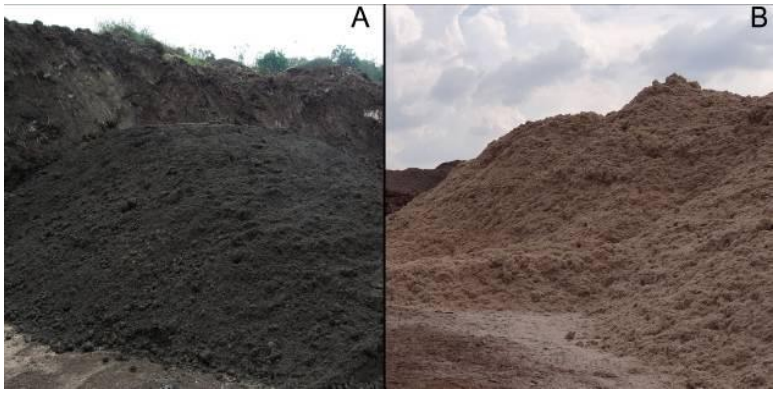

Figure 1 Piles of SFC (1A) and SBG (1B) at Site I.

quality of processing, the variety of sugarcane, the maturation stage of the sugarcane, the type of soil, the procedure used for juice clarification, and the age of the SFC and SBG (Prado et al., 2013).

Fungal Isolation

The dilution plate method outlined by Piasai and Manoch (2009) was used to isolate fungi. A total of 1 g of each SFC and SBG sample was mixed aseptically in 9 mL of sterilized distilled water and shaken vigorously. Appropriate serial dilutions were prepared and 0.1 mL of the diluent was transferred to sterilized Petri plates containing potato dextrose agar (PDA) and Czapek agar (CZA) with 50 mg per liter of Rose Bengal and three antibiotics (i.e., 50 mg of streptomycin per liter, 50 mg of penicillin per liter, and 50 mg of ampicillin per liter). Triplicates of each sample were incubated at 25±2^{o}C for 7 days and examined daily. After incubation, the fungal isolates were examined on the plates under a compound microscope.

Fungal Identification

Morphological identification was performed on the basis of colony characteristics such as diameter, color, and texture on 6 different culture media (creatine sucrose agar (CREA), Czapek yeast extract agar (CYA), CZA, yeast extract sucrose agar (YES), malt extract agar (MEA) and Sabouraud dextrose agar (SDA)). Microscopic characteristics were assessed on slide cultures using the guidelines from previous publications as identification keys (Raper and Fennell, 1965; Pitt, 1979; Ramirez, 1982; Samson et al., 2010). Pure cultures were maintained in the BIOTEC Culture Collection. Voucher slides (i.e., herbarium materials) were deposited at the BIOTEC Bangkok Herbarium.

Statistical Analyses

The percent abundance for each taxon was calculated according to the method proposed by Pinruan et al. (2007) using the following formula:

$$\text{Percentage abundance of taxon A} = \frac{\text{Occurrence of taxon A}}{\text{Occurrence of all taxon}} \times 100$$

The diversity of fungi, as measured using the Shannon-Weaver (*H)* and Evenness (*J*) indices, was assessed using the "Species diversity and richness" computer program, version 2.2 (Henderson and Seaby, 1998).

Results and Discussion

Diversity of Fungi on Two Different Lignocelluloses at Sugar Refineries

The samples yielded 79 fungal occurrences from 23 species in 11 genera (Table 2). All fungal isolates were also confirmed by comparing ITS rDNA and partial β-tubulin sequences (data not shown). The isolates represent 8 *Aspergillus* species (34.78% of all species), three *Curvularia* species (13.04%), two *Penicillium* species (8.69%), two *Humicola* species (8.69%), two *Trichoderma* species (8.69%) and one species (4.34%) in each of the following genera: *Chaetomidium*, *Paecilomyces*, *Cladosporium*, *Mucor*, *Neosartorya* and *Talaromyces*. All fungal isolates were separated into pure cultures, including

Table 2 Percent occurrence (OP) of different fungal species isolated from sugarcane filter cake and sugarcane bagasse obtained from three sugar mill sites in Thailand.

Fungus	Number of isolates	Percent occurrence[1/]								
		SFC[2/]			Overall percent occurrence on SFC	SBG[2/]			Overall percent occurrence on SBG	OP[3/]
		Site I	Site II	Site III		Site I	Site II	Site III		
Aspergillus fischerianus	1	-	-	-	-	-	-	6.6	2.6	1.3
A. flavus	7	23	4.7	-	9.7	13.3	-	6.6	7.8	8.8
A. fumigatus	28	-	9.5	100	21.9*	33.3	12.5	86.6	50*	35.4*
A. nidulans	2	-	-	-	-	13.3	-	-	5.2	2.5
A. niger	1	-	-	-	-	6.6	-	-	2.6	1.3
A. quadrilineatus	1	-	-	-	-	6.6	-	-	2.6	1.3
A. terreus	10	46	-	-	14.6*	26.6	-	-	10.5*	12.6*
A. tubingensis	1	7.7	-	-	2.4	-	-	-	-	1.3
Chaetomidium sp.1	1	7.7	-	-	2.4	-	-	-	-	1.3
Cladosporium cladosporioides	2	15.4	-	-	4.8	-	-	-	-	2.5
Curvularia clavata	1	-	-	-	-	-	12.5	-	2.6	1.3
C. eragrostidis	1	-	-	-	-	-	12.5	-	2.6	1.3
C. lunata	1	-	-	-	-	-	12.5	-	2.6	1.3
Humicola sp.1	1	-	4.7	-	2.4	-	-	-	-	1.3
Humicola sp.2	1	-	4.7	-	2.4	-	-	-	-	1.3
Mucor sp.1	1	-	4.7	-	2.4	-	-	-	-	1.3
Neosartorya ferenczii	2	-	9.5	-	4.8	-	-	-	-	2.5
Penicillium citrinum	1	-	-	-	-	-	12.5	-	2.6	1.3
P. glabrum	1	-	-	-	-	-	12.5	-	2.6	1.3
Paecilomyces variotii	11	-	42.8	-	21.9*	-	25	-	5.2	13.9*
Talaromyces spectabilis-like sp.	1	-	4.7	-	2.4	-	-	-	-	1.3
Trichoderma virens	2	-	9.5	-	4.8	-	-	-	-	2.5
T. viride	1	-	4.7	-	2.4	-	-	-	-	1.3
Total no. of species at each site	#	5	10	1	#	6	7	3	#	#
Total no. of fungal isolates at each site	#	13	21	7	#	15	8	15	#	#
Total no. of species on each lignocellulose	#		14		#		13		#	#
Total no. of fungal isolates on each lignocellulose	#	41	#	38	#	#		6.6	2.6	1.3
Shannon-Weaver (*H*)	#	1.37	1.90	0	#	1.61	1.90	6.6	7.8	8.8
Evenness (*J*)	#	0.44	0.60	0	#	0.51	0.60	86.6	50*	35.4*
Total genera in the study	#					11				
Total species in the study	#					23				
Total no. of fungal isolates	#					79				

[1/] Asterisk (*) = The most dominant species found on each or both substrates (with >10% abundance); Bold = Overall percent occurrence on both lignocelluloses (OP).
[2/] Site I = (1) the Eastern Sugar & Cane Co., Ltd; Site II = (2) T. N. Sugar Industry Co., Ltd; Site III = (3) Buri Ram Sugar Factory Co., Ltd.
[3/] OP: Overall percent occurrence including both lignocellulosic materials;# = Data not presented;- = No fungal occurrence.
[6/] Report on thermophilic anaerobic digestion of SFC, no fungi isolated.
[7/] Report on for thermophilic anaerobic digestion of SFC with fungal isolation.

41 isolates (51.89%) from SFC and 38 isolates (48.10%) from SBG. The average percent occurrence values for the recorded fungi ranged from 2.4-21.9% for the SFC and 2.6-50% for the SBG.

The three most dominant species found on both lignocellulosic substrates were *A. fumigatus* (28 isolates), *P. variotii* (11 isolates) and *A. terreus* (10 isolates), with overall percent occurrence values of 35.4, 13.9 and 12.6%, respectively. In contrast, fifteen species (65.21% of the total species) were represented by only one record (i.e., *A. fischerianus*) and can be considered infrequent. Among the fungal

isolates from SFC, 14 species from 9 genera were isolated, with the most common being *P. variotii*, (21.9%), *A. fumigatus* (21.9%), *A. terreus* (14.6%). Among the fungal isolates from SBG, 13 species from 4 genera were isolated, with the most predominant taxa being *A. fumigatus* (50%) and *P. variotii* (10.5%).

SFC residues from Site II supported the highest number of fungi (10 species), whereas those from Site III supported the lowest number of fungi (one species) (Table 2). On SFC residues from Site II, isolates of *P. variotii* were the most dominant and frequently isolated (42.8%), followed by *A. fumigatus* (9.5%), *Neosartorya ferenczii* (9.5%) and *Trichoderma virens* (9.5%). On SBG residues from Site II, *P. variotii* was the most dominant and frequently isolated taxon (25%). The *H* and *J* indices were highest for SFC materials from Site II (*H*=1.90 and *J*=0.60) and for SBG materials from Site II (*H*=1.90 and *J*=0.60). The lowest indices were observed for SFC materials from Site III. *Aspergillus fumigatus* AG85 was identified as the most dominant representative species found in this study based on macroscopic and microscopic features and this species was cultured on 6 synthetic media at 25°C for 7 days to study fungal features and colony morphology (Figure 2).

This study reports the initial record of the fungal communities associated with two self-heating lignocellulosic materials at three sugar mills and is the first comparison of fungal diversity between these substrates that originated from sugarcane in Thailand. *Aspergillus* (8 species) was frequently isolated and was the predominant genus. The incidence of occurrence of all other genera/species varied among the samples and micro-locations. Additionally, the species of *Aspergillus* found in this study are more common in warm climates (i.e., not only in Thailand, but also in other tropical countries) and several of these species are more thermotolerant than other species (Klich, 2002). In contrast, some of the observed species are considered to be rare in the study environment. For example, *Penicillium* species were less abundant (1.3%). Although *Penicillium* spores are likely to be found dispersed in the air in all environments, these species are more common in regions where low temperatures prevail (Domsch et al., 2007).

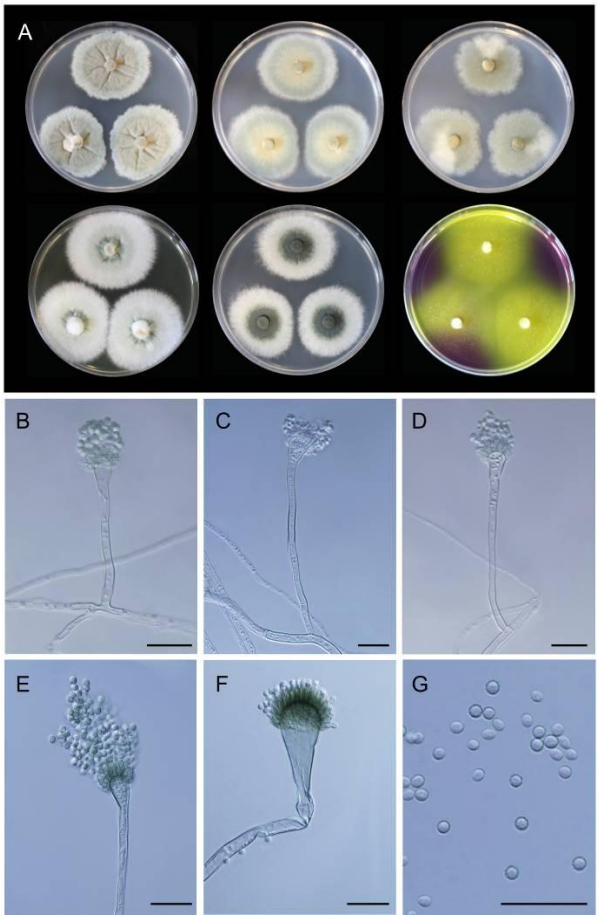

Figure 2 The most common species, *Aspergillus fumigatus* AG85, found in SFC and SBG. A. Colonies after incubation for 7 days at 25°C on 6 types of media (from left to right, all obverse, CYA, MEA, CZA; second row, YES, SDA, CREA). B–F. Conidiophores. G. Conidia. Scale bars = 20 µm.

Some species were considered rare taxa occurring at frequentcies from 1.3-2.5%, such as *Aspergillus nidulans*, *A. niger*, *Cladosporium cladosporioides*, *Penicillium citrinum, Trichoderma viride* and *T. virens*. This finding can be explained by the fact that these fungi were recorded in compost and self-heating organic materials at mesophilic temperatures, according to the review by Ryckeboer et al. (2003). However, some fungi (e.g., *Humicola* spp.) are considered rare taxa, but were found at both thermophilic and mesophilic temperatures. This fungus was found on alkaline soil and produces some chemicals that are antagonistic to fungal plant pathogens and to fungi reported as human pathogens (Manoch, 2012). The results from the present study showed that the *Humicola* spp. found on SFC at Site II (Table 2)

correlated with the alkaline soil with a pH of 7.12 (Table 1). Some fungi reported here, including *A. niger, A. terreus, A. fumigatus, P. glabrum* and *Cladosporium cladosporoides,* were similar to those reported by Cortés-Espinosa et al. (2006), who studied the selection, identification, and application of fungi isolated from SBG in Mexico. In addition to those fungi reported from SBG and SFC, Anastasi et al. (2005) isolated these fungi from compost and vermicompost. For other samples, *A. flavus, A. terreus P. variotii* and *A. niger* were found as fungal contaminants on acid preserved cereals, species, and rye bread. These fungi are cosmopolitan, saprotrophic and plant parasitic species (Manoch and Piasai, 2012).

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Not surprisingly, the overlapping fungi observed in this study are not different from those observed in previous studies (Table 3). This result suggests that the identified fungal species may depend on the sample origin (SFC and SBG), but are not related necessarily to differences in geographical locations and countries (Table 3). Similar findings were reported from Egypt (Sherief et al., 2010), which concluded that the genus *Aspergillus* (*A. terreus, A. flavus, A. ornatus, A. niger, A. wentii, A. orchraceus, A. viridi-nutans* and *A. fumigatus*) was the most frequently observed genus. Other species, including cellulolytic enzyme-producing *Trichoderma viride* and *Penicillium chrysogenum,* were also recorded, however, this work was performed using rice straw for bio-ethanol production and these differences were likely due to differences in the chemical composition of the substrates (rice straw versus sugarcane).

The present study also found that three rare species of SBG-originating fungi, *Curvularia clavata, C. eragrostidis*, and *C. lunata*, were found only at Site II on SBG and were less abundant. Nonetheless, these fungi were not found in SFC. Based on the review by Ryckeboer et al. (2003), *Curvularia* spp. are considered to be mesophilic fungi. The results of the present study support his conclusion as the fungi were found only on SBG at Site II, where the average temperature was 36.02°C, whereas the average temperature of the SFC at Site II was 48.65°C (Table 1).

Only two isolates of *Neosartorya fischeri* were found in the present study, both from Site III on SBG. In Thailand, this species was previously reported as a soil fungus that produces new metabolites (aszonalenin and meroditerpene) (Eamvijarn et al., 2013). Additionally, *Talaromyces spectabilis* was also considered to be a rare taxon. This species is able to spoil either several foods or feedstuffs and is often found in heat-treated products (Udagawa and Suzuki, 1994; Houbraken et al., 2008) and only 1 isolate identified tentatively as a *T. spectabilis*–like sp. was recovered from SFC in Site II. This fungus demonstrated that the fungal origin may correlate with the temperature of the collected SFC samples. Although several factors have affected the diversity and abundance of fungi

Table 3 Fungal taxa isolated from sugarcane filter cake and sugarcane bagasse in published studies.

Place and reference	Lignocellulosic materials	Habitat origin	Fungal species
Cortés-Espinosa et al. (2006)	SBG	México	*Aspergillus terreus, A .fumigatus, A. niger, Penicillium glabrum,* and *Cladosporium cladosporioides*
Basso et al. (2010)	SBG	Brazil	*Acremonium cellulolyticus, Aspergillus fumigatus, Moniliophthora perniciosa, Paecilomyces variotii, Penicillium verruculosum,* and *Trichoderma* sp.
Naik et al. (2012)	SBG	Southern India	*Trichoderma* spp. and *Coprinus* spp.
Sandhu el al. (1980)	SBG	Northern India	*Acrophialophora fusispora, Aspergillus flavus, A. fumigatus, A. caespitosus, A. candidus, A. nidulans, A. nidulans* var. *echinulatus, A. niger, A. oryzae, A. terreus, A. terreus* var. *aureus, Absidia corymbifera, Absidia spinosa, Humicola grisea* var. *thermoidea, Mucor pusillus, Paecilomyces variotii, Penicillium* sp., *Phialophora* sp., *Sporotrichum thermophile*, and *Thermomyces lanuginosus*
Pandey et al. (2013)	SFC	India	*Curvularia lunata, C. robusta, Chaetomium globosum,* and *Fusarium oxysporum*
Patil et al. (2013)	SFC	India	*Aspergillus niger, A. awamori, Penicillium crysogenum,* and *Tricoderma viridiae*

in our study, the moisture content of the fungal sample may influence their distribution and diversity. The greater species diversity, richness and evenness, especially at Site II, is generally thought to indicate a broader fungal community. Indeed, Site II, with high humidity, showed a greater number of fungal species and fungi on SFC than the other sites. In addition, the temperature and other characteristics including the pH and type of nutrients and organic content in each substrate type (data not shown) are likely to influence the fungal populations, fungal number, fungal species, and the fungal diversity.

Fungi Found on Either Both Lignocellulosic Materials or From Multiple Sites

Ten species (43.47%) were found only on SFC and 9 species (39.13%) were found exclusively on SBG, whereas four fungi (i.e., *A. flavus, A. fumigatus, A. terreus* and *P. variotii)* were common to both lignocellulosic materials. On SFC, 4 and 8 fungi were found exclusively at Sites I (28.57%) and II (57.14%), respectively. For mycoflora obtained from SFC only *A. flavus* (7.14%), occurred at both Sites I and II and only *A. fumigates* (7.14%) occurred at both Sites II and III. In contrast, four species from SBG (30.76%) were found only at Sites I, 6 (46.15%) only at Site II, and one only at Site III (7.69%). A single overlapping fungal species was obtained from SBG at Sites II and III. *A. flavus* (7.69%). In addition, a single species, *A. fumigates* (7.69%) was common to all sites (Figure 4).

Comparison of the Three Most Common Fungi from Two Lignocellulosic Materials in Thailand to Those from Published Studies

Except for SFC from Site I, *A. fumigatus* was the most frequently recovered species from both SFC and SBG in the current study. This species is considered a ubiquitous airborne saprophyte and is typically found in soil and decaying organic matter, such as waste substrates, compost heaps, and lignocellulose residues, where it plays an essential role in carbon and nitrogen recycling (Anastasi et al., 2002). In addition, this species is also an opportunistic causal agent of invasive and allergic aspergillosis in both humans and animals and was frequently reported in the previous studies (Rhodes,

2006; Pitt, 1994). We isolated fungal isolates of *A. fumigatus* from both substrates, with average temperatures of 46.06°C for the SFC and 36.03°C for the SBG. This result confirms earlier findings that this fungus is thermotolerant and thrives at temperatures greater than 37°C, exhibiting increased germination efficiency and growth rates (Domsch et al., 2007; Pitt, 1994). The second mostcommon species in the current study, *P. variotii*, is also considered a cosmopolitan taxon. This fungus has been isolated from self-heated substrates and is considered a thermotolerant and thermoresistant fungus (Samson, 1974)

According to Luangsa-ard et al. (2004), *P. variotii*, was isolated together with its meiosporic state from mixed deciduous soil in Thailand, suggesting that this taxon is primarily thermotolerant to thermophilic. The third most prevalent species, *A. terreus*, was the most frequently recovered from both lignocellulosic materials at Site I. This species is considered to be both ubiquitous and thermotolerant, consistent with Ryckeboer et al. (2003), and with other reports this species was identified as both mesophilic and thermophilic based on isolates collected from composting and self-heating organic substrates. For example, Singh and Sandhu (1982) reported that this species is a thermophilous fungus based on growth response at different incubation temperatures. Additionally, Pitt (1994) demonstrated that *A. terreus* exhibited tolerance to high temperatures, and this factor likely contributes to the high frequency at which it is found on both types of lignocellulosic materials in these environments.

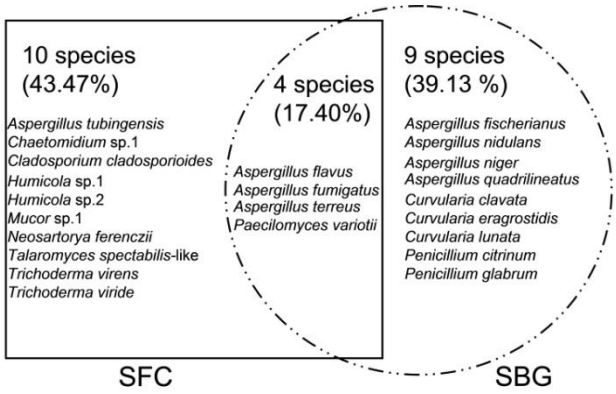

Figure 3 Distribution and percentage of fungi by origin. The numbers within the square and circle denote the number of fungal species found on sugarcane filter cake and sugarcane bagasse. The numbers in the overlapping region denote the number of species common to both lignocellulosic materials.

Conclusions

The recorded percentages of fungi at the different sites were as follows: SFC at Site II supported the most fungi, with 16.59% of all isolates (21 isolates representing 10 species), while SFC at Site III supported the lowest number of fungi (5.53%), with 7 isolates representing 1 species. *Aspergillus* accounted for the most species (8), constituting 37.78% of the total fungal diversity in all species. Based on the overall percent occurrence of fungi on the SFC sample, the most dominant species with occurrence values >10%, were *A. fumigatus* (21.95%), *A. terreus* (14.6%), and *P. variotii* (21.9%), although these fungi were found at different sites. The most

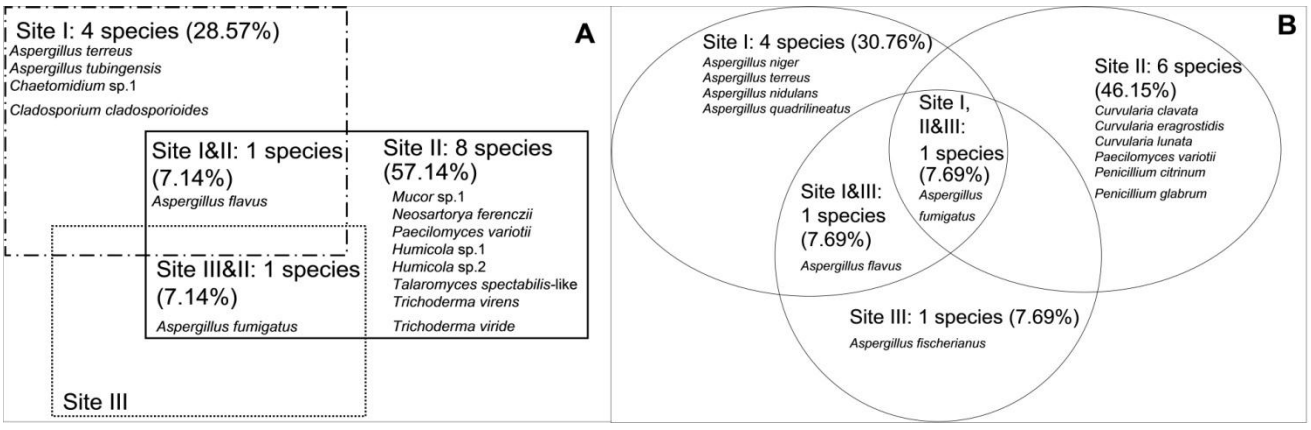

Figure 4 Distribution and percentage of fungi found on sugarcane filter cake (4A) and sugarcane bagasse (4B) from the three selected sugar refining industry sites. The fungal names within each square denote the fungal distribution at that site. The fungal names in the overlapping regions denote species common to the different site.

dominant fungi on SBG at the 3 different sites were *A. fumigatus* (50%), which was found at all sites, and *A. terreus* (10.5%), which was found only at Site I. Based on the overall percent occurrence for both lignocellulosic substrates (>10%), the most frequently observed taxa in this study was *A. fumigatus* (35.4%), followed by *P. variotii* and *A. terreus* (13.9 and 12.6%, respectively). The species diversity on the SFC at Site II was the highest, and the fungal communities on the two types of lignocelluloic materials at this site exhibited little overlap with the other sites.

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