

Comparison of dichloran rose bengal chloramphenicol and Sabouraud dextrose agar with cycloheximide and chloramphenicol for airborne mold sampling

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Abstract The more the mold species isolated on a culture medium, the more the sampling environment is represented accurately. According to the sampling purpose, it is crucial to use the best culture medium for mold. However, no study is available regarding the comparison of dichloran rose bengal chloramphenicol (DRBC) and Sabouraud dextrose agar with cycloheximide and chloramphenicol (SDA-CHX-CHL) culture media in terms of their application for airborne sampling, isolation, and identification of fungi. Airborne mold samples were impacted onto both DRBC and SDA-CHX-CHL, simultaneously using single-stage Andersen sampler. The limit of detection (LOD) value for airborne mold count was 7 CFU m^{-3} (1 colony growth on the Petri dish). The total mold counts (TMC) ranged between <7 and 504 CFU m^{-3} (med 56 CFU m^{-3}) and <7 and 1218 CFU m^{-3} (med 259 CFU m^{-3}), collected on SDA-CHX-CHL and DRBC, respectively. Significantly higher TMC were observed on DRBC than on SDA regardless of the sampling environment (i.e., indoor or outdoor) ($p < 0.05$). Among the most predominant mold

genera, observation frequencies of *Penicillium* spp. and *Aspergillus* spp. on both culture media were found to be more than 70%. Observation frequencies of *Cladosporium* spp., *Alternaria* spp., and yeast were found to be higher in samples collected on DRBC than those on SDA-CHX-CHL. Finally, DRBC was found to be superior to SDA in terms of both number of colonies and number of genera isolated from the air.

Keywords Airborne mold sampling · Chloramphenicol (CHL) · Culture medium · Dichloran rose bengal chloramphenicol (DRBC) agar · Indoor air · Outdoor air · Sabouraud dextrose agar (SDA)

1 Introduction

Scientists and several governmental and private organizations have been working to determine “safe” indoor/outdoor airborne mold levels particularly since the 1980s. Additionally, new green/sustainable building rating systems promote lower indoor mold levels to gain sufficient credits to receive a “green” certificate. However, critical debates on setting acceptable and safe concentration limits to airborne mold exposure have still been continuing due to the lack of information regarding human dose–response and different susceptibility/sensitivity levels of individuals to airborne mold (Bascom et al. 1994; Rao et al. 1996).

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The use of standard sampling, enumeration, and identification techniques is crucial to obtain representative and accurate results. Many studies are available in the literature investigating indoor, outdoor, or occupational mold exposure levels where different culture media have been used. Numerous culture media have been used to cultivate airborne mold to date. The most popular culture media for airborne sampling are malt extract agar (MEA), dichloran glycerol (DG18) agar, rose bengal agar (RBA), rose bengal chloramphenicol (RBC) agar, dichloran rose bengal chloramphenicol (DRBC) agar, and Sabouraud dextrose agar (SDA). One of the most preferred bioaerosol sampling methods, NIOSH method 0800 (1998), recommends MEA for fungi and other culture media for specific target fungi. A limited number of studies have been carried out in different environments by using more than one culture media to compare the performance of the culture medium in terms of airborne mold sampling (e.g., Burge et al. 1977; Morring et al. 1983; Sunesson et al. 1995; Dillon et al. 1996; Wu et al. 2000; Ren et al. 2001; Duchaine et al. 2002; Godish and Godish 2007; Nieguitsila et al. 2011). These studies indicate that selection of culture medium is crucial for isolation, enumeration, and identification of mold species collected from the air.

Airborne molds can be found ubiquitously in the environment due to the abundance of substrate availability, such as plants, foods, human and pet activities and favorable environmental conditions such as temperature and relative humidity for mold propagation (Morring et al. 1983). Choosing the best culture medium for this purpose is important. The more the mold species isolated on a culture medium, the more the sampling environment is represented accurately. Sabouraud agar was found just before the 1900s to culture pathogenic molds and yeasts that cause skin lesions (Sabouraud 1896). The reason why DRBC and SDA were chosen in this study is DRBC and SDA are widely used culture media recommended for both molds and yeasts and considered to be satisfactory as general purpose enumeration media (Rosenthal and Furnari 1957; King et al. 1979; Pitt and Hocking 1997; Pitt et al. 2009; Mentese et al. 2009, 2012a, b, 2015). However, no study is available regarding the comparison of DRBC and SDA-CHX-CHL culture media in terms of their airborne sampling, isolation, and identification performances. This study would be

beneficial to determine the choice of culture medium for airborne mold sampling purposes.

2 Material and method

2.1 Sample collection and analysis

Airborne mold samples were collected daily from indoor ($n = 88$) and outdoor environments ($n = 26$) in Canakkale, Turkey, from August 13 to August 27, 2013 within the context of *Canakkale Health Air Study* (Mentese et al. 2015). Canakkale is a small college city, located on the farthestmost west coast of Turkey. Its climate has a transient character between the Mediterranean and Black Sea regions. All indoor environments were selected randomly, and any known dampness in the indoor sampling sites was not recorded prior to the study.

Indoor airborne mold samples were collected during the daytime at 1.5 m height in the center of the living room of the private homes, and outdoor samples were collected minimum 2 m away outside the buildings and any potential source as described in NIOSH (1998) and Mentese et al. (2009, 2012a, b, 2015). Airborne mold samples were impacted onto both DRBC and SDA-CHX-CHL culture media, simultaneously by a single-stage Andersen sampler (SKC Inc., USA). Sampling flow rate was 28.3 mL min^{-1} and sampling time was 5 min. Airborne mold samples were incubated at 25°C for 4–10 days. After the incubation period, enumeration of mold colonies' grown on the plate was carried out by using a light microscope ($10\times$ and $40\times$ magnification, Primo Star, Zeiss, Germany). Mold counts were calculated in colony forming unit per drawn air in m^3 (CFU m^{-3}). The limit of detection (LOD) value for airborne mold count was 7 CFU m^{-3} (1 colony growth on the Petri dish). Additionally, both field and laboratory blanks for both SDA-CHX-CHL and DRBC culture media were kept for each sampling day. No contamination was observed on the culture media. Also, exact values of temperature and relative humidity (RH) were monitored online (435-2, Testo Inc., Germany). Airborne molds were identified under the light microscope on the level of genus. Each isolated mold sample was identified according to their shape by using morphological atlases under the

microscope (Sutton et al. 1998; Barnett and Hunter 2003; Samson et al. 2010; Larone 2011).

All agar plates were ready to use and sterile (Salubris Co., Turkey). SDA-CHX-CHL contains (in 1 L) peptic digest of meat (5 g), glucose (40 g), pancreatic digest of casein (5 g), cycloheximide (0.05 g), chloramphenicol (0.04 g), and agar (15 g) with a final pH of 6.8 ± 0.2 at 25 °C. DRBC contains (in 1 L) peptic digest of animal tissue (5 g), glucose (10 g), monopotassium phosphate (1 g), magnesium sulfate (0.5 g), rose bengal (0.0025 g), dichloran (0.002 g), chloramphenicol (0.1 g), and agar (15 g) with a final pH of 5.6 ± 0.2 at 25 °C. Both culture media were tested for quality control purposes by *E. coli* ATCC 25922 as a negative control and with *C. albicans* ATCC 10231 as a positive control.

Before choosing the culture medium of SDA-CHX-CHL for this study, SDA with and without CHX-CHL was used to sample airborne mold in parallel for comparison. Since it was not possible to identify and/or count the mold growth on SDA due to excessive and very rapidly growing bacteria and/or mold species on the SDA plate, mold species could not be identified or counted thoroughly. Thus, SDA-CHX-CHL was used in this study instead of SDA.

2.2 Statistical analyses

Statistical analyses were performed using SPSS 19.0 software. Distribution of mold counts was investigated using the Kolmogorov–Smirnov test. Correlations between the mold counts collected with DRBC and SDA-CHX-CHL media were examined by analysis of variance (ANOVA) tests. Paired *t* test and Wilcoxon signed-rank test were applied to mold count pairs to test the statistically significant differences between the pairs in terms of their mean and median values, respectively. Values with $p < 0.05$ were considered statistically significant for all tests.

3 Results

3.1 Temperature and humidity

Median (med) values of both indoor and outdoor temperature and RH were around 29 °C and 50%, respectively. Temperature values were in the range of mesophilic conditions (i.e., 20–35 °C), differences in

temperature between outdoor and indoor sampling sites were noticeably low (med $\Delta t_{\text{out-in}} = 0.8$ °C), and humidity levels were considered to be moderate and acceptable for human comfort (ASHRAE 2013).

3.2 Mold counts collected on DRBC and SDA-CHX-CHL media

Distribution of mold counts of SDA-CHX-CHL and DRBC was found to be lognormally distributed with 95% confidence by Kolmogorov–Smirnov test. Since no statistically significant difference between indoor and outdoor mold samples was found ($p > 0.05$) and climatic conditions (i.e., temperature and RH) were similar in both indoor and outdoor air throughout the study, all airborne mold samples were analyzed statistically without a categorization. As shown in Table 1, TMC levels ranged between <7 and 504 CFU m^{-3} and <7 and 1218 CFU m^{-3} collected by SDA-CHX-CHL and DRBC, respectively. Median values of all samples were 56 and 259 CFU m^{-3} collected by SDA-CHX-CHL and DRBC, respectively. Thus, it can be said that TMC levels were observed to be markedly higher on DRBC than on SDA, regardless of the environment (i.e., indoor or outdoor).

3.3 Mold growth on DRBC and SDA-CHX-CHL media according to mold genus

Total number of observed mold genera ($n = 114$) was calculated by summing up the number of observed mold genera in each sample (see Table 1). Accordingly, a total of 281 and 523 mold colonies were isolated from SDA-CHX-CHL and DRBC culture media, respectively. Similar to TMC levels, total number of mold genera observed on DRBC was found to be higher than those on SDA-CHX-CHL (i.e., with twofolds higher median value). Some of the sample pairs were selected to show the visual comparison of mold growth on DRBC and SDA-CHX-CHL culture media collected from parallel samplings (Fig. 1). As shown in Fig. 1, both the number of mold genera and number of mold colonies grown on DRBC were higher than those grown on SDA-CHX-CHL.

Table 2 shows whether any mold genus was observed (1) only on SDA-CHX-CHL or (2) only on DRBC or (3) on both culture media at the same time, from each parallel sample regardless of the colony

Table 1 Total number of isolated colonies and TMC levels (CFU m^{-3}) collected simultaneously by SDA-CHX-CHL and DRBC culture media

Parameter	Sum	Min	Max	Med	Mean	SD
<i>Number of isolated genera^a</i>						
SDA-CHX-CHL	–	<1 ^d	6	3.00	2.93	1.20
DRBC	–	<1 ^d	8	6.00	5.57	1.32
<i>Total number of isolated colonies from all samples^b</i>						
SDA-CHX-CHL	281	–	–	–	–	–
DRBC	523					
<i>Number of isolated genera ratio^c</i>						
$n_{\text{DRBC}}/n_{\text{SDA-CHX-CHL}}$	–	0	6.0	2.0	2.14	1.18
<i>TMC (CFU m^{-3})</i>						
SDA-CHX-CHL	–	<7 ^d	504	56	70	10.6
DRBC	–	<7 ^d	1218	259	315	29.7

$N = 114$ for each culture medium

^a n_i = number of isolated genera on a plate

^b $\sum_{i=1}^{114} n_i$

^c Calculated from each parallel sample pair

^d No colony growth was observed on the culture medium

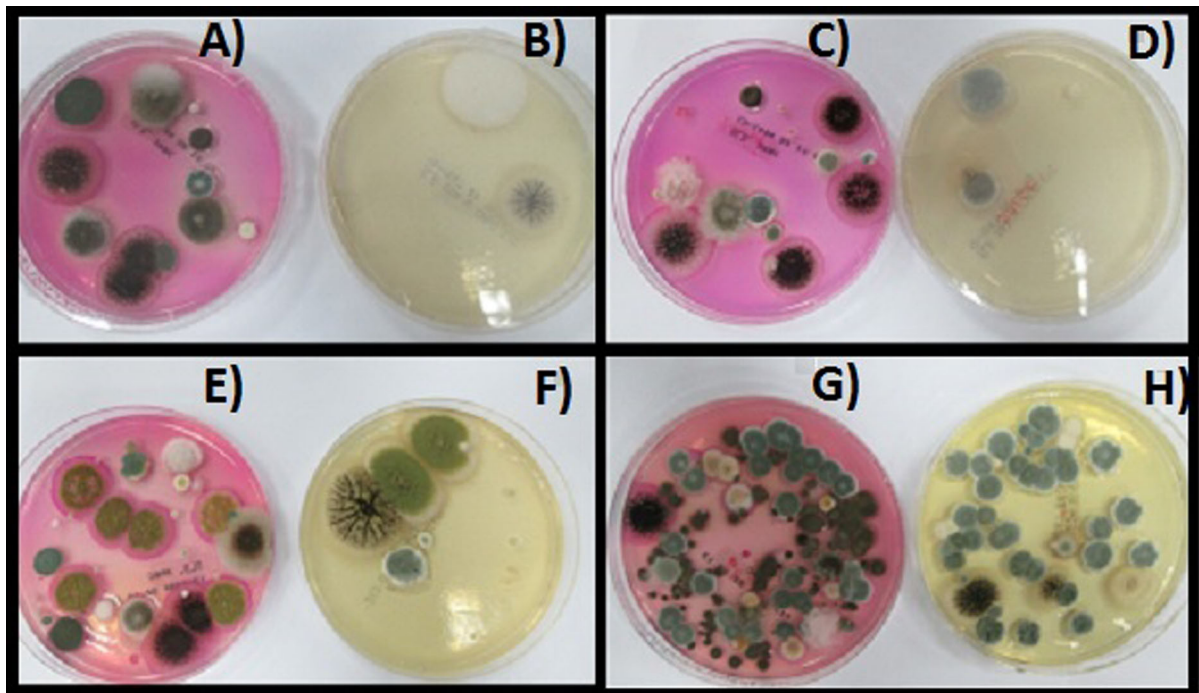
**Fig. 1** Typical views of airborne mold sample pairs collected by DRBC (a, c, e, g) and SDA-CHX-CHL (b, d, f, h)

Table 2 Relative observational frequencies of individual mold genus collected simultaneously by SDA-CHX-CHL and/or DRBC media

Mold genus	Relative observation frequency (%)		
	Only SDA-CHX-CHL	Only DRBC	SDA-CHX-CHL and DRBC
<i>Penicillium</i> spp.	6.1	16.7	74.6
<i>Aspergillus</i> spp.	6.1	16.7	73.7
<i>Cladosporium</i> spp.	0.0	63.2	32.5
<i>Alternaria</i> spp.	6.1	57.0	21.1
Sterile hyphae + NI ^a	7.0	36.0	9.6
Yeast	0.0	33.3	7.0
<i>Acremonium</i> spp.	0.0	28.9	3.5
<i>Mucor</i> spp.	9.6	9.6	0.0
<i>Rhizopus</i> spp.	1.8	4.4	3.5
<i>Chaetomium</i> spp.	0.9	0.9	0.0
<i>Fusarium</i> spp.	0.0	3.5	0.0
<i>Scopulariopsis</i> spp.	0.0	2.6	0.0
<i>Drechslera</i> spp.	0.0	1.8	0.0
<i>Geotrichum</i> spp.	0.0	0.9	0.0
<i>Cunninghamella</i> spp.	0.0	0.9	0.0
<i>Trichoderma</i> spp.	0.0	0.9	0.0

$\sum n_i/n\%$, where n_i observation number of each mold genus among the all samples; $n = 114$; predominant mold genera were shown in bold

^a NI not identified

counts of each mold genus on the samples. Among the most predominant mold genera, observation frequencies of *Penicillium* spp. and *Aspergillus* spp. on both culture media were found to be more than 70%. *Cladosporium* spp., *Alternaria* spp., and yeast were observed in 95.7, 78.1, and 40.0% of the samples collected on DRBC media, respectively, and in 32.5, 27.2, and 7% of the samples collected on SDA-CHX-CHL medium, respectively. Also, *Penicillium* spp., *Aspergillus* spp., *Alternaria* spp., yeast, and *Acremonium* spp. occurred more frequently on DRBC than on SDA-CHX-CHL. No mold genus was found to be more frequent on SDA-CHX-CHL than on DRBC medium. Moreover, once mold growth was observed on DRBC medium for *Cladosporium* spp., yeast, and *Acremonium* spp., no colony growth was observed on SDA-CHX-CHL culture medium at the same time.

Total colony counts (CFU m⁻³) of individual mold genus collected simultaneously by SDA-CHX-CHL and DRBC culture media from all samples ($n = 114$) together with the colony count ratios of the observed individual mold genus on both media are given in Table 3. When we compared the concentrations of individual mold genus, mold concentrations were always found to be higher for all mold genera on samples collected with DRBC than on those with

SDA-CHX-CHL medium. The ratios of colony counts of the individual mold genus on DRBC compared to SDA-CHX-CHL varied between 1.23 and 25.5. The highest ratios were found for *Acremonium* spp. (25.50) and *Cladosporium* spp. (22.38), while the lowest ratio was observed for *Penicillium* spp. (1.23) among the most predominant mold genera. As shown in Table 2, among the least frequent mold genera (relative frequency <5%), *Fusarium* spp., *Scopulariopsis* spp., *Drechslera* spp., *Geotrichum* spp., *Cunninghamella* spp., and *Trichoderma* spp. occurred only on DRBC.

3.4 Associations between the mold counts collected by DRBC and SDA-CHX-CHL

Neither TMC levels nor concentrations of the most prevalent mold genera collected by both culture media had normal distribution. To find the associations between the mold counts collected by DRBC and SDA-CHX-CHL culture media, paired sample tests were applied at 95% confidence level (see Table 4). Paired *t* test and Wilcoxon signed-rank tests showed that individual concentrations of *Alternaria* spp., *Aspergillus* spp., *Cladosporium* spp., *Penicillium* spp., and yeast and TMC observed on DRBC were

Table 3 Total numbers of colony counts (CFU m⁻³) of each individual mold genus collected simultaneously by SDA-CHX-CHL and DRBC from all samples

Genus	Total colony counts (CFU m ⁻³)		
	SDA-CHX-CHL	DRBC	[DRBC]/[SDA-CHX-CHL] ratio
<i>Aspergillus</i> spp.	3318	6055	1.83
<i>Penicillium</i> spp.	2772	3409	1.23
<i>Cladosporium</i> spp.	931	20,832	22.38
<i>Alternaria</i> spp.	406	2723	6.71
Yeast	273	1449	5.31
Sterile hyphae + NI ^a	189	805	4.26
<i>Acremonium</i> spp.	28	714	25.5
<i>Mucor</i> spp.	77	105	1.36
<i>Rhizopus</i> spp.	42	77	1.83
<i>Fusarium</i> spp.	0	42	–
<i>Scopulariopsis</i> spp.	0	35	–
<i>Drechslera</i> spp.	0	14	–
<i>Cunninghamella</i> spp.	0	7	–
<i>Geotrichum</i> spp.	0	7	–
<i>Trichoderma</i> spp.	0	7	–

n = 114; predominant mold genera were shown in bold

^a NI not identified

Table 4 Paired sample comparison at 95% confidence levels of the most prevalent mold genera counts and TMC between DRBC and SDA-CHX-CHL

Variable	Mean diff. ± SD ^a	<i>z</i> ^{b,c}	<i>p</i> ^b
<i>Alternaria</i> spp.	−2.90 ± 2.97	−7.86	0.00
<i>Aspergillus</i> spp.	−3.43 ± 10.66	−5.34	0.00
<i>Cladosporium</i> spp.	−24.94 ± 21.22	−9.04	0.00
<i>Penicillium</i> spp.	−0.87 ± 3.64	−3.01	0.03
TMC	−35.21 ± 24.86	−9.20	0.00

n = 114

^a Paired *t* test was applied

^b Wilcoxon signed-rank test was applied

^c *z* value based on possible negative ranks

found to be statistically significantly different than those observed on SDA-CHX-CHL (*p* < 0.03).

4 Discussion

Many studies are available in the literature focusing on indoor, outdoor, or occupational exposure levels to airborne mold where different culture media have been used. When studies conducted with different culture media in different countries in last decade were considered, it can be seen that different culture media

such as SDA, DRBC, PeDA, MEA, RBA, DG18, and PDA are more common for airborne mold sampling (e.g., Mentese et al. 2009, 2012a, b, 2015; Hoppe et al. 2012; Madureira et al. 2014; Ponce-Caballero et al. 2013; Rosenbaum et al. 2010; Gent et al. 2012; Kawasaki et al. 2010; Kim et al. 2011; Hoseinzadeh et al. 2013). There is no clear consistency in the type of culture medium used, even in the studies conducted in the same or close geographical regions in the literature.

Most of the studies which compare culture media in terms of mold isolation efficiency are based on food samples. However, it is crucial to use standard sampling, enumeration, and identification techniques to obtain accurate results in terms of representing the sampled environmental conditions for airborne mold. No study was found investigating the efficiency for airborne mold using DRBC and SDA-CHX-CHL together. Thus, only studies which compare the airborne mold sampling efficiency of culture media other than DRBC versus SDA-CHX-CHL are discussed here.

Few studies are available in the literature in which multiple culture media have been used for airborne mold sampling, yet some of them did not compare the performances of the culture media. Table 5 shows the studies found worldwide investigating the mold isolation performances of culture media for samples

Table 5 Selected studies conducted worldwide by using different culture media

City, country	Sampling environment	Culture media	Remarks	Reference
Canakkale, Turkey (this study)	Homes and outdoors	DRBC versus SDA with CHX and CHL	Higher mold counts on DRBC	Mentese et al. (2015)
Manisa, Turkey	Ambient air	RBA with CHL versus MEA	No comparison	Kalyoncu (2010)
Burgundy, French	Poultry farmhouse	MEA versus DG18	More species were observed on DG18	Nieguitsila et al. (2011)
Värmland, Sweden ^a	Children's homes	MEA versus DG18	No difference in mold spore counts	Holme et al. (2010)
Styria, Austria ^a	Homes	MEA versus DG18 with CHL	Higher mold counts on MEA	Haas et al. (2014)
El-Beida, Libya	Homes and outdoors	PDA versus MEA with CHL	No comparison	El-Gali and Abdullrahman (2014)
Isfahan, Iran	Ambient air	SDA versus PDA	No comparison	Chadeganipour et al. (2010)
Shenzhen, China	Technological building in Shenzhen University	PeDA, PDA versus Czapek Dox Agar	No comparison	Li et al. (2010)
Taiwan	Hospital	MEA versus DG18	Higher mold counts and more species on DG18	Wu et al. (2000)
Minnesota, USA	Hospital	Inhibitory mold agar, Littman oxgall agar, RB streptomycin versus SDA	Authors chose RB streptomycin	Morring et al. (1983)
Northeast USA	Infant bedrooms	MEA versus DG18	Higher mold levels on DG18	Ren et al. (2001)
USA	Living rooms	MEA-DG18	No difference for 1–10-min sampling	Godish and Godish (2007)
Canada	Sawmills	SDA, RBA versus CZA with CHL	No significant effect of media and SDA showed invalid results	Duchaine et al. (2002)

CHL chloramphenicol, DG18 dichloran glycerol, MEA malt extract agar, PDA potato dextrose agar, PeDA peptone dextrose agar, RBA rose bengal agar, SDA Sabouraud dextrose agar

^a Only fungal spore concentrations were measured

collected from the air. Li et al. (2010) collected air samples on PDA, PeDA, and Czapek agar using the open-plate method at different altitudes in Shenzhen University, China. However, the authors did not compare the airborne mold sampling performances of those three culture media. Holme et al. (2010) collected air samples from the kitchen, living room, and bedrooms in Värmland, Sweden, using MEA and DG18. However, the authors mentioned only the mold spore concentrations and found no large difference in spore concentrations between the two culture media. There are a few more studies which have been conducted with more than one culture media without any comparison of the culture media (Chadeganipour et al. 2010; Kalyoncu 2010; El-Gali and

Abdullrahman 2014). Mold recovery from 3 different culture media (i.e., SDA, RBA, and Czapek solution (CZA)) with CHL was researched in 17 sawmills in Canada. There was no significant effect of culture media in terms of concentration and the mold species recovered, and also SDA was found to have nonusable enumeration (i.e., invalid results) (Duchaine et al. 2002), similar to our findings.

Also antimicrobial agents (e.g., chloramphenicol) have not been used in all of the studies, which could have influenced both identification and counting efficiency of airborne mold due to rapid bacteria/mold growth on the culture medium. Adding antibiotics (chloramphenicol and cycloheximide here) to the agar plate increased the enumeration and identification

performances of the SDA culture medium. Chloramphenicol and cycloheximide are antibiotics which inhibit the growth of some bacteria as well as some pathogenic fungi (Ajello 1957; MacFaddin 1985; Mentese et al. 2009). Chloramphenicol and cycloheximide are used due to in vivo inhibitory effects on the synthesis of mitochondrial enzymes. Chloramphenicol causes a significant decrease in the activity of enzymes which are “markers” of all submitochondrial structures, while the effect of cycloheximide depends on the amount of the inhibitor and the time (Turska et al. 1976). Similar to our preliminary study results, SDA agar with and without cycloheximide–chloramphenicol was used for isolates of dermatophytes in parallel (Rosenthal and Furnari 1957) and it was found that SDA-CHX-CHL was much superior to SDA and SDA-CHX-CHL is recommended for routine diagnostic mycology.

5 Conclusion

Choosing the best culture medium in terms of performance for airborne mold sampling, isolation, enumeration, and identification is crucial to reach the best representative results for the sampled environment. In this study, two culture media (DRBC vs. SDA-CHX-CHL) which have been commonly used for airborne mold sampling were compared. It was found that both mold counts and the number of mold genera isolated from the air were found to be higher in samples collected on DRBC culture medium than those on SDA-CHX-CHL culture medium, regardless of the environment (i.e., indoor air or outdoor air), indicating that DRBC was found to be superior to SDA in terms of both number of colony and number of species isolated from the air. Moreover, some of the mold genera commonly found in the air samples, such as *Cladosporium* spp., yeast, and *Acremonium* spp., as well as less frequent mold genera such as *Fusarium* spp., *Scopulariopsis* spp., *Drechslera* spp., *Geotrichum* spp., *Cunninghamella* spp., and *Trichoderma* spp. did not occur on SDA-CHX-CHL, while they occurred on DRBC. Finally, adding an antibiotic agent to SDA enabled better counting and identification of mold species.

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References

- Ajello, L. (1957). Cultural methods for human pathogenic fungi. *Journal of Chronic Diseases*, 5(5), 545–551.
- American Society of Heating, Refrigerating and Air-Conditioning Engineers (ASHRAE). (2013). ASHRAE Standard 62.1-2013.
- Barnett, H. L., & Hunter, B. B. (Eds.). (2003). *Illustrated genera of imperfect fungi* (4th ed.). St. Paul, MN: The American Phytopathological Society (APS) Press.
- Bascom, R., Kesavanathan, J., & Swift, D. L. (1994). Human susceptibility to indoor contaminants. *Occupational Medicine (Philadelphia, Pa.)*, 10(1), 119–132.
- Burge, H. P., Solomon, W. R., & Boise, J. R. (1977). Comparative merits of eight popular media in aerometric studies of fungi. *Journal of Allergy and Clinical Immunology*, 60(3), 199–203.
- Chadeganipour, M., Shadzi, S., Nilipour, S., et al. (2010). Airborne fungi in Isfahan and evaluation of allergenic responses of their extracts in animal model. *Jundishapur Journal of Microbiology*, 3(4), 155–160.
- Dilon, K., Heinsohn, P., & Miler, D. (Eds.). (1996). *Field guide for the determination of biological contaminants in environmental samples* (pp. 37–40). Fairfax, VA: AIHA Publications, American Industrial Hygiene Association.
- Duchaine, C., Mériaux, A., & Comtois, P. (2002). Usefulness of using three different culture media for mold recovery in exposure assessment studies. *Aerobiologia*, 18(3–4), 245–251.
- El-Gali, Z. I., & Abdullrahman, E. M. (2014). Seasonal distribution of indoor and outdoor fungi in the air of El-Beida City, Libya. *New York Science Journal*, 7(6), 94–100.
- Gent, J. F., Kezik, J. M., Hill, M. E., et al. (2012). Household mold and dust allergens: Exposure, sensitization and childhood asthma morbidity. *Environmental Research*, 118, 86–93.
- Godish, D., & Godish, T. (2007). Relationship between sampling duration and concentration of culturable airborne mould and bacteria on selected culture media. *Journal of Applied Microbiology*, 102(6), 1479–1484.
- Haas, D., Habib, J., Luxner, J., et al. (2014). Comparison of background levels of culturable fungal spore concentrations in indoor and outdoor air in southeastern Austria. *Atmospheric Environment*, 98, 640–647.
- Holme, J., Hägerhed-Engman, L., Mattsson, J., et al. (2010). Culturable mold in indoor air and its association with moisture-related problems and asthma and allergy among Swedish children. *Indoor Air*, 20(4), 329–340.
- Hoppe, K. A., Metwali, N., Perry, S. S., et al. (2012). Assessment of airborne exposures and health in flooded homes undergoing renovation. *Indoor Air*, 22(6), 446–456.
- Hoseinzadeh, E., Samarghandie, M. R., Ghiasian, S. A., et al. (2013). Evaluation of bioaerosols in five educational hospitals wards air in Hamedan, during 2011–2012. *Jundishapur Journal of Microbiology*, 6(6), e10704.

- Kalyoncu, F. (2010). Relationship between airborne fungal allergens and meteorological factors in Manisa City, Turkey. *Environmental Monitoring and Assessment*, 165, 553–558.
- Kawasaki, T., Kyotani, T., Ushioji, T., et al. (2010). Distribution and identification of airborne fungi in railway stations in Tokyo, Japan. *Journal of Occupational Health*, 52(3), 186–193.
- Kim, K. Y., Kim, Y. S., Kim, D., et al. (2011). Exposure level and distribution characteristics of airborne bacteria and fungi in Seoul metropolitan subway stations. *Industrial Health*, 49(2), 242–248.
- King, A. D., Hocking, A. D., & Pitt, J. I. (1979). Dichloran rose bengal medium for enumeration and isolation of moulds from foods. *Applied and Environmental Microbiology*, 37, 959–964.
- Larone, D. H. (2011). *Medically important fungi, a guide to identification* (5th ed.). Washington: ASM Press.
- Li, L., Lei, C., & Liu, Z. G. (2010). Investigation of airborne fungi at different altitudes in Shenzhen University. *Natural Science*, 2(5), 506–514.
- MacFaddin, J. F. (1985). *Media for isolation-cultivation identification—Maintenance of medical bacteria* (Vol. 1). Baltimore: Williams and Wilkins.
- Madureira, J., Pereira, C., Paciência, I., et al. (2014). Identification and levels of airborne fungi in Portuguese primary schools. *Journal of Toxicology & Environmental Health Part A: Current Issues*, 77(14–16), 816–826.
- Mentese, S., Arisoy, M., Rad, A., et al. (2009). Bacteria and fungi levels in various indoor and outdoor environments in Ankara, Turkey. *Clean—Soil, Air, Water*, 37(6), 487–493.
- Mentese, S., Mirici, N. A., Otkun, M. T., et al. (2015). Association between respiratory health and indoor air pollution exposure in Canakkale, Turkey. *Building and Environment*, 93(1), 72–83.
- Mentese, S., Rad, A. Y., Arisoy, M., et al. (2012a). Multiple comparisons of organic, microbial, and fine particulate pollutants in typical indoor environments: Diurnal and seasonal variations. *Journal of the Air & Waste Management Association*, 62(12), 1380–1393.
- Mentese, S., Rad, A. Y., Arisoy, M., et al. (2012b). Seasonal and spatial variations of bioaerosols in indoor urban environments, Ankara, Turkey. *Indoor and Built Environment*, 21(6), 797–810.
- Morring, K. L., Sorenson, W., & Attfield, M. D. (1983). Sampling for airborne fungi: A statistical comparison of media. *The American Industrial Hygiene Association Journal*, 44(9), 662–664.
- Nieguitsila, A., Arne, P., Durand, B., et al. (2011). Relative efficiencies of two air sampling methods and three culture conditions for the assessment of airborne culturable fungi in a poultry farmhouse in France. *Environmental Research*, 111, 248–253.
- NIOSH. (1998). *Method 0800—Bioaerosol sampling (indoor air), culturable organisms: Bacteria, fungi, thermophilic actinomycetes*. Washington: NIOSH.
- Pitt, J. I., & Hocking, A. D. (1997). *Fungi and food spoilage* (2nd ed.). London: Blackie Academic and Professional.
- Pitt, J. I., Hocking, A. D., & Diane, A. (2009). *Fungi and food spoilage* (3rd ed.). Heidelberg: Springer.
- Ponce-Caballero, C., Gamboa-Marrufo, M., López-Pacheco, M., et al. (2013). Seasonal variation of airborne fungal propagules indoor and outdoor of domestic environments in Mérida, Mexico. *Atmósfera*, 26(3), 369–377.
- Rao, C. Y., Burge, H. A., & Chang, J. C. (1996). Review of quantitative standards and guidelines for fungi in indoor air. *Journal of the Air & Waste Management Association*, 46(9), 899–908.
- Ren, P., Jankun, T., Belanger, K., et al. (2001). The relation between fungal propagules in indoor air and home characteristics. *Allergy*, 56(5), 419–424.
- Rosenbaum, P. F., Crawford, J. A., Anagnost, S. E., et al. (2010). Indoor airborne fungi and wheeze in the first year of life among a cohort of infants at risk for asthma. *Journal of Exposure Science & Environmental Epidemiology*, 20(6), 503–515.
- Rosenthal, S. A., & Furnari, D. (1957). The use of a cycloheximide–chloramphenicol medium in routine culture for fungi. *Journal of Investigative Dermatology*, 28(5), 367–371.
- Sabouraud, R. (1896). La question des teignes. In *Annales de dermatologie 3rd series*, Vol. VII, pp. 87–135.
- Samson, R. A., Houbaken, J., Thrane, U., et al. (2010). *Food and indoor fungi*. Utrecht, The Netherlands: CBS-KNAW Fungal Biodiversity Centre.
- Sunesson, A., Vaes, W., Nilsson, C., et al. (1995). Identification of volatile metabolites from five fungal species cultivated on two media. *Applied and Environmental Microbiology*, 61(8), 2911–2918.
- Sutton, D. A., Fothergill, W. A., & Rinaldi, M. G. (1998). *Guide to clinically significant fungi*. Baltimore: Williams and Wilkins.
- Turska, E., Konopka, K., & Turski, W. (1976). The effect of chloramphenicol and cycloheximide on the activity of enzyme “markers” of mitochondrial substructures of the rat liver. *Acta Biologica et Medica Germanica*, 36(9), 1231–1236.
- Wu, P. C., Su, H. J. J., & Ho, H. M. (2000). A comparison of sampling media for environmental viable fungi collected in a hospital environment. *Environmental Research*, 82(3), 253–257.