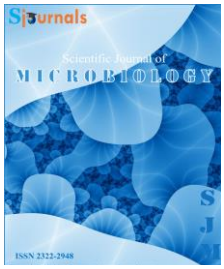
Scientific Journal of Microbiology (2021) 7(2) 181-187
ISSN 2322-2948
doi: 10.14196/sjm.v7i2.1651



Contents lists available at Sjournals
Scientific Journal of **Microbiology**
Journal homepage: www.sjournals.com

Original article

Molecular characterization of potential allergenic molds (*Aspergillus*, *Penicillium* and *Fusarium*) in two buildings receiving the public in Abidjan – Côte d'Ivoire

M'boh Epi Reine Elisabeth N'Gou[a,b,*], David Coulibaly N'Golo[a], Chiaye Claire Antoinette Yapo-crezoit[a], Ama Valerie Bonouman[a], Solange Kakou[a], Ossey Bernard Yapo[c], Allico Joseph Djaman[a,b], Mireille Dosso[a]

[a]*Institut Pasteur of Côte d'Ivoire (Biology of Immunity Unit, Molecular Biology Platform, Mycology Laboratory, Biochemistry Laboratory, Microbiology Laboratory).*
[b]*Université Félix Houphouët Boigny of Côte d'Ivoire (Biology and Health Laboratory), 22 BP 582 Abidjan 22.*
[c]*Central Environmental Laboratory, Ivorian Anti-Pollution Centre (CIAPOL).*

*Corresponding author: reinemboh@hotmail.com

ARTICLE INFO

Article history,
Received 15 February 2021
Accepted 23 March 2021
Available online 29 March 2021
iThenticate screening 18 February 2021
English editing 21 March 2021
Quality control 28 March 2021

Keywords,
Allergy
Moulds
Aspergillus
Penicillium
Fusarium
PCR

ABSTRACT

Aspergillus, Penicillium and *Fusarium* species play an important role in environmental allergy. These molds are potential density in the indoor environment. This study aimed to characterize the strains *Aspergillus, Penicillium* and *Fusarium* using a molecular method. The beta-tubulin and elongation factor TEF-α genes were utilized to differentiate the species. A total of 405 environmental species (*Aspergillus, Penicillium* and *Fusarium*) were isolated, purified and initially identified by colony morphology. Subsequently, DNA was extracted and; PCR was performed from 351 isolates. The results were then compared to morphological characteristics. Of the 351 isolates tested, 167 were *Aspergillus*, 103 were *Penicillium* and 81 were *Fusarium*. The beta-tubulin and elongation factor TEF-α genes were found to be the most suitable for differentiating these three genera among them; the beta-tubulin gene was used for molecular identification of *Aspergillus, Penicillium* and the elongation factor TEF-α gene for characterizing *Fusarium* species.

1. Introduction

Many different spores and mycelial fragments grow indoor environment where available nutrients, humidity and temperature conditions ideal. People are continuously in contact with airborne molds via inhalation (Pei-Chih et al., 2000), and airborne molds are known to induce allergies disease such as chronic bronchitis, sunisitis, asthma, persensitivity reactions, pneumonitis and aspergillosis allergic (Plewa-Tutaj and Lonc, 2014; Ozkara et al., 2007; El-Morsy, 2006; Ceylan et al., 2006; Oliveira et al., 2005; Pepeljnjak and Segvic, 2003). The sensitivity rate is 5-20% in allergy clinics people (Twaroch et al., 2015) with type I to IV hypersensitivity responses (Simon-Nobbe et al., 2008). *Penicillium*, *Aspergillus, Alternaria, Cladosporium, Fusarium* was found to be the predominant indoor genus associated with respiratory allergies (Anaya et al., 2016; Plewa-Tutaj and Lonc, 2014; Horner et al., 1995). A recent study in Abidjan identified the genera *Penicillium*, *Aspergillus* and *Fusarium* as the predominant indoor molds (N'Gou et al., 2021). Given the allergic and biological importance of *Penicillium*, *Aspergillus* and *Fusarium*, their spread and diversity, and similarity to each other, precise identification of these sections and species is clearly necessary for any environment research. Furthermore, more allergen extracts of these three molds will be included in a panel of skin test reagents (Horner et al., 1995). However, the use of morphological criteria for identification of molds species is very difficult. Moreover, as environmental factors and conditions influence morphological identification, molecular methods are needed to identify *Aspergillus, Penicillium, Fusarium* sections and to differentiate them from one another (Bialek et al., 2005; Husain et al., 2004). Thus, this study intended molecular characterization of 03 potential allergenic strains: *Penicillium, Aspergillus* and *Fusarium* in Abidjan, Côte d'Ivoire.

2. Materials and methods

2.1. Sampling

Air, surface and dust samples from two professional establishments were collected by Passive air sampling method (NF EN ISO 16000-19) and swabbing (NF ISO 16000-21) (N'Gou et al., 2021).

2.2. Culture and morphological identification

Strains were isolated from cultures on Sabouraud Chloramphenicol medium. The allergenic strains *Penicillium, Aspergillus* and *Fusarium* were identified with the mycological existing identification keys (Samson et al., 2010; Klich, 2002).

2.3. Molecular characterization

All the following steps from extraction to PCR were carried out at the Molecular Biology platform of the Institut Pasteur de Côte d'Ivoire.

DNA extraction was performed from pure culture. Strains were submitted to a lysis buffer (200mM Tris-HCl lysis buffer, pH 8.5; 250mM NaCl; 25mM EDTA; 0.5% SDS) and proteinase K at 65°C and 500 trs/ min in a incubator block (Thermomixer, Eppendorf). The lysate cell were concentrated in Sodium acetate solution on cold temperature. DNA elution was done to the column. The purity and quantity of DNA were assessed using a Nanodrop instrument (NanoDrop One^{c}, version 1.3.1 Database version 1, Thermo Scientific).

PCR reactions were realized in a final volume of 25 µL containing: DNA template (5 µL), 5X HOT FIREPol (Biosolis), Blend Master Mix Ready to Load (4 µL), Primer Foward (25 µM; 0.5 µL), Primer Reverse (25 µM; 0.5 µL), Sterile Ultra Pure Water (15 µL). Amplifications were done using primers as described by Glass and Donaldson (1995) and O'donnell et al. (1998) (Table 1).

The β-tubulin gene of *Penicillium* and *Aspergillus* strains and the Elongation Factor gene (TEF-α) of *Fusarium* were amplified according to different programs. The amplification of the β-tubulin gene was performed on (02) steps using the following program: first step (5 cycles) and the 2nd step (35 cycles), with denaturation 94°C for 1 min, annealing temperature of primers at 68°C, a decrease of the hybridization temperature of 1°C / cycle (first step) and 64°C (2nd step) for 90 s and an extension at 72°C for 2 min. A final extension at 72°C for 10 min. Elongation Factor (TEF-α) of the Fusarium amplification included initial denaturation at 95°C for 3 min, followed by denaturation at 95°C for 45s, annealing temperature of primers at 58°C (5 cycles), 56°C (5 cycles), 52°C (30 cycles),

for 45s and extension at 72°C for 2 min. A final extension at 72°C for 8 minutes. Amplifications were performed using a Thermocycleur Gene Amp PCR System 9700.

Table 1
Details of the primers used in present study.

Name	Sequences (5' – 3')	Target	Strains	Amplicon size (pb)	References
Bt2a Bt2b	GGTAACCAAATCGGTGCTGCTTTC ACCCTCAGTGTAGTGACCCTTGGC	β-tubulin	*Aspergillus fumigatus*	550	Glass and Donaldson, 1995
			Aspergillus flavus	550	Nasri et al., 2015
			Aspergillus niger	555	Glass and Donaldson, 1995
			Aspergillus ochraceus	584	Glass and Donaldson, 1995
			Aspergillus terreus	574	Glass and Donaldson, 1995
			Aspergillus clavatus	561	Glass and Donaldson, 1995
			Aspergillus versicolor	422	Glass and Donaldson, 1995
			Aspergillus	422 - 600	Pasqualetti et al., 2020; Sanchez Espinosa et al., 2021; Laforgue et al., 2009
			Penicillium chrysogenum	480	Glass and Donaldson, 1995
			Penicillium sp	450 - 500	Johnston, 2008; Laforgue et al., 2009
EF1F EF1R	ATGGGTAAGGAGGACAAGAC GGAAGTACCAGTGATCATGTT	Elongation Factor (TEF-α)	*Fusarium spp*	700 - 750	Geiser et al., 2004; O'Donnell et al., 1998

2.4. PCR products revelation

Amplicons were revealed on a 2% agarose gel stained with SyberSafe after an electrophoresis.

3. Results and discussion

Identification based on morphological characteristics of the isolated *Aspergillus, Penicillium* and *Fusarium* species indicated that, out of the 405 environmental isolates, 190 were *Aspergillus*, 128 were *Penicillium* and 87 were *Fusarium* (Tabe 2).

Amplification with the primers Bt2a - Bt2b and EF1F-EF1R gave fragments between 350 and 800 bp allowing, to distinguish the species *Aspergillus fumigatus, Aspergillus flavus, Aspergillus niger, Aspergillus ochraceus, Aspergillus terreus, Aspergillus clavatus, Aspergillus versicolor, Penicillium chrysogenum, Aspergillus sp, Penicillium sp* and *Fusarium sp*.

Comparison of morphological identification with that of molecular characterization showed that 10 of the species identified as Aspergillus flavus by microscopy did not belong to this species. Two species identified respectively as *Aspergillus fumigatus* and *Aspergillus sp*. based on strain morphology were not. 10 species identified by microscopy as Penicillium sp, according to morphological aspect of the colonies were not members of these species. 6 species identified on phenotypic criterion as *Fusarium sp* were not members of the species. PCR results for *Aspergillus, Penicillium* and *Fusarium* strains are reported in Table 2.

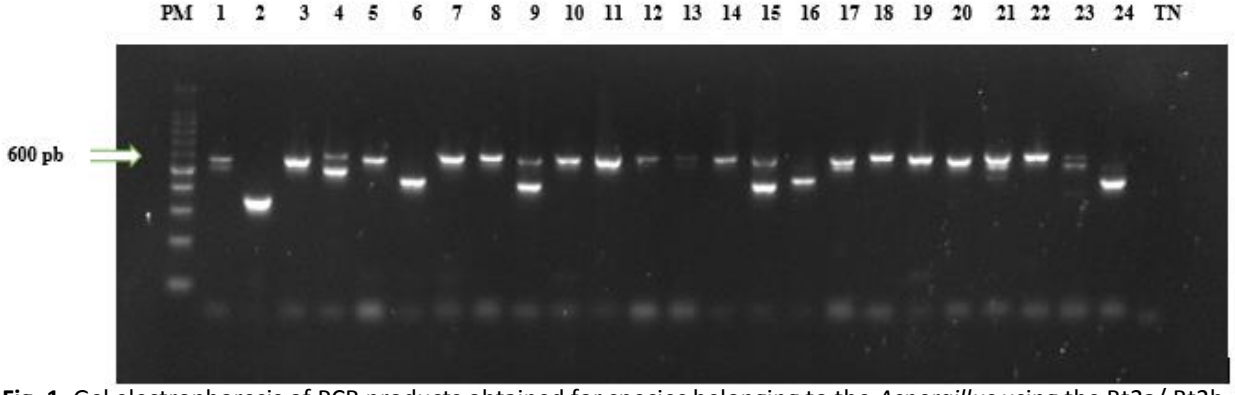


Fig. 1. Gel electrophoresis of PCR products obtained for species belonging to the *Aspergillus* using the Bt2a/ Bt2b primers. Lane 1: 100 bp DNA size marker (Fermentas, Germany), Lane 2 – 25: the isolates of *Aspergillus*, Lane 26: negative control.

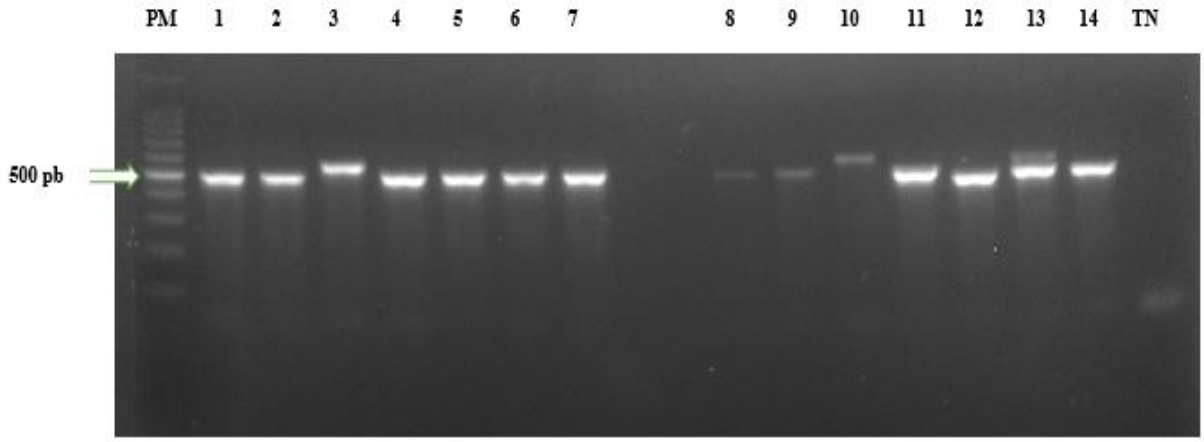


Fig. 2. Gel electrophoresis of PCR products obtained for species belonging to the *Penicillium* using the Bt2a/ Bt2b primers. Lane 1: 100 bp DNA size marker (Fermentas, Germany), Lane 2 – 15: the isolates of *Penicillium*, Lane 16: negative control.

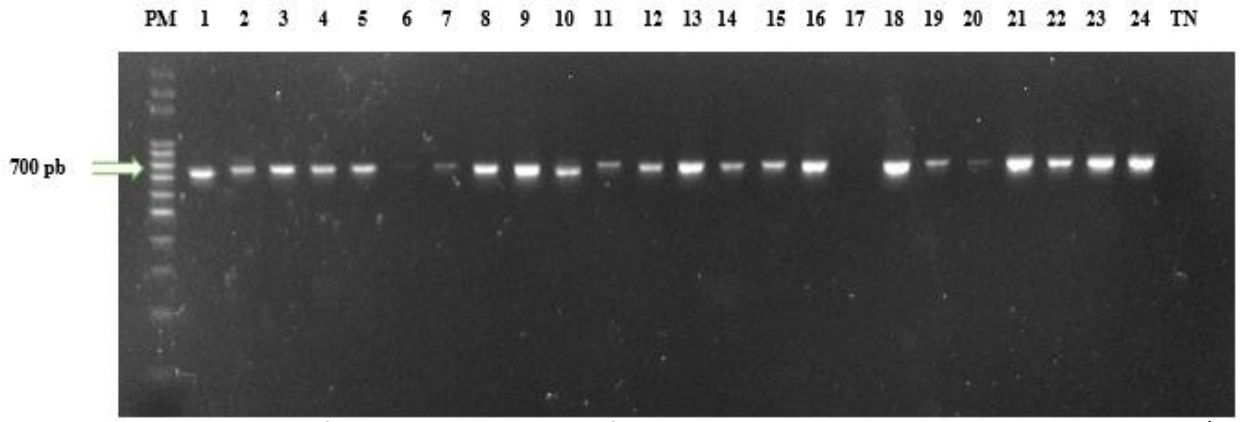


Fig. 3. Gel electrophoresis of PCR products obtained for species belonging to the *Fusarium* using the EF1/EF2 primers. Lane 1: 100 bp DNA size marker (Fermentas, Germany), Lane 2 – 25: the isolates of *Fusarium*, Lane 26: negative control.

Table 2

Results of the molecular identification.

	Identification		Discordance identification
Strain	**Phenotypical**	**Molecular**	**Ratio (%)**
A. fumigatus	27	21	8.70
A. flavus	57	38	20.83
A. niger	15	14	0
A. ochraceus	13	13	0
A. terreus	3	3	0
A. clavatus	1	1	0
A. versicolor	8	4	0
Aspergillus sp	66	59	3.28
Penicillium sp	54	19	34.48
P. chrysogenum	74	74	0
Fusarium sp	87	75	7.41
Total	405	321	8.55

Aspergillus, Penicillium and *Fusarium* are among the important genus implicated in allergic diseases (Anaya et al., 2016; Plewa-Tutaj and Lonc, 2014; Horner et al., 1995). A identification of *Aspergillus, Penicillium* and *Fusarium* species various is very subtantial for allergic diagnostic. The molecular technique is high, sensible and rapid method for the moulds specific identification on genetic data (Verscheure et al., 2002). It's important to combine molecular biological techniques with classical mycological methods to improve species identification (Bougnoux and Espinasse, 2003).

Two genes (beta-tubulin and Elongation Factor (TEF-α)) were used to precisely differentiate among the *Aspergillus, Penicillium* and *Fusarium* isolates collected.

In a previous study, many authors stated that beta-tubulin is important for the identification and differentiation in *Aspergillus* species (Shokouhi et al., 2011; Yaguchi et al., 2007; Balajee et al., 2005; Hong et al., 2005) but the β-tubulin gene is known to have more variation in *Penicillium* and it's currently considered as reliable alternative marker for differentiation of the *Penicillium* species (Houbraken and Samson, 2011). Microscopic identification of all *Aspergillus* and *Penicillium* species on morphology criterion was showed to be very problematic and often impossible. Relying on sequences size, β–tubulin, allowed for accurate and rapid recognition of the isolates in our study like Abastar et al. had demonstrated (Abastabar et al., 2014; Frisvad and Samson, 2004).

In the present study, a combination of the morphological identification key and TEF1-α gene amplication were used to identify the *Fusarium* isolates. There are limits to the use of morphological characters for identification the *Fusarium* complex. The authors as Bakar et al. (2013); Kvas et al. (2009) and Geiser et al. (2004) are identified many species of Fusarium with TEF1-α gene and establishing phylogenetic and taxonomic profile of *Fusarium* spp isolates (Kristensen et al., 2005; Geiser et al., 2004).

Rapid and accurate detection of potentially allergenic pathogenic moulds at the species is essential for the prevention and treatment of allergies (Tsui et al., 2000). Molecular approaches focusing on a partial β-tubulin sequence and elongation factor 1α (TEF-1α) are required to clarify the complete spectrum of the species.

4. Conclusion

Morphological identification is essential to distinguish the cultural characters of moulds. In this study, the β-tubulin and Elongation Factor (TEF-α) allowed the determination of sequence sizes corresponding to the different isolates of *Penicillium, Aspergillus* and *Fusarium.* Thus, molecular characterisation is proving to be an efficient tool to confirm the morphological identification of molds.

Conflicts of interest

The authors have no conflicts of interest to disclose.

References

Abastabar, M., Mirhendi, H., Rezaei-Matehkolaei, A., Shidfar, M.R., Kordbacheh, P., Makimura, K., 2014. Restriction analysis of beta-tubulin gene for differentiation of the common pathogenic dermatophytes. J. Clin. Lab. Anal., 28, 91-96.

Anaya, M., Sofıa, F.B., Gamez, E., Castro, M., Molina, A., Valdès, O., 2016. Viable fungi in the air of indoor environments of the National Archive of the Republic of Cuba. Aerobiol., 32, 513-527.

Bakar, A.A.I., Izzati, M.Z.N.A., Kalsom, U.Y., 2013. Diversity of Fusarium species associated with post-harvest fruit rot disease of tomato. Sains Malaysiana, 42, 911-920.

Balajee, S.A., Gribskov, J.L., Hanley, E., Nickle, D., Marr, K.A., 2005. Aspergillus lentulus sp. Nov. a new sibling species of A. fumigatus. Eukaryot Cell, 4, 625-632.

Bialek, R., Konrad, F., Kern, J., 2005. PCR based identification and discrimination of agents of mucormycosis and aspergillosis in paraffin wax embedded tissue. J. Clin. Pathol., 58, 1180-1184.

Bougnoux, M.E., Espinasse, F., 2003. Nouvelles applications des techniques de biologie moléculaire en mycologie médicale. Revue Française des Laboratoires, 351, 67-71.

Ceylan, E., Ozkutuk, A., Ergor, G., Yucesoy, M., Itil, O., Caymaz, S., 2006. Fungi and indoor conditions in asthma patients. J. Asthma, 43, 789-794.

El-Morsy, E.S.M., 2006. Preliminary survey of indoor and outdoor airborne microfungi at coastal buildings in Egypt. Aerobiol., 22, 197-210.

Frisvad, J.C., Samson, R.A., 2004. Polyphasic taxonomy of Penicillium subgenus Penicillium. A guide to identification of food and airborne terverticillate Penicillia and their mycotoxins. Stud. Mycol., 49, 174.

Geiser, D.M., Jiménez-Gasco, M.M., Kang, S., Makalowska, I., Veeraraghavan, N., Ward, T.J., Zhang, N., Kuldau, G.A., O'Donnell, K., 2004. FUSARIUM-ID v. 1.0: A DNA sequence database for identifying Fusarium. Eur. J. Plant Pathol., 110, 473-479.

Glass, N.L., Donaldson, C.G., 1995. American society for microbiology development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Appl. Environ. Microbiol., 61, 1323-1330.

Hong, S.B., Go, S.J., Shin, H.D., Frisvad, J.C., Samson, R.A., 2005. Polyphasic taxonomy of *Aspergillus fumigatus* and related species. Mycol., 97, 1316-1329.

Horner, W.E., Helbling, A., Salvaggio, J.E., Lehrer, S.B., 1995. Fungal allergens. Clin. Microbiol. Rev., 8, 161-179.

Houbraken, J., Samson, R.A., 2011. Phylogeny of Penicillium and the segregation of Trichocomaceae into three families. Stud. Mycol., 70, 1-51.

Husain, S., Kwak, E.J., Obman, A., 2004. Prospective assessment of Platelia Aspergillus galactomannan antigen for the diagnosis of invasive aspergillosis in lung transplant recipients. Am. J. Transplant., 4, 796-802.

Johnston, C.L., 2008. Identification of Penicillium species in the South African litchi export chain. Department of Microbiology and Plant Pathology, Faculty of Natural and Agricultural Sciences, University of Pretoria, Pretoria, South Africa, 123p.

Klich, M.A., 2002. Identification of common Aspergillus species. Utrecht, The Netherlands: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands, 116p.

Kristensen, R., Torp, M., Kosiak, B., Holst-Jensen, A., 2005. Phylogeny and toxigenic potential is correlated in Fusarium species as revealed by partial translation elongation factor 1 alpha gene sequences. Mycol. Res., 109, 173-186.

Kvas, M., Marasas, W.F.O., Wingfield, B.D., Wingfield, M.J., Steenkamp, E.T., 2009. Diversity and evolution of Fusarium species in the Gibberella fujikuroi complex. Fungal Divers., 34, 1-21.

Laforgue, R., Guérin, L., Pernelle, J.J., Monet, C., Dupont, J., Bouix, M., 2009. Evaluation of PCR-DGGE methodology to monitor fungal communities on grapes. J. Appl. Microbiol., 107, 1208-1218.

N'Gou, M.E.R.E., Yapo-Crezoit, C.C.A., Bonouman, A.V., Kouao, D., Djaman, A.J., Dosso, M., 2021. Biodiversité des Moisissures - Abidjan - Côte d'Ivoire. Moulds Biodiversity in Abidjan - Côte d'Ivoire. Revue française d'allergologie. 61(5), 351-356.

Nasrı, T., Hedayatı, M.T., Abastabar, M., Pasqualotto, A.C., Armakı, M.T., Nabılı, M., 2015. PCR-RFLP on β-tubulin gene for rapid identification of the most clinically important species of Aspergillus. J. Microbiol. Meth., 117, 144-147.

NF EN ISO 16000-19 Novembre, 2014. Air intérieur - Partie 19: stratégie d'échantillonnage des moisissures.

NF ISO 16000-21 Février, 2014. Air intérieur-Partie 21: détection et dénombrement des moisissures - Échantillonnage à partir de matériaux.

O'Donnell, K., Kistler, H.C., Cigelnik, E., Ploetz, R.C., 1998. Applied biological sciences multiple evolutionary origins of the fungus causing Panama disease of banana: Concordant evidence from nuclear and mitochondrial gene genealogies. Proc. Natl. Acad. Sci. USA, 95, 2044-2049.

Oliveira, M., Ribeiro, H., Abreu, I., 2005. Annual variation of fungal spores in atmosphere of Porto: 2003. Ann. Agr. Environ. Med., 12, 309-315.

Ozkara, A., Ocak, I., Korcan, S.E., Konuk, M., 2007. Determination of fungal air spora in Afyonkarahisar, Turkey. Mycotaxon, 102, 199-202.

Pasqualetti, M., Giovannini, V., Barghini, P., Gorrasi, S., Fenice, M., 2020. Diversity and ecology of culturable marine fungi associated with Posidonia oceanica leaves and their epiphytic algae Dictyota dichotoma and Sphaerococcus coronopifolius. Fungal Ecol., 44, 100906.

Pei- Chih, W., Huey-Jen, S., Chia-Yin, L., 2000. Characteristic of indoor and outdoor airborne fungi at suburban and urban homes in two Season. Sci. Total Environ., 253, 111-118.

Pepeljnjak, S., Segvic, M., 2003. Occurrence of fungi in air and on plants investigation of different climatic regions in Croatia. Aerobiol., 19, 11-19.

Plewa-Tutaj, K., Lonc, E., 2014. Molecular identification and biodiversity of potential allergenic molds (Aspergillus and Penicilium) in the poultry house: first report. Aerobiol., 30, 445-451.

Samson, R.A., Houbraken, J., Thrane, U., Frisvad, J.C., Andersen, B., 2010. Food and indoor fungi. Centraalbureau voor Schimmelcultures (CBS-KNAW Fungal Biodiversity Centre: Utrecht, The Netherlands). 390p.

Sanchez Espinosa, K.C., Almaguer Chavez, M., Duarte-Escalante, E., Rojas Flores, T.I., Frias-De-Leon, M.G., Reyes-Montes, M.D.R., 2021. Phylogenetic identification, diversity, and richness of aspergillus from homes in Havana, Cuba. Microorganisms, 9, E115.

Shokouhi, G.R., Mirhendi, S.H., Kordbachcheh, P., Nikaein, M., Matehekolaei, A.R., Abbastabar, M., 2011. Morphological and genotypic identification of some environmental isolates of Aspergillus in Iran based on beta-tubulin gene sequencing. J. Isfahan Med. Sch., 166, 1-10.

Simon-Nobbe, B., Denk, U., Pöll, V., Rid, R., Breitenbach, M., 2008. The spectrum of fungal allergy. Int. Arch. Allergy Immunol., 145, 58-86.

Tsui, K.M., Hyde, K.D., Hodgkiss, I.J., 2000. Biodiversity of fungi on submerged wood in Hong Kong streams. Aquat. Microb. Ecol., 21, 289-298.

Twaroch, T.E., Curin, M., Valenta, R., Swoboda, I., 2015. Mold allergens in respiratory allergy: From structure to therapy. Allergy Asthma Immunol. Res., 7, 205-220.

Verscheure, M., Lognay, G., Marlier, M., 2002. Revue bibliographique: les méthodes chimiques d'identification et de classification des champignons. Biotechnol. Agron. Soc. Environ., 6, 131-142.

Yaguchi, T., Horie, Y., Tanaka, R., Matsuzawa, T., Ito, J., Nishimura, K., 2007. Molecular phylogenetics of multiple genes on Aspergillus section Fumigati isolated from clinical specimens in Japan. Nippon Ishinkin Gakkai Zasshi. 48, 37-46.

How to cite this article: N'Gou, M.E.R.E., N'Golo, D.C., Yapo-crezoit, C.C.A., Bonouman, A.V., Kakou, S., Yapo, O.B., Djaman, A.J., Dosso, M., 2021. Molecular characterization of potential allergenic molds (*Aspergillus*, *Penicillium* and *Fusarium*) in two buildings receiving the public in Abidjan – Côte d'Ivoire. Scientific Journal of Microbiology, 7(2), 181-187.