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Cladosporium fungi isolated from Bali Dog's skin

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Abstract
Fungal diseases in animals are often ignored, but the economic losses due to fungal infections are quite disturbing, damage to skin, fur and the potential for zoonoses need to be considered. A lot of fungi are widespread in the environment but many of their species are not well understood especially their roles in human and animal health. This research aims to study further about the identification process using morphology and molecular method of *Cladosporium* fungi that found in dog's skin. The sample for this study was a fungal isolate from the skin of a Bali dog which was suspected to be infected with a fungus. Identification is carried out by microscopic and macroscopic observations using Saboraud Dextrose Agar (SDA) media. The molecular methods are using the PCR ITS1 and ITS4 primers and GeneJET PCR Purification Kit. The result showed that 30% of the samples are identified as *Cladosporium* fungi, with the specific species is *Cladosporium tenuissimum*.

Keywords: Bali dog's skin, *Cladosporium*, *Cladosporium tenuissimum*, fungi

Introduction
Fungal infections in beloved animals, especially dogs, still occur frequently. The system of keeping dogs in the wild, especially in Bali, Indonesia is still a trigger for disease infections, apart from the dangerous rabies, fungal diseases can also be transmitted between dogs or spread to humans. Fungal infections in dogs are dominated by several agents, including *Microsporum* and *Trichophyton*, including *M. canis*, *M. gypseum*, and *T. mentagrophytes* (Miller *et al*. 2013) [1]. Apart from that, there are several opportunistic fungi such as *Aspergillus* and *Curvularia* which are also often found on dog skin, with a prevalence of 54.84% and 12.9% respectively in Balinese Kintamani dogs species, as well as *Penicillium* fungi with a total of 6.45% (Sudipa *et al*, 2022) [2]. In Bali local dogs the prevalence of *Curvularia* fungus was found about 19% (Sudipa *et al*, 2021) [3]. With its low mortality, fungal diseases are often ignored, but the economic losses due to fungal infections are quite disturbing, damage to skin, fur and the potential for zoonoses need to be considered (Kotnik, 2007) [4].

The *Cladosporium* fungi role is not well understood in human and animal. Their small conidia are easily dispersed, making them one of the most common air-borne microorganisms (De Hoog *et al*. 2011) [5]. They are among the most important allergenic fungi linked to allergic rhinitis and respiratory arrest in asthmatic patients (Sellart-Altisent *et al*. 2007) [6]. Some species are described as a cause of opportunistic phaeohyphomycosis, including subcutaneous and deep infections in humans and animals (Sandoval-Denis *et al*. 2015) [7]. *Cladosporium* species are not producing damages, and they are of great interest for specific medical effects, such as beneficial human health effects (Almatar & Makky, 2016) [8], agro-industrial applications (Uma *et al*, 2012) [9] and in the degradation of keratin containing wastes from natural environment (Nwadiaro, 2015) [10]. Species identification in *Cladosporium* has always relied on the morphology of the conidiogenous apparatus together with data on host ranges. Traditionally, those dematiaceous fungi showing branched acropetal chains of aseptate to septate conidia were included in *Cladosporium*, which has made it a large and complex group of fungi difficult to differentiate (Bensch *et al*. 2012) [11]. The widespread of *Cladosporium* in the environment lead to some infection potential question to animals.

To begin with, it is necessary to know the detailed species of the fungus found so that its handling and infection potential can be known early. So far, fungal identification has only been carried out by morphological identification by looking at the shape of the colony, color, shape, size of the hyphae and microscopically by looking at the shape of the mycelium which has been stained with lactophenol cotton blue (Gaddeyya *et al*., 2012) [12]. However, morphological identification often encounters difficulties such as: the length of time needed for identification, the need for high identification skills, and the large number of similar morphologies within one species (Liu *et al*., 2000) [13]. Molecular identification is the answer to this problem, but is still rarely carried out on fungi of animal origin. Molecular identification of fungal DNA can provide certainty of identification to the species level (Landeweert *et al*., 2003) [14], besides that it can be done quickly, efficiently, reproducibly, and provides high specificity for distinguishing between species and subspecies of fungi compared to morphological and biochemical tests (Liu *et al*., 2000) [15]. By knowing specifically, the fungal species that are often found on dog's skin using molecular identification techniques, the characteristics of the fungus found can be studied further, whether the fungus is normal flora that is found on the skin or other infections, zoonotic potential can also be seen through specific identification.

Materials and Methods

Sample

The sample for this study was a fungal isolate from the skin of a Bali dog which was suspected to be infected with a fungus that attacks the skin, hair and nails of dogs. It is characterized by a superficial skin infection confined to the keratinized epithelium (Ganguly *et al*., 2017) [16]. Some symptoms include local wet spots in various areas of the dog's body, crusty skin on the nails, skin folds, armpits and anal area.

Culture Method

Identification is carried out by microscopic and macroscopic observations, a skin swab is taken from the skin of an infected dog using the Mackenzie method, a sterile toothbrush is gently rubbed from the dog's skin onto Saboraud Dextrose Agar (SDA) media (Abdalla&Wisal, 2018) [17], SDA is mixed with gentamicin to prevent bacterial growth. After 3-5 days of incubation of the media at 25-27 °C, the media will be examined based on the morphological characteristics of the colony: mycelial aspect (cotton or thin), border shape (regular or irregular), colony coloration (black, moss green, or gray), the back side of the colony (with or without dark pigmentation), and the absence or presence of conidia (Santos *et al*. 2018) [18]. Microscopic identification will be carried out using clear tape pressed into the colony and placed onto a slide with one drop of Methylene Blue dye. The slides will be examined using a light microscope (400X) to identify the growing fungus.

Molecular Method

Molecular examination of fungi can be carried out using the PCR method with universal primers, using ITS1 and ITS4 (White *et al*., 1990)[19] and Gene JET PCR Purification Kit. The process followed by electrophoresis, sequencing and analysis using the Bioinformatics method Basic Local Alignment Search Tool (BLAST) (Sandy *et al*. 2015) [20] and MEGA11 program. All ITS sequences were aligned using CLUSTALW in MEGA to build a phylogenetic tree using the Neighbor-Joining method with a bootstrap replication value of 1000 (Tamura *et al*. 2013) [21].

Results and Discussion

From 20 SDA media plate that used in this research, 6 (30%) media showed a green colony and branched conidiophores (Fig.1) that identified as *Cladosporium* fungi according to Nam *et al* (2015) [22] that found the *Cladosporium* fungi formed olivaceous-green to olivaceous-brown, velvet-like colonies with apically and laterally branched conidiophores and lemon-shaped conidia. De Hoog (2000) [23] also identified *Cladosporium* colonies are olive-green to olive-brown and appear velvety or powdery. This research result also aligns with Bensch *et al* (2010) [24] findings that showed the *Cladosporium* colonies are olive-grey to dull green, velvety and tufted. The edges of the colony can be olive-grey to white, and feathery. The colonies are diffuse and the mycelia form mats and rarely grow upwards from the surface of the colony. There are three species that are morphologically very similar and often misidentified which are *C. tenuissimum* that has rarely reported, *C. cladosporioides* and *C. tenuissimum*. These are common saprobic species isolated from numerous substrates.

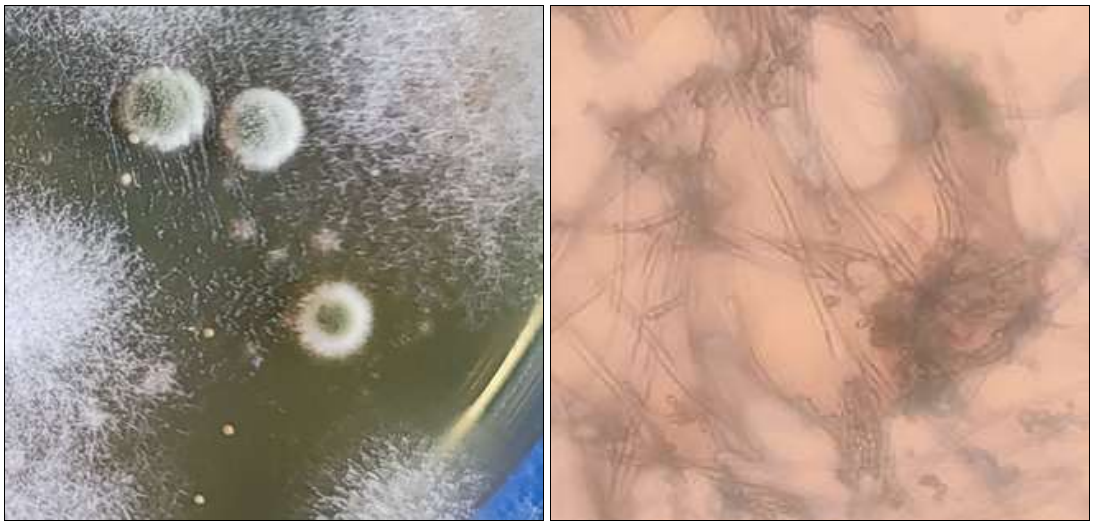

Fig 1: *Cladosporium* colony on SDA media and their microscopic view

The result of BLAST test showed that the species are *Cladosporium tenuissimum*, with 99.39% similarity. *Cladosporium anthropophilum* also resembles *C. tenuissimum*, a species previously described as human opportunistic pathogen (De Hoog *et al*. 2011) [25]. However both are genetically well differentiated (99.3%, 87.7% and 89.9% similarity for ITS, tef1 and actA, respectively) and, morphologically, *C. anthropophilum* shows longer terminal conidia (3.5–9 µm long (av. (± SD) 5.6 (± 1.2)) vs (2–)2.5–5(–6) µm long (av. (± SD) 3.7 ± 1.0)) in *C. tenuissimum*) and shorter intercalary conidia (4.5–11 µm long (av. (± SD) 6.9 (± 1.8)) vs 4–12(–17) µm long (av. (± SD) 8.1 (± 2.7)) in *C. tenuissimum*) (Bensch *et al*. 2012) [26]. The antagonistic capability of *C. tenuissimum* was confirmed by inhibiting the germination of propagules of other rust fungi, as *Peridermium pini* and *Cronartium flaccidum* (Moricca *et al*, 2001) [27], and, also the germination and mycelial growth of several fungi like, *Alternaria alternata*, *Botrytis cinerea*, *Mucor sp.*, *Rhizoctonia solani* (Moricca *et al*, 2005) [28].

Cladosporium species are present in the human mycobiome but are rarely pathogenic to humans. They have been reported to cause infections of the skin and toenails as well as sinuses and lungs, with more common symptoms including nasal congestion, sneezing, coughing, and itchy eyes. Mold sensitization is also a major risk factor for developing upper and lower respiratory diseases such as allergic rhinitis and allergic asthma (Simon *et al*, 2008)[29]. *Cladosporium* fungi is rarely reported found in animal, specifically in dog's skin. These findings are expanded the list of *Cladosporium* species as potential opportunistic fungi (De Hoog *et al*, 2015) [30]. These fungi maybe accidentally found in dog skin because most of the samples are stray or free roaming dog that have contact with soil that contain *Cladosporium*. Fungi of the genus *Cladosporium* are cosmopolitan organisms. Their spores can be found in air, soil and water. However, some genera are pathogens of various plants and people (Ogórek, 2012) [31]. They can manifest in the form of food allergy, contact allergy, allergies to antibiotics or an allergic reaction in the case of fungal infection existing in the body of focus. It should be noted that fungal spores are among the most widely represented biological molecules in atmospheric air. They considerably outweigh the number of pollen grains present in the air (Lipiec, 2002) [32]. While it's considered a seasonal outdoor mold and is found worldwide mainly on plants, in soil, and on food, it can also colonize a host of indoor surfaces (Piecková & Jesenská, 1999) [33] In fact, *Cladosporium* is among the top three most common indoor airborne fungi (along with *Penicillium* and *Aspergillus*). Further study of these species is needed to uncover their role in animal health.

Conclusion

The result of this research showed that 30% of the samples are identified as *Cladosporium* fungi, with the specific species is *Cladosporium tenuissimum* that validated using molecular test.

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Conflict

The authors declare no conflicts of interest regarding the publication of this paper.

Reference

1. Miller WH, Craige EG, Karen LC. Muller & Kirk's Small Animal Dermatology. 7th ed. Missouri (US): Elsevier; c2013.
2. Sudipa PH, Gelgel KTP. Prevalence and types of fungi in Kintamani Bali dogs. Int J Vet Sci. 2022;11(3):378-383. https://doi.org/10.47278/journal.ijvs/2021.131
3. Sudipa P, Gelgel K, Jayanti P. Identifikasi dan Prevalensi Jamur Curvularia pada Anjing dan Kucing di Kabupaten Badung, Bali Tahun 2020. Indonesia Medicus Veterinus; c2021. p. 432-440. doi:10.19087/imv.2021.10.3.432
4. Kotnik T. Dermatophytoses in Domestic Animals and Their Zoonotic Potential. Slovenian Vet Res. 2007;44(3):63-73.
5. De Hoog GS, Guarro J, Gené J, *et al*. Atlas of clinical fungi. CD-ROM version 3.1. 2011. CBS-KNAW Fungal Biodiversity Centre, Utrecht, Netherlands.
6. Sellart-Altisent M, Torres-Rodríguez JM, Gómez de Ana S, *et al*. Nasal fungal microbiota in allergic and healthy subjects. Rev Iberoam Micología. 2007;24(2):125-130. [In Spanish.]
7. Sandoval-Denis M, Sutton DA, Martin-Vicente A, *et al*. Cladosporium species recovered from clinical samples in the United States. J Clin Microbiol. 2015;53(9):2990–3000.
8. Almatar M, Makky E.A. *Cladosporium cladosporioides* from the perspectives of medical and biotechnological approaches. 3 Biotech. 2016;6:4.
9. Uma C, Gomathi D, Ravikumar G, Kalaiselvi M, Palaniswamy M. Production and properties of invertase from a *Cladosporium cladosporioides* in SmF using pomegranate peel waste as substrate. Asian Pac J Trop Biomed. 2012;2(2):S605–S611.
10. Nwadiaro P, Ogbonna A, Ponchang W, Adekojo D. Keratinolytic activity of *Cladosporium* and *Trichoderma* species isolated from barbers' landfill. Int J Biosci. 2015;6:104–115.
11. Bensch K, Braun U, Groenewald JZ, *et al*. The genus Cladosporium. Stud Mycol. 2012;72:1–401.
12. Gaddeyya G, Niharika PS, *et al*. Isolation and identification of soil mycoflora in different crop fields at Salur Mandal. Adv Appl Sci Res. 2012;3(4):2020-2026.
13. Liu D, Coloe S, Baird R, Pedersen J. Application of PCR to the identification of dermatophyte fungi. J Med Microbiol. 2000;49(6):493-497.
14. Landeweert R, Leeflang P, Kuyper TW, Hoffland E, Rosling A, Wernars K, *et al*. Molecular identification of ectomycorrhizal mycelium in soil horizons. Appl Environ Microbiol. 2003;69(1):327-333.
15. Liu D, Coloe S, Baird R, Pedersen J. Application of PCR to the identification of dermatophyte fungi. J Med Microbiol. 2000;49(6):493-497.
16. Ganguly S, Para P, Shoukat S. Mycological investigation of dermatophytosis in Dog: A case study. J Entomol Zool Stud. 2017;5(1):970-971. https://doi.org/10.22271/j.ento.5.1.203.1.
17. Abdalla, Wisal G. An overview of canine dermatophytosis. South Asian J Res Microbiol.

2018;2(2):1-16.
18. Santos PRRD, Leão EU, Aguiar RWDS, Melo MPD, Santos, GRD. Morphological and molecular characterization of *Curvularia lunata* pathogenic to Andropogon grass. Bragantia. 2018;77:326-332.
19. White TJ, Bruns T, Lee S, Taylor J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols: a guide to methods and applications. Academic Press, San Diego; c1990. p. 315–322.
20. Sandy YA, Djauhari S, Sektiono AW. Identifikasi Molekuler Jamur Antagonis *Trichoderma harzianum* Diisolasi Dari Tanah Pertanian Di Malang, Jawa Timur. Jurnal HPT, 2015, 3(3). ISSN: 2338 - 4336. http://repository.ub.ac.id/id/eprint/130177
21. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol; c2013. doi:10.1093/molbev/mst197
22. Nam MH, Park MS, Kim HS, Kim TI, Kim HG. *Cladosporium cladosporioides* and *C. tenuissimum* Cause Blossom Blight in Strawberry in Korea. Mycobiology. 2015;43(3):354–359. https://doi.org/10.5941/MYCO.2015.43.3.354
23. De Hoog, Gerrit S. Atlas of clinical fungi (2. ed.). Netherlands: Amer Society for Microbiology, 2000, 1-1126. ISBN 9070351439.
24. Bensch K, Groenewald JZ, Dijksterhuis J, Starink-Willemse M, Andersen B, Summerell BA, *et al*. Species and ecological diversity within the *Cladosporium cladosporioides* complex (Davidiellaceae, Capnodiales). Stud Mycol. 2010;67:1-94.
25. Moricca S, Ragazzi A, Mitchelson KR, Assante G. Antagonism of the two-needle pine stem rust fungi *Cronartium flaccidum* and *Peridermium pini* by *Cladosporium tenuissimum* in vitro and in planta. *Phytopathology*. 2001;91(5):457–468.
26. Moricca S, Ragazzi A, Assante G. Biocontrol of Rust Fungi by *Cladosporium tenuissimum*. In *Rust Diseases of Willow and Poplar*; A4913:AMA:Pei:First Proofs: 17-Nov-2004; Pei, M.H., McCracken, A.R., Eds.; ©CAB International: Wallingford, UK; c2005. p. 213-229.
27. Simon-Nobbe B, Denk U, Pöll V, Rid R, Breitenbach M. The spectrum of fungal allergy. Int Arch Allergy Immunol. 2008;145(1):58-86. doi: 10.1159/000107578. Epub 2007 Aug 20. PMID: 17709917. Available from: https://www.karger.com/Article/FullText/107578.
28. De Hoog GS, Chaturvedi V, Denning DW, *et al*. Name changes in medically important fungi and their implications for clinical practice. Journal of Clinical Microbiology. 2015;53(4):1056–1062.
29. Ogórek R, Lejman A, Pusz W, Miłuch A, Miodyńska P. Characteristics and taxonomy of *Cladosporium* fungi. Mikologia Lekarska. 2012;19(2):80-85.
30. Lipiec A. Grzyby w etiologii chorób alergicznych. Alergol Współcz. 2002;1:10-14.
31. Piecková E, Jesenská Z. Microscopic fungi in dwellings and their health implications in humans. Ann Agric Environ Med. 1999;6(1):1-11. PMID: 10384209. Available from: https://pubmed.ncbi.nlm.nih.gov/10384209.

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