Contemporary Engineering Sciences, Vol. 11, 2018, no. 94, 4691 - 4699
HIKARI Ltd, www.m-hikari.com
https://doi.org/10.12988/ces.2018.88433

Identification of Fungi Dermatophytes in the Coastal Area of District of Riohacha, La Guajira

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Abstract

The epidemiology of diseases caused by microorganisms has received considerable attention, but a limited number of studies have determined the presence of microbial contaminants in beach sand. To identify presence of dermatophytes in the coastal area of the District of Riohacha, Colombia, we cultured a total of 16 parallel dry and wet sand samples (back beach- dry sand- and front beach-wet sand-); using hair brushing method, for isolation of dermatophytes, Riddel microculture technique, for sporulation, and double coverslip technique for identification of genus and species. Samples were collected from 8 sites during March 2016 (dry season). We observed growth of 4 anamorphic filamentous fungi species belonging to 3 different genera in 75% of the samples. The most frequently encountered genus was *Fusarium* sp. (31.25%), followed by *Aspergillus* sp. (12.5%), and *Dermatophyte* sp. (6.25%). The most relevant species was *Aspergillus flavus* (12.5%), the rest of species were distributed homogeneously with a percentage of 12.5%. These results demonstrate that high organic loads as well as human and animal intervention causes an impact in keratinophilic fungi and dermatophytes presence in beach sand.

Keywords: beach sand, anamorphic filamentous fungi, dermatophyte fungi, health risk, Riohacha

1 Introduction

Beaches represent an economic and social value, since they are destinations for leisure and recreation; thus significant concern exists to preserve its ecological balance [3] and [10]. Conventional tourism is perhaps the most common use of beaches around the world, but the constant use of any natural resource generates a negative impact to the environment [7].

Diversity in beach sand composition and texture favors the growth and proliferation of microbes [27] by generating ideal conditions for their growth [1]. Bacterial pathogens are not the only microorganism present in sand; pathogens include different groups of fungi (yeasts, pathogenic fungi, allergenic fungi and fungi dermatophytes) which represent a potential risk to the health of bathers. Bathers are in direct contact with the sand [14] specifically [15], infants and children are most vulnerable to the attack of these pathogens; symptoms include diarrhea and gastrointestinal disorders [5].

Fecal contamination on beaches is largely due to discharge from known point sources, particularly non- treated wastewater, and due to direct contact between sand and water through waves, wind, waste, and debris. Authors in [27] expand on contamination sources mentioning other human activities as main causes of beach sand contamination, examples of these activities are: deterioration or rupture of sewer lines, inadequate treatment of wastewater, surface runoff of contaminated water from gutters and pipes from urban centers and farms (agriculture).

Beach users are greatly responsible for beach sand contamination. In many cases they are carriers of microorganisms that remain in the sand. They also deposit organic waste including those from commercial activities such as fishing, which provide food and moisture necessary for the conservation and development of yeasts, bacteria, molds and keratinophilic fungi- fungi like *Aspergillus sp., Fusarium sp., Scytalidium sp. and Scopulariopsis sp*, among others-, that can infect skin and nails [28].

Keratinophilic fungi are anamorphic or conidial, meaning that they have asexual reproduction, these characteristic have contributed to elucidate fungal taxonomy [31]. [8] Indicate in their paper that fungal anamorphisms rapidly reproduces spores or conidia, requiring lower energy consumption compared to sexual propagules. Isolation of these fungi from soil samples have been generally done by the hair bait technique, introduced by Vanbreusegiiem in 1952 [21].

Colombia is one of the Caribbean destinations with the highest touristic activity, and the country's beaches are rapidly becoming essential parts of local economies [10] and [4]. For this reason, efforts have been made to stablish the environmental quality of the country’s beaches, but it has not been sufficient due to scarcity of data and absence of standardized monitoring mechanisms, as well as the lack of characte-

rization and records of keratinophilic fungi and dermatophytes distribution. Both groups represent a risk to the health of bathers in touristic beaches.

Beach sand in Riohacha can be a vector and ideal habitat for the proliferation of keratinophilic fungi and dermatophytes, because all of its multiple influences, its touristic character, and its great economic contribution to the province. These influences are: commercial (settlement of foreigners and bathers, street sales, preparation and direct retailing of sea products), recreational, harbor area (arrival of small boats to the dock), environmental (discharge of municipal wastewater, deposits of sediment from multiple activities, partial mixing with Rancheria's river water or complete opening of Rancheria's river mouth, and beach topography-induced stagnation of rain water).
Riohacha lacks researches that document presence of fungi in beach sand. This is a disadvantage to establish beach-society relationships. Bathers' exposure to beach sand contaminated with keratinophilic fungi and dermatophytes increase their risk to develop skin diseases [4]. Presence and proliferation of these pathogens harms touristic activity in the province leading to socioeconomic problems and burdens provincial public health department. Thus, it is advisable for both the government and beach environmental quality to invest in the prevention and mitigation of this problem.

In 2015, Riohacha city was declared special touristic and cultural district. In Colombia, these types of districts have tourism based economy, which requires high quality standards on its beaches. University of La Guajira, the highest public education institution in the province, undertook a pilot research to study whether there is presence of keratinophilic fungi and dermatophytes in approximately 1 km Riohacha's coastline. This study aims to provide baseline information to regional touristic institutions, health sector and to contribute to the growing body of research on fungi in beach sand.

2 Materials and methods

2.1 Sampling

We analyzed 16 samples in the coastal zone of Riohacha's District. This city is located on the Caribbean Sea shores of Southwestern La Guajira's province, over the western margin of Ranchería' River mouth, at 11° 33' 59 "north latitude and 72° 54' 37" west longitude. Parallel samples were collected in March 2016 (dry season) from 8 sampling sites along a transect taken across the beach, at a depth of 2cm using sand collection method and a grid of $1m^2$. After collection, samples were placed in sealed bag with sterile spoons, labeled and transported to the laboratory in a cold environment. In order to obtain a representative sample in 1 km of beach, samples were collected considering the influence of bathers, estuarine activities, fishing, and presence of pets and occasional discharges of wastewater from known point-sources.

2.2 Mycological analysis

Presence of keratinophilic fungi and dermatophytes was determined using hair brushing method of Vanbreusegiiem [35]. Sands samples were deposited in sterile Petri dishes, adding sterilized human hair, (Sterilization was performed in an autoclave at 120 °C for 45 min) and moistened with sterilized distilled water every 15 days for 2 months. Petri dishes were left at room temperature.

Sporulation of anamorphic fungi was achieved using Riddel microculture technique [18] and [36], on Potato Dextrose Agar (PDA) [2], when fungal growth was observed, these strains were placed with a flamed scalpel on the sides, corners and center of 1cm^2 of PDA squares and humidified with 3-5 mL of glycerinated water. Procedure was performed in a laminar flow cabin. In order to identify the fungal groups present in the samples, wet mount of fungal growth was carried out through the double coverslip method [37].

3 Results and Discussion

Out of 16 sand samples tested, 11 showed presence of keratinophilic fungi, and only 1 showed presence of dermatophytes (Table 1). We found 4 species belonging to 3 different genus. *Fusarium sp*. was the most frequently found genus (31.25%). *Fusarium sp*. is a group of filamentous fungi widely distributed in soil and plants, these are considered opportunistic pathogens able to cause systemic infections in immunocompromised patients [33]. Some of its species produce toxins that affect man and animals, but only 12 of the 100 species of *Fusarium* described are considered pathogenic for humans. The most relevant pathogenic species of this genus are *F. solani*, *F. oxysporum* and *F. verticillioides*,(listed in decreasing order of frequency) [20]. *Aspergillus sp* and *Aspergillus flavus* were the following isolates with greater frequency (12.5%), followed by *Dermatophyte sp, Aspergillus niger* and *Fusarium verticillioides* (6.25%).

Table 1. Anamorphic fungi distribution in Riohacha's District coastal zone

Sand Samples	Back-Beach (Dry Sand)	Front-Beach (Wet Sand)	Total	Distribution Percentage
Number of sand samples	8	8	16	
Number of positive samples	5	7	12	
Distribution (%)	62,5	87,5	75	
Dermatophytes sp	1	-	1	6,25
Aspergillus sp	-	2	2	12,5
Aspergillus flavus	1	1	2	12,5
Aspergillus niger	-	1	1	6,25
Fusarium sp	2	3	5	31,25
Fusarium verticillioides	1	-	1	6,25

In most cases, keratinophilic fungi and dermatophytes enter the environment through point and non-point sources, that include wastewater discharges, surface runoff and seweage [16], [25] and [17] and other factors such as humidity, wildlife, domestic animals, morphology of beaches, streams, waste and water containing pathogenic microorganisms and parasites [30], [28] and [32]. Species of keratinophilic fungi differ from one environment to another despite being endemic in most regions [13] and [18].

Fungi identified in this study are similar to those found in the beach sand of Madeira archipelago, Portugal [27]. But, we found an increase in potentially pathogenic and allergenic fungi, being *Penicillium sp*. the most frequent genus identified; followed by *Aspergillus sp.* and *Fusarium sp.*; some of these species may contain compounds in their spores that can cause irritation and sensitivity [23]. Our findings may be explained by the excessive rainfall that occurred in 2010. Excessive rainfall events caused sewer lines damage, that lead to waste being transported to ocean shores [28],

Other studies showed similar results to those reported in this research: on the Pakistan Coasts, the most commonly reported species were otomycosis causing agents such as *Aspergillus niger* (31.59%) and *Aspergillus flavus* (21.40%) [9], [26] and [6]. In Liguria, Italy [29] *Candida sp.* and *Aspergillus sp.* were the most frequently found genus, and displayed high sensitivity to critical environmental situations like large number of bathers visiting beaches. In India, species of dermatophytes and keratinophilic fungi were also reported, being *Aspergillus Flavus* the most frequent species [22]. Likewise, on the beaches of Olinda, Brazil, [11] researchers found that *Penicillium sp*. and *Aspergillus sp.* were the most common genus in both water and sand; In the coastal waters of northern Greece, filamentous fungi such as *Aspergillus niger* and *Aspergillus flavus* represented 57.4% of isolated species, followed by *Penicillium sp*. (68.5%) and *Alternaria sp*. (23.9%) [2], their presence was associated with significant numbers of total coliforms, fecal coliforms and enterococcus during high bathing season. Filamentous fungi counts were not significant in these seasons. In Mexican beaches, the species most frequently reported were *Aspergillus fumigatus*, *Aspergillus flavus-furcatis*, followed by *Fusarium solani*, and *Fusarium semitectum*. These species behaved as saprophytic and potentially pathogenic to beach users [12].

Aspergillus sp. and *Fusarium sp.* the most common non-dermatophyte keratinophilic fungi isolated on the beaches of different geographic regions [34], [24] and [19]. *Aspergillus niger, Aspergillus Flavus* and *Fusarium solani* are the most representative species worldwide. Identification of *Dermatophyte sp*. in Riohacha's back-beach (dry sand) indicates animal, and human intervention as well as high levels of residual organic load (keratinized substrates) in these beaches, given that back-beach zone accumulates pollution during floods, maintaining viable fungal spores, that are capable to parasitize keratinized tissue on skin, hair and nails of man and animals.

Acknowledgements. The authors give special recognition to PICHIHÜEL research group, for providing physical space and tools needed to fulfill this research. We would also like to give thanks to Msc Erisbel Samón Legrá for his support in the identification of the fungal groups.

References

[1] A.M. Abdelzaher, M.E. Wright, C. Ortega, H.M. Solo-Gabriele, G. Miller, S. Elmir, X. Newman., H. Shih, J.A. Bonilla, T.D. Bonilla, C.J. Palmer, T. Scott, J. Lukasik, V.J. Harwood, S. McQuaig, C. Sinigalliano, M. Gidley, L.R.W. Plano, X. Zhu, J.D. Wang, L.E. Fleming, Presence of pathogens and indicator microbes at a nonpoint source subtropical recreational marine beach, *Appl, Environ, Microbiol*, **76** (2010), no. 3, 724–732. https://doi.org/10.1128/aem.02127-09

[2] M. Arvanitidou, K. kanellou, V. Katsouyannopoulos and A. Tsakris, Occurrence and densities of fungi from northern Greek coastal bathing waters and their relation with faecal pollution indicators, *Water Research*, **36** (2002), no. 20, 5127-5131. https://doi.org/10.1016/s0043-1354(02)00235-x

[3] N. Beharry-Borg, R. Scarpa, Valuing quality changes in Caribbean coastal waters for heterogeneous beach visitors, *Ecol, Econ*, **69** (2010), no. 5, 1124-1139. https://doi.org/10.1016/j.ecolecon.2009.12.007

[4] C. Botero, G. Anfuso, N. Rangel-Buitrago and I. Correa, Coastal erosion monitoring in Colombia: overview and study cases on Caribbean and Pacific coasts, in *Coastal Erosion Monitoring*, 2013, 199-213.

[5] J. Brandão, B. Wergikosky, C. Rosado, G. Noronha, L. Rosado, C. Veríssimo et al, Qualidade Microbiológica de Areias de Praias Litorais: relatório final, *Portugal: Instituto do Ambiente*, (2002), 19-47

[6] M. Cattana, M. Fernández, F. Rojas, M. Sosa, G. Giusiano, Otomicosis por Aspergillus niger, Fac, M Imágenes Biomédicas.

[7] O. Cervantes, I. Espejel, E. Arellano, S. Delhumeau, Users' perception as a tool to improve urban beach planning and management, *Environ. Manag*, **42** (2008), no. 2, 249-264. https://doi.org/10.1007/s00267-008-9104-8

[8] G. Cole, B. Kendryck, Conidiogenesis and Conidiomatal Ontogeny, in *Biology of Conidial Fungi*, Acafemic Press, 1981, 271-327. https://doi.org/10.1016/b978-0-12-179502-3.50016-9

[9] I. Fozia, M. Suhail, H. Abro, Keratinophilic fungi from the soil of District, Jamshoro, Sindh, Pakistan, *Pakistan Journal of Botany*, **39** (2007), 1377-1382.

[10] B. Gavio, S. Palmer-Cantillo, J.E Mancera, Historical analysis (2000-2005) of the coastal water quality in San Andres Island, Sea Flower Biosphere Reserve, *Mar., Pollu., Bull.*, **60** (2010), no. 7, 1018-1030. https://doi.org/10.1016/j.marpolbul.2010.01.025

[11] D.N.F. Gomes, M.A.Q. Cavalcanti, M.J.S. Fernandez, D.M.M. Lima, J.Z.O. Passavante, Filamentous fungi isolated from sand and water of "Bairro Novo" and "Casa Caiada" beaches, Olinda, Pernambuco, Brazil, *Brazilian Journal of Biology*, **68** (2008), no. 3, 577-582. https://doi.org/10.1590/s1519-69842008000300016

[12] M. González, R. Hanlin, T. Herrera, M. Ulloa, Fungi colonizing hair-baits from three coastal beaches of Mexico, *Mycoscience.* **41** (2000), no. 3, 259-262. https://doi.org/10.1007/bf02489680

[13] A.K. Gupta, Y. Kohli, R. Batra, In vitro activities of posaconazole, ravuconazole, terbinafine, itraconazole and fluconazole against dermatophyte, yeast and non-dermatophyte species, *Med. Mycol.*, **43** (2005), 179–185. https://doi.org/10.1080/13693780410001731583

[14] C.D. Heaney, E. Sams, A.P. Dufour, K.P. Brenner, R.A. E. Haugland, S. Chern, S. Wing, D.C. Marshall, Love, M. Serre, R. Noble, T.J. Wade, Fecal indicators in sand, sand contact, and risk of enteric illness among beachgoers, *Epidemiology*, **23** (2012), 95-106. https://doi.org/10.1097/ede.0b013e31823b504c

[15] C.D. Heaney, E. Sams, S. Wing, S. Marshall, K. Brenner, A.P. Dufour, T.J. Wade, Contact with beach sand among beachgoers and risk of illness, *Am. J. Epidemiol*, **170** (2009), no. 2, 164–172. https://doi.org/10.1093/aje/kwp152

[16] D. Kay, J. Crowther, C.M. Stapleton, M.D. Wyer, L. Fewtrell, A. Edwards, C.A. Francis, A.T. McDonald, J. Watkins, J. Wilkinson, Faecal indicator organism concentrations in sewage and treated effluents, *Water Res.*, **42** (2008), no. 1-2, 442-454. https://doi.org/10.1016/j.watres.2007.07.036

[17] A. Keswani, D. Oliver, T. Gutiérrez, R. Quillian, Microbial hitchhikers on marine plastic debris: Human exposure risks at bathing waters and beach environments, *Marine Environmental Research*, **118** (2016), 10-19. https://doi.org/10.1016/j.marenvres.2016.04.006

[18] J. Khaled, H. Golah, A. Khalel, N. Alharbi, R. Mothana, Dermatophyte and non-dermatophyte fungi in Riyadh City, Saudi Arabia, *Saudi Journal of Biological Sciences*, **22** (2015), 604-609. https://doi.org/10.1016/j.sjbs.2014.12.006

[19] Min Lee, Joon Park, Hyun Chung, Jae Jun, Yong Bang, Distribution of Soil Keratinophilic Fungi Isolated in Summer Beaches of the East Sea in Korea, *Kor. J. Med. Mycol.*, **16** (2011), 44-50. https://doi.org/10.17966/kjmm.2011.16.2.44

[20] J. Leslie, B. Summerell, Practical approaches to identification, *The Fusarium* laboratory manual, 1st ed.,Blackwell Publishing, Ames, IA, USA, 2006, 101-110. https://doi.org/10.1002/9780470278376.ch11

[21] M. Marples, The distribution of keratinophilic fungi in soils from New Zealand, and from two polynesian Islands, *Mycopathologia Et Mycologia Applicata*, **25** (1965), no. 3-4, 361-372. https://doi.org/10.1007/bf02049922

[22] Y. Maruthi, D. Chaitanya, K. Hossain, A. Sravani, S. Jagadish, The Occurrence of Keratiophilic Fungi and Related Dermatophytesfrom sewage water discharge points, *European Journal of Experimental Biology*, **1** (2012), 13-16.

[23] M. Matavulj, N. Vulikiã, I. Gojkoviã, M. Karaman, Conditionally pathogenic fungi in recreational Waters, *Proc. Nat. Sci. Matica Srpska Novi Sad*, 109 (2005), 149-160. https://doi.org/10.2298/zmspn0519149m

[24] F. Migahed, Distribution of Fungi in the Sandy of Egyptian Beaches, *Pakistan Journal of Biological Sciences*, **6** (2003), no. 10, 860-866. https://doi.org/10.3923/pjbs.2003.860.866

[25] D.M. Oliver, K.D.H. Porter, A.L. Heathwaite, T. Zhang, R.S. Quilliam, Impact of low intensity summer rainfall on E. coli-discharge event dynamics with reference to sample acquisition and storage, *Environ. Monit. Assess.*, **187** (2015), 426. https://doi.org/10.1007/s10661-015-4628-x

[26] A.A. Padhye, *Fungi Pathogenic to Man and Animals*, Handbook of Microbiology, Palm Beach, FL, 1982.

[27] E. Pereira, C. Figueira, N. Aguiar, R. Vasconcelos, S. Vasconcelos, G. Calado, J. Brandão, S. Prada, Microbiological and mycological beach sand quality in a volcanic environment: Madeira archipelago, Portugal, Sci. *Total Environ.*, **461-462** (2013), 469–479. https://doi.org/10.1016/j.scitotenv.2013.05.025

[28] R. Cunha, M. Wergikoski, B. Ferreira, R. Rodrigues et al, Pathogenic fungi: An unacknowledged risk at coastal resorts? New insights on microbiological sand quality in Portugal, *Marine Pollution Bulletin*, **62** (2011), 1506-1511. https://doi.org/10.1016/j.marpolbul.2011.04.008

[29] V. Salvo, M. Fabiano, Mycological assessment of sediments in Ligurian beaches in the Northwestern Mediterranean: Pathogens and opportunistic pathogens, *Journal of Environmental Management*, **83** (2006), 365–369. https://doi.org/10.1016/j.jenvman.2006.04.001

[30] M.Z. Sato, M. Di Bari, C.C. Lamparelli, T.A. Cristina, M.C.L.S. Coelho, E.M. Hachich, Sanitary quality of sands from marine recreational beaches of São Paulo, Brazil, *Brazilian Journal of Microbiology*, **36** (2005), 321–326. https://doi.org/10.1590/s1517-83822005000400003

[31] K. Seifert, W. Gams, The Taxonomy of Anamorphic Fungi, *Systematics and Evolution*, Springer, 2001, 307-347. https://doi.org/10.1007/978-3-662-10376-0_14

[32] H. Solo-Gabriele, V. D. Harwood, Kay, R. Fujioka, M. Sadowsky, R. Whitman, J.C. Brandao, Beach sand and the potential for infectious disease transmission: observations and recommendations, *Journal of the Marine Biological Association of the United Kingdom*, **96** (2015), 101-120. https://doi.org/10.1017/s0025315415000843

[33] C. Tapias, J. Amaro, Género Fusarium, *Rev. Chilena Infecto.*, **31** (2014), 85-86. https://doi.org/10.4067/s0716-10182014000100012

[34] K. Ulfing, The occurrence of keratinolytic fungi in waste and waste-contaminated hábitats, *Revista Iberoamericana de Micología*, **699** (2000), 44-50.

[35] R. Vanbreuseghem, Technique biologique pour 1isolement des dermatophytes du sol, *Annals of the Societe Belge. Medical Tropicale*, **32** (1952), 173-178.

[36] P. Velez, M. González, E. Rosique-Gil, J. Cifuentes, M. Reyes-Montes, S. Apello-García, R. Hanlin, Community structure and diversity of marine ascomycetes from coastal beaches of the southern Gulf of Mexico, *Fungal Ecology*, **6** (2013), 513-521. https://doi.org/10.1016/j.funeco.2013.10.002

[37] B. Volkmann-Kohlmeyer, J. Kohlmeyer, How to prepare truly permanent microscope slides, *Mycologia*, **10** (1996), 107- 108. https://doi.org/10.1016/s0269-915x(96)80068-1

Received: August 27, 2018; Published: November 12, 2018