Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Scholars Research Library

Annals of Biological Research, 2012, 3 (5):2173-2180
(http://scholarsresearchlibrary.com/archive.html)

Annals of Biological Research
Scholars Research Library
ISSN 0976-1233
CODEN (USA): ABRNBW

Oral fungal diversity (mycobiome) in healthy individuals from rural and urban areas from the neovolcanic axis in Puebla, Mexico

Munguia-Pérez R.[1, 2*], Martínez-Contreras R. D[2]., Xicotencatl-Rosas S[2]., Duarte-Escalante E.[3], Castañeda-Roldan E. I.[1, 2].

[1]Posgrado en Ciencias Ambientales, [2]Centro de Investigaciones en Ciencias Microbiológicas. Instituto de Ciencias de la Benemérita Universidad Autónoma de Puebla, México. [3]Depto. de Microbiología y Parasitología, Facultad de Medicina, Universidad Nacional Autónoma de México.

ABSTRACT

The relation between human beings and their oral microflora initiates little after birth and lasts throughout life. Oral microbes, like fungi, are a complex community. Environmental fungi, mold, and yeast enter the oral cavity through air, food and fomites, among others. The oral microbiome and its fungal component (mycobiome) are critical health and disease component; however little is known and it has not been characterized yet. In this study there have been cultivated an identified fungi from saliva samples from a hundred healthy subjects who live in the State of Puebla, determining oral fungal biodiversity using Shannon´s index. The species richness in the cultivable basal mycobiome conformed by 241 filamentous and yeast fungi including 16 genera and 29 species (environmental, pathogenic and opportunistic); of which 81.25% corresponded to phylum Ascomycota, 12.5% Zygomycota and 6.25% Basidiomycota. Being Candida albicans (18.67%), Penicillium sp (12.03%) and Cladosporium sp (9.96%) the dominant species. Obtaining a Shannon diversity index H´: 0.99. This study identified the cultivable basal mycobiome in healthy subjects of various populations from the neovolcanic axis in the state of Puebla, which is proposed as an indicator of the risk in development of fungal infections.

Key words: fungal diversity, oral cavity, mycobiome.

INTRODUCTION

Fungi are cosmopolitan organisms that colonize and remain in various natural environments that include from the ground to the oral human cavity. However, its distribution varies according to the various regions in the world, weather and local specific environments [35]. In the human body, some of them may be encountered colonizing the skin and annexes, oral cavity and mucosal. Nonetheless, the ones in the environment can enter through abrasion, implantation, inhalation or drinking water, contact with fomites, dust particles, bioaerosols or contaminated food [4, 27]. Other interactions between fungi and human health are respiratory allergies, sick building syndrome and establishment of endemic areas. Exposition to spores or its metabolites may have potentially negative effects in health both public and environmental [29]. The oral cavity it is a complex habitat for microorganisms in healthy subjects, and to understand the nature of this interaction it is required to know the microbial community in the oral cavity of healthy individuals. It has been reported that the endogenic fungal flora plays an important role in nutrition, carcinogenesis and resistance to colonization by opportunistic microorganisms [10]; the mycobiome is the fungal component that resides in the oral cavity and generally are saprobiotic fungi, but some of them can be pathogenic and/or opportunistic, therefore they are considered critical components in the generation of mycosis, and they have been proposed as triggering indicators for having influence in oral diseases, especially in opportunistic mycosis, principally candidosis, which are exacerbated in individuals with some immunocompetence or inmunocompromised, but can be present in healthy individuals in 25 to 30% [19]. The species of *Candida*

constitute the oral mycobioma of humans and its opportunism is influenced by many factors like immunological state, hygiene, dental amalgams, nutritional, cultural, geographic and environmental state, among others, as it has been described by Ghannoum *et al*., (2010) [16]. Also, social and economic factors, lifestyle and globalization, are critical in the association of certain species of *Candida* that reside in the oral cavity of individuals from various geographic areas, with no hygiene habits, which have more morbidity as it has been demonstrated by Samaranayake *et al*., (1982) [34]. The state of Puebla is in the center of the Mexican republic; it has an area of 33,995 km^2 and part of the state is within the neovolcanic axis, which confides it geographic characteristics that favor the existence of microclimates, with dry areas in the southwest, temperate in the center and tropical in the northeast and southeast; inhabited mostly by farmers with low education level and low income *per capita*, giving origin to urban and rural areas with various lifestyles and different levels of marginalization [9, 17]. Therefore, the objective of this study was to describe and quantified the biodiversity of the basal cultivable mycobiome on the oral cavity of healthy individuals in rural and urban populations in the neovolcanic axis in Puebla (PNA).

MATERIALS AND METHODS

Ethics report

An informed consent was obtained from all the participants. The protocol was approved by the ethics committee of the Environmental Sciences postgraduate program from the Science Institute of University of Puebla.

Participants

They were included 100 healthy volunteers, without signs or symptoms of disease, over 18 years old, nonsmokers, and without any antifungal medication. The participants were from four rural areas, Chignahuapan, Emilio Portes Gil (EPG), Tehuitzingo y Tepeaca (20 individuals from each place) and 20 individuals, were from de city of Puebla, corresponding to an urban area. The samples of each individual were obtained in the time period from October 2010 to September 2011. The five geographic areas are found within the PNA (Figure 1).

Social and economic conditions

The four rural areas analyzed, were marginalized areas, with little resources, well water consumption, latrines and almost null hygienic measures. The urban non marginalized area (Puebla city), with a modern lifestyle (electricity, drinking water and all the services). Data from the National Population Council (CONAPO) and National Institute of Statistics and Geography (INEGI) was consulted to know the marginalization indexes and the economic activities respectively, from the studied geographic areas [9, 17].

Sampling from the oral cavity

Samples were obtained from saliva of the participants; they were collected using volumetric technics, under fasting conditions, without having a prior cleaning, approximately at the same hour. For the sampling, the individuals were asked to deposit the saliva in plates containing Dextrose Sabouraud Agar (Bioxon®, México), short after; they were transferred to the laboratory in containers with a temperature of 4°C, and they were seeded by surface extension. Later the plates were incubated at 28°C for 20 days, keeping a daily journal.

Identification

For the identification of filamentous fungal, reseedings were realized, until axenic cultures were obtained, and from these, microcultures technique were made, which were stained with cotton blue and identified through a microscopic test (40x), following taxonomic keys described in specialized texts [6, 12, 30]. Isolated yeasts were identified phenotypically and through a standard identification system API® 20C AUX (Biomerieux, México), the *Candida* species were reseeded in a chromogenic agar (CHROMAgar Candida PPM®, México) and in the API® Candida system (Biomerieux, México).

Biodiversity index

The Shannon index was used [21] $H' = -^{S}\sum_{i=1} p_i \log_2 p_i$ from S to n_i where: S= number of species (species richness) p_i = proportion of individuals from species *i* in regard to total of individuals (relative abundance of species *i*): n_i/N, n_i = number of individuals from species *i*, N = total of individuals from all the species.

Statistic analysis

A descriptive analysis of the data was realized with Statgraphics plus 5.1. The variables were presented with their absolute frequency and percentage. The Kruskal-Wallis test was applied to the biodiversity indexes and a statistical significance level of $P<0.05$ was used.

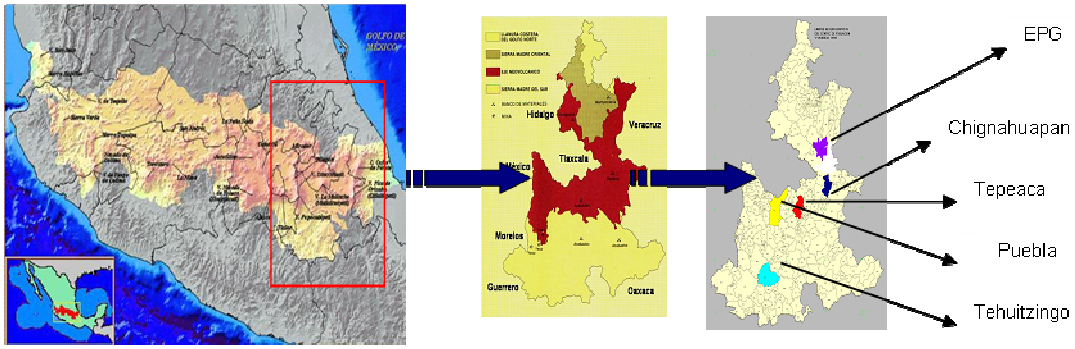

Figure1. Geographic location of the sampling areas within the neovolcanic axis in Puebla
***Taken from*: INEGI, 2011**

RESULTS

Demographic data from the participants

The demographic characteristics of the participants were the following: age average of 36.47±9.91 years, 70 women and 30 men, nonsmokers, 80 individuals from rural areas (Chignahuapan, Emilio Portes Gil, Tehuitzingo y Tepeaca) and 20 individuals from urban area (Puebla), with high marginalization rates like area Emilio Portes Gil (0.413) to very low in the city of Puebla (-1.62), as shown in table 1.

Table1. Demographic and social characteristics from the participants

Location	Chignahuapan		EPG		Puebla		Tehuitzingo		Tepeaca		Total
Genus	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	
No. of individuals	8	12	0	20	8	12	9	11	5	15	100
Age (years)	44.2±15.6	48.5±19.1	-	43.7±15.1	23.7±2.2	22.6±1.3	30.7±11	34.4±15.2	47.6±5.02	32.86±10.06	36.47±9.91
Marginalization index [a]	-1.12 (low)		0.413 (high)		-1.62 (very low)		-0.715 (medium)		-1.318 (low)		-
Economic activity [b]	Farming		Farming		University population		Farming		Farming		-
Type of population	Rural		Rural		Urban		Rural		Rural		-

a: CONAPO, 2011.
b: INEGI, 2011.

Mycobiome

The species richness in the basal cultivable mycobiome was formed by a total of 241 filamentous fungi isolates and yeast fungi (Figure 2), included in 16 genera and 29 species (environmental, pathogenic and opportunistic); of which 81.25% corresponded to phylum Ascomycota, 12.5% to phylum Zygomycota and 6.25% to phylum Basidiomycota. The predominant fungi were found in the phylum Ascomycota (Figure 2), family Saccharomycetaceae, of which *Candida albicans* (18.67%) was the most abundant, followed by *Penicillium* sp (12.03%) y *Cladosporium* sp (9.96%) as shown in table 2.

Table2. Fungal richness presents in the oral cavity (mycobioma) from healthy individuals in the PNA areas.

Location	Chignahuapan	EPG	Puebla	Tehuitzingo	Tepeaca	Total	% of frequency
No. of samples	20	20	20	20	20	100	-
No. of isolates	28	38	43	53	79	241	-
Shannon biodiversity index (H')	2.37	1.93	1.72	1.44	0.95	0.99	-
Acremonium sp. (A)	-	2	-	-	2	4	1.66
Alternaria sp. (A)	2	3	8	-	3	16	6.6
Aspergillus candidus (A)	-	-	-	-	1	1	0.41
Aspergillus flavus (A)	-	-	-	-	1	1	0.41
Aspergillus humicola (A)	-	-	-	-	1	1	0.41
Aspergillus janus (A)	-	-	-	-	5	5	2.07
Aspergillus oryzae (A)	-	-	-	-	1	1	0.41
Aspergillus sp. (A)	2	-	2	2	-	6	2.49
Candida albicans (A)	4	12	5	5	19	45	18.67
Candida glabrata (A)	-	-	1	1	3	5	2.07
Candida kruzei (A)	-	2	9	3	9	23	9.54

A:

Candida parapsilosis (A)	7	-	-	-	-	7	2.9
Candida tropicalis (A)	-	7	3	3	6	19	7.88
Cladosporium sp. (A)	4	2	9	8	1	24	9.96
Cunninghamella sp. (Z)	-	-	-	1	-	1	0.41
Fonsecaea pedrosoi (A)	-	-	-	12	-	12	4.98
Fusarium sp. (A)	-	-	-	1	-	1	0.41
Geotrichum sp. (A)	-	-	1	-	-	1	0.41
Paecilomyces sp. (A)	-	1	-	-	3	4	1.66
Penicillium carneum (A)	-	-	-	-	3	3	1.24
Penicillium claviforme (A)	-	-	-	-	1	1	0.41
Penicillium frequentans (A)	-	-	-	-	1	1	0.41
Penicillium marneffei (A)	-	-	-	-	1	1	0.41
Penicillium sp. (A)	5	3	1	10	10	29	12.03
Rhizopus sp. (Z)	-	-	4	1	-	5	2.07
Rhodotorula rubra (B)	1	1	-	1	-	3	1.24
Scopulariopis sp. (A)	2	3	-	5	7	17	7.05
Trichoderma sp. (A)	1	1	-	-	1	3	1.24
Verticillium sp. (A)	-	1	-	-	-	1	0.41

Ascomycota, B: Basidiomycota, Z: Zygomycota

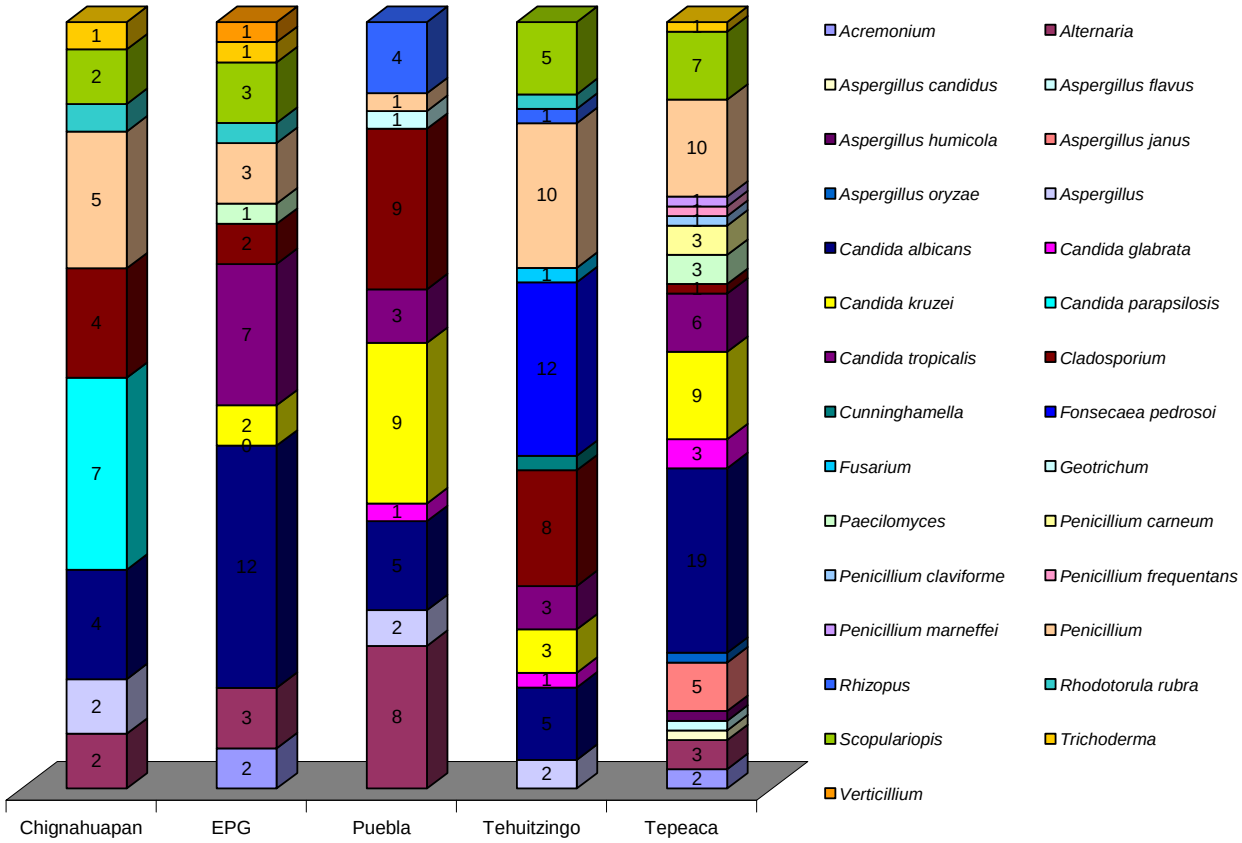

Figure2. Oral mycobiome richness distribution from the individuals in the NAP represented by location and by number of isolates of each fungus.

Figure3. Venn diagram that shows the dominant genera (*C. albicans* 18.67 %, *Cladosporium* 9.69 % y *Penicillium* 12.03 %) in the oral cavity mycobiome of the participants in each community.

Mycobiome biodiversity

The fungal diversity in the oral cavity of healthy individuals in each one of the studied locations, represented by the Shannon index of biodiversity (H´), is shown in table 2. It was found a total diversity of H´=0.99, being the Chignahuapan location, with 28 isolates, included in 8 genera and 9 species, the one with more diversity (H'=2.37), where 87.5% corresponded to Ascomycetes and 12.5% to Basidiomycetes. In the EPG location, with 38 isolates, included in 10 genera and 12 species, the biodiversity index was H´=1.93 and 90% of the isolates were Ascomycetes and 10% Basidiomycetes. The locality in Puebla, with 43 isolates, included in 7 genera and 10 species, the biodiversity index was H'=1.72, 85.70% corresponded to Ascomycetes and 14.30% to Zygomycetes. In the locality of Tehuitzingo, with 53 isolates, included in 9 genera and 12 species, the biodiversity index was H'=1.44, 66.66% corresponded to Ascomycetes, 22.22% to Zygomycetes and 11.11% to Basidiomycetes. Whereas in the Tepeaca locality, with 79 isolates, got the lowest biodiversity index (H'= 0.95), and 100% of these were Ascomycetes.

On the Venn diagram (Figure 3), constructed with the superposition of the mycobiome of each sampled locality, showed that *C. albicans* (18.67 %), *Penicillium* sp (12.03%), and *Cladosporium* sp (9.96%), were the predominant genus and species in the five geographic areas. The Shannon probability, which indicates the probability of finding a microorganism for every 100 microorganisms (Figure 4), was calculated for each species and/or genus present in the oral cavity mycobioma of the participant individuals, and the higher probabilities corresponded to *Candida albicans* (0.31), followed by the genus *Penicillium* sp (0.25), and the species *Candida kruzei* (0.25) and *Cladosporium* sp (0.22).

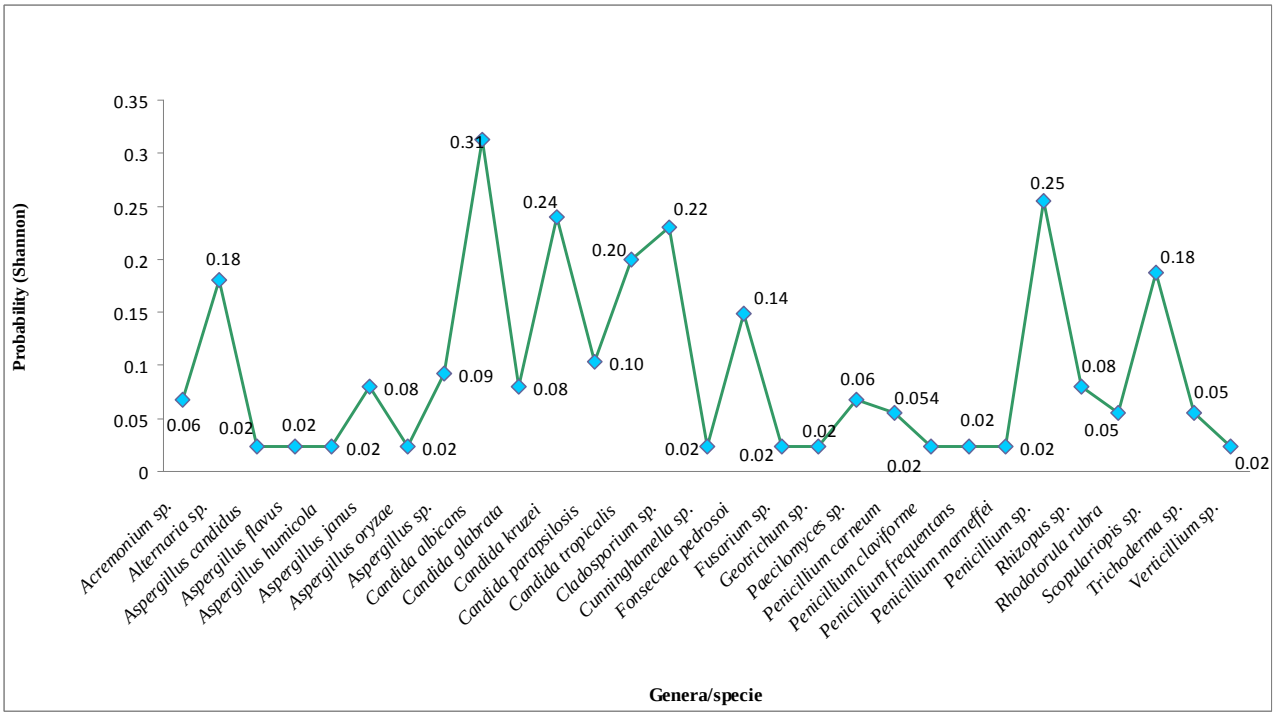

Figura 4. Graphic of the Shannon probability frequency of the mycobiome richness in the oral cavity of healthy individuals in PNA.

DISCUSSION

The mycobiome in the oral cavity of humans has not been yet established, which definitely constitutes a great challenge, since the knowledge of how it is formed, may be transcendental in understanding the role that fungi play in the relation with their host and the rest of the mycobiome, both in health and disease. Nowadays, there are few studies that address this issue, for most of them are focus on the study of bacterial microbiome [36]. In México, the situation is not different, since there is not information about the mycobiome on its population, and the present work constitutes the first input on the components of the mycobiome, considering rural and urban populations, since there is work indicating that various diets and lifestyles, among others, influence its composition [25].

In the present work, the mycobiome of the participant individuals was constituted by 241 cultivable fungi, forming these, the basal fungal richness in the oral cavity, showing that the fungal component is not limited to few species, particularly *Candida*, like it was thought [10], but it is formed by a great number of fungal cultivable genera, as it was observed in this study, as well as non-cultivable genera, like it has been described in other papers [16]. Other fungi, besides *Candida*, have been reported to be in the oral cavity, among which are *S. cerevisiae, Penicillium, Geotrichum, Aspergillus* and *Scopulariopsis* [35].

On the other hand, from the cultivable fungi obtained, *C. albicans* (18.67%), *Cladosporium* (9.96%) and *Penicillium* (12.03%) dominated, coinciding the first two with the frequency observed in the recent work of Ghannoum *et al*., (2010), whom also did a study in healthy individuals. In this way, our work reassures that the distribution and profile of the fungal species in the oral cavity of the participant individuals is complex.

Also, the results of the species distribution in the different rural areas included in this work, revealed a variation in the distribution of these, being Chignahuapan the location with the highest diversity (H´ = 2.37) and Teutzingo the lowest (H´ = 0.95), a finding that also corroborates the variations found in the microbiome diversity in the saliva of individuals from different geographic regions, with various lifestyles, reported recently by Nasidze *et al.*, (2009) [26].

On the other hand, the dominance of *C. albicans* found in individuals from the regions included on the PNA was not a surprise, since other studies reported up to 40 and 50% of this fungus isolates in the oral cavity of healthy subjects [2, 5, 38]. Another relevant input of this study, was the species diversity of *Candida* identified, among these, *C. kruzei*, *C tropicalis*, *C. parapsilosis* and *C. glabrata*, a fact that agrees with other studies made [33, 36], in which it has been analyzed the distribution of the *Candida* species, associated with environmental factors like urbanization,

industrialization, globalization, climate change, also social and geographic factors that promote this distribution [32].

Some authors, like McMichael (2004) [22], Méndez-Tovar *et al*., (2003, 2006) [23, 24] and Madhavi *et al*., (2011) [20], have reported that the indiscriminate use of antifungal medications like azoles, has originated the exacerbation of species of *Candida*, mainly in urban populations that generally do not present marginalization. Instead, in rural marginalized populations, what determinates this distribution is the lack of personal hygiene, cultural and nutritional factors like high ingestion of carbohydrates and diseases like diabetes, obesity and malnutrition, a fact that also coincides with our results. On the other hand, it is possible that the pathogenicity of these agents is controlled by other fungi that form the mycobiome, besides of the intervention of the immune system of the individual [19].

It is also possible for relationships like symbiosis and antagonism to be present and control them, but it is necessary to investigate using a greater number of samples and through longitudinal studies [13]. It is important to point out that the presence of fungi like *Acremonium*, *Aspergillus*, *F. pedrosoi* and *Fusarium*, in the oral cavity of the participant individuals was unexpected, despite that these are ubiquitous in the environment, that is to say, can be found in plants, soil, water or air and usually are no associated with infections, nor they are part of the oral flora of healthy individuals [7, 8, 18]. Other fungi identified in this study were *Alternaria* (6.6%), important in respiratory allergies in individuals from urban populations, and *Rhodotorula rubra*, which has also been documented on healthy individuals [11, 15]. It has been found that this last one may cause some risk in persons with a subjacent disease like cancer o infect other people with some degree of malnutrition [3, 14]. Also, due to its ubiquity in nature, the finding of these two fungi in the participant individuals of this study, should not be consider as a strange event, due to that probably they are acquired through breathing, food intake or fomites, like it has been suggested by Romeo, *et al*., (2010) [31].

The importance of this study was to identify the mycobiome present in the oral cavity of healthy individuals from the neovolcanic axis in Puebla, México, which was until this moment not described. Besides, the knowledge of mycobiome associated with the human body, under a variety of conditions, may be a great potential for explaining how does it impacts on health and disease, since to date there had not been any systematic effort to enumerate and describe the fungi on the human body and the interaction between them and with their host. Thus, Panichakul *et al*., (2002) [28], suggest that it is possible that the presence of a fungus on the oral cavity, may be the first step into predispose the host to an opportunistic infection. This consideration indicates that the colonization of the oral cavity by *Candida* represents a risk of infection in immunocompromised individuals [34]. In this way, the comprehension of the interactions between the various fungal species, as well as the mycobiome with other members of the oral microbiome would also proportionate a bigger understanding about the pathogenicity and changes in the diversity of these organisms [1, 37]. The results of this work demonstrate that the fungal component of the oral cavity in healthy individuals from the rural and urban community was complex, formed by various genera of fungi, of which *Candida albicans* was predominant and its dominance was related to oral hygiene, diet and social condition.

REFERENCES

[1] JA Aas, JP Bruce, NS Lauren, I Olsen, FE. Dewhirs. *Journal of Clinical Microbiology*. **2005**, 43(11):5721-5732.
[2] JAO Alli, IO Okonko, NN Odu, AF Kolade, JC Nwanze, *Journal of Microbiology and Biotechnology Research,* **2011,** 1(3):176-184.
[3] AA Al-Sufyani, MS Hassan. *Innate Immunity*, **2011**, 17(2):339-350
[4] D Anupam, R Kansal, AK Asthana, A Pandey, M Madan, *Annals of Biological Research*, **2011**, 2(2):111-115.
[5] M Ávila, DM Ojcius, O Yilmaz, *DNA and Cell Biology*, **2009**, 28(8):405-411.
[6] JA Barnett, RW Payne, D Yarrow, Yeast: Characteristics and identification. Cambridge, Cambridge University Press, **1983**. pp. viii 811.
[7] M Breitenbach, SN Birgit, **2002**, *Chem Immunol*. 81: 48–72.
[8] J Cai, Y Jianhong-Du, Y Fan, QY Lihong, LJ Kennedy, F John, *Carbohydrate Polymers*, **2006**. 65(2): 211-217.
[9] Consejo Nacional de Población. (CONAPO). http: // www.conapo.gob.mx
[10] M Contreras, F Costello, G Hidalgo, M Magris, R Knigth, M Dominguez-Bello, *Microbiology*, **2010**, 156: 3282-3287.
[11] M Cuenca-Estrella, A Gómez-López, E Mellado, MJ Buitrago, A Monzón, JL Rodríguez-Tudela, *Antimicrobial Agents and Chemotherapy*, **2003,** 47(7): 2339-2341.
[12] GS De Hoog, J Guarro, J Gené, MJ Figueras. Atlas of Clinical Fungi 2da ed., Utrecht-Reus, Centraalbureau voor Schimmenlcultures and Universitat Rovirai Virgili, **2000**. pp. 749.
[13] W Delgado, JM Aguirre, *Rev Iberoam Micol*, **1997**, 14: 14-22.
[14] D Dennis, D Robertson, L Curtis, J Black, *Toxycology and Industrial Health*, **2009**, 25(9-10): 669-680.

[15] ML Elias, Ak Soliman, FJ Mahoney, AZ Karam El-Din, RA El-Kebbi, TF Ismail, MM Wasfy, AM Mansour, YA Sultan, G Pimentel, KC Earhart, *J Egypt Public Health Assoc*, **2009**, 84(1-2):169-81.
[16] MA Ghannoum, RJ Juveric, PK Mukherjee, C Fan, S Masoumeh, N Ammar, PM. Gillevet, *Plos Phatogens*, **2010**, 6 (1): 1-8.
[17] Instituto Nacional de Estadística y Geografía. **(**INEGI). http://www.inegi.gob.mx
[18] HF Jenkinson, RJ Lamont, *TRENDS in Microbiology*, **2005**, 13(12):589-595.
[19] M Karaman M, KM Bayrakal, HA Bagrıyanık, O Yılmaz, IH Bahar, *Mikrobiyol Bul*, **2011**, 45(2):336-43.
[20] S Madhavi, MV Rama Rao, K Jyothsna, *Annals of Biological Research*, **2011**, 2(3):88-93.
[21] A Magurran, Diversidad Ecológica y su Medición. Ed. Vedra; España, **1983**. pp. 124 – 125.
[22] AJ McMichael, *Phil. Trans. R. Soc. Lond. B*, **2004**, 359:1049-1058.
[23] LJ Méndez-Tovar, A Anides-Fonseca, A Vázquez-Hernández, M Galindo-González, M Díaz-Madrid, A Berdón-Castro, P Manzano-Gayosso, B Millan-Chiu, F Hernández-Hernández, R López-Martínez, *Gac Med Méx*, **2006**, 142 (5):381-386.
[24] LJ Méndez-Tovar, A Lemini-López, F Hernández-Hernández, P Manzano-Gayosso, R Blancas-Espinosa, R López-Martínez, *Gac Méd Méx*, **2003**, 139 (2):118-122.
[25] I Nasidze, J Li, R Schroeder, JL Creasey, M Li, *PLoS ONE*, **2011**, 6(8):1-7.
[26] I Nasidze, L Jing, D Quinque, K Tang, M Stoneking, *Genome Res*, **2009**, 19: 636-643.
[27] J Neetuu, S Meenakshi, *Blackwell Verlag GMBH*, **2009,** (1):1-7.
[28] T Panichakul, R Chawengkirttikul, C Sansanee, C Chaiyaroj, S Sirisinha, *J. Trop. Med. Hyg*, **2002**, 67(4): 443–447.
[29] PC Paquet, G.E Piérard-Franchimont, P Piérard, P Quatresooz, *Arch Dermatol Res*, **2008**, 300:167–171.
[30] C Ramirez, Manual and atlas of the Penicillia. Amsterdam, Elsevier Biomed Press, **1982**. pp. 227-252.
[31] J Romeo, J Warnberg, A Marcos, *Proceedings of the Nutrition Society*, **2010**, 69:341–346.
[32] M Ruhnke, A Eigler, I Tennagen, B Geiseler, E Engelmann, M Trautmann, *J Clin Microbiol*, **1994**, 32: 2092-2098.
[33] IH Sahand, JL Maza, E Eraso, M Montejo, MD Moragues, JM Aguirre, G Quindós, J Pontón, *J. Med Microbiol*, **2009**, 58:1437–1442.
[34] LP Samaranayake, TW Macfarlane, *J. Med. Microbiol*, **1982**, 15 (51):511-517.
[35] M Sellart-Altisent, JM Torres-Rodríguez, S Gómez de Ana, E Alvarado-Ramírez, *Rev Iberoam Micol*, **2007**, 24: 125-130.
[36] J Xu, TG Mitchell, *Clinical Infectious Diseases*, **2003**, 36:221-224.
[37] Y Yun-Liang, H Li-Yun, W An-Huei, L Hsiu- Jun, *Mycopathologia*, **2011**, 172:131-139.
[38] E Zaura, JF Bart-Keijser, SM Huse, W Crielaard, *BMC Microbiology*, **2009**, 9:259-271.