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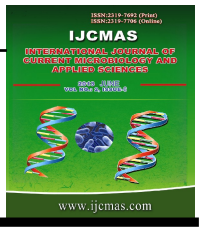

Original Research Article

Identification of indoor airborne microorganisms in residential rural houses of Uttarakhand, India

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Keywords

Indoor air; Fungal and bacterial species; DNA isolates; Polymerase chain reaction (PCR).

A B S T R A C T

Indoor air contains a complex mixture of microorganisms, microorganism fragments, and by products such as molds, bacteria, endotoxins, mycotoxins, and volatile microbial organic compounds. Airborne bacteria can be toxic, allergenic, and/or infectious. This research attempts to uses a molecular approach for detecting microbes and fungi found in indoor environment of residential houses. In this study three bacterial and three fungal species has been isolated from sample collected from indoors in rural residential house of Uttarakhand. A Polymerase chain reaction (PCR) based method for the specific detection of microbes was developed. DNA isolated from collected sample served as a template for PCR amplification with specific primer pairs targeting the 16S rRNA gene. The PCR amplification products were sequenced and the obtained sequences were analyzed. The results of this work confirm the presence of the *Brevibacillus brevis*, *Arthrobacter* sp. FB24 and *Bacillus cereus* bacterial species and *Neosartorya fischeri*, *Aspergillus clavatus* and *Trichoderma reesei* fungal species in indoor air.

Introduction

Microbes are omnipresent, appearing in the aerosphere, hydrosphere, lithosphere and ontosphere (on biota). Airborne microbes have been found to be associated with certain respiratory illness and allergy. Indoor air contains a complex mixture of microorganisms, microorganism fragments, and byproducts such as molds, bacteria, endotoxins, mycotoxins, and volatile microbial organic compounds. Airborne bacteria can be toxic, allergenic, and/or infectious. Bacteria, fungi, viruses, protozoans, and microscopic animals have been linked to poor indoor air quality (Fabian *et al*., 2005). Exposure to these microbial fragments and metabolites may result in adverse health effects. Elevated concentrations of bacteria are associated with an increasing probability of epidemics and food pollution and may be responsible for a number of respiratory and dermatological infections and diseases (Aydogdu *et al*., 2008). The PCR based DNA analyses was developed lately as the technique for identification of specific bacterial or fungal species in indoor air

(Simsekli *et al.,* 1999; Chew *et al.,* 2003**;** Kampfer *et al.,* 2010)**.** In PCR, a sequence of DNA, usually a portion of a gene was selected and multiple copies were produced by an enzymatic reaction. The gene sequence selected can also be from a microbial functional gene. The sequence chosen should be specific for the microorganism or a group of microorganisms (Peccia and Hernandez, 2006). These template DNA sequences was targeted, amplified and quantified by designed DNA primers. The DNA primers used to detect bacteria or fungi were commonly sequences from the 16S or 18S rRNA gene, respectively (Cruz-Perez *et al.,* 2001). Examination and characterization of typical microbes and fungus in indoor environment can be helpful in identifying associations between domestic microbial and fungal characteristics their clinical diagnosis and in prevention of allergic and other airborne respiratory diseases.

Materials and Methods

Sampling and isolation methods

Research samples were taken from indoor air in different houses. The rural residential houses were made of mud, wood and stone having poor hygienic condition. The indoor air was sampled by using Gravitational Petri Plate Method for monitoring of airborne microorganisms in indoor (Sen and Asan, 2009). External variations like wind, rainfall, vegetation etc. did not affect indoor air monitoring. For monitoring of indoor contaminants a set of sterilized petridishes treated with nutrient agar and potato dextrose agar were exposed at a time to capture and cultivate the available bacteria and fungi in the room. This petridishes treated with NA and PDA was exposed in the center of the room at a height of 1.0m to 1.5 m (breathing height) from the surface for the period of one minute. All the doors and window were closed while taking the sample so as to let the micro flora settle down freely without any disturbance from the air current into petri-dishes. The bacterial cultures were incubated at 25°C for 48 h, while the fungal plates were incubated at 25°C for up to 5 days depending on the growth of colonies (Green and Scarpino, 2002). Large varieties of heterotrophic bacteria and fungus seem to be the first colonizers in NA and PDA respectively.

Purification and maintenance of pure culture

After 5 days the mixed culture of fungus and bacteria were obtained in their respective culture. Numerous fungal and bacterial colonies were found in mixed culture. Isolation of pure bacterial and fungal culture was done from their respective mixed culture. The streak plate was performed in order to obtained pure colonies of bacteria on the surface of the nutrient agar and the plates were incubated in inverted position in incubator at 32°Cfor 24 to 72 hr. This results the growth and multiplication of the inoculated microorganisms in the form of isolated colonies. After 2 days of incubation the growing colonies were observed which were more and more sparsely distributed towards the streak. A number of bacterial and fungal colonies were obtained from indoor air sampling in the studied households from each plate as shown in Figure.1 and Figure.2 respectively. Pick up well separated colonies and transfer than to agar slants as pure culture.

The culture of the fungus obtained was purified by single spore isolation and was maintained on PDA at 27^{0}C for 5 to 7

days. To keep the culture viable, sub culturing was done at an interval of 15 days and preserved at low temperature (5 ± 1°C) in the refrigerator.

DNA extraction

The bacterial and fungal genomic DNA was extracted for molecular characterization and species identification studies. To obtain sequence information of individual members of the bacterial and fungal communities, the DNA extracted from the indoor air was amplified by PCR. The detection of bacteria is mainly based on the PCR-amplification of sequences of the small subunit 16S ribosomal RNA (rRNA) genes (Gurtner *et al*., 2000). The molecular identification of fungi to species level has been based mostly on the use of variable ribosomal DNA (rDNA) internally transcribedspacer (ITS) regions (Anderson *et al*., 2003). ITS and Gm primers were use for sequencing of PCR products of fungus and bacteria . The annealing temperature of Gm primers for the amplification of bacterial DNA was 51°C to 54°C with a product size of nearly 750b.p. as shown in Figure.3 and the annealing temperature for ITS primers for the amplification of fungal DNA was 54°C and products size was approximately 550b.p as shown in Figure.4. The amplified product sequence was obtained by direct sequencing, using the DNA sequencing service of Delhi University. Nucleotide sequence similarities were determined by using BLAST, version 2.0 (National Center for Biotechnology Information databases).

Results and Discussion

The sequencing of PCR product provides the more reliable phylogenetic identification of microorganisms. The molecular approach used in this study led to the identification of 3 bacterial species and 3 fungal species in indoor environment of house. The obtained sequences were subjected to homology search for species identification using blast. The sequence similarity to the nearest relative was determined as an indication of identity. Table 1 shows the phylogenetic affinities of bacterial coding sequences and fungal coding sequences. The PCR products of bacterial DNA B1, B4, B5 showed 98% identity with *Brevibacillus brevis* and B2 showed 95% identity with *Arthrobacter* sp. FB24 whereas B3 showed to 99% identity with *Bacillus cereus* respectively. *Bacillus cereus* is known to cause fatal food poisoning in various studies. High levels of *Bacillus* species in the indoor air generally indicate dampness of building and lack of adequate maintenance of the building or house. Bacteria grow more rapidly than fungi and may be the first microorganism to cause problems in moisture damaged houses. Kampfer *et al.*, (2010) also isolated the gram positive, rod-shaped, non-endospore-forming, orange-pigmented (coloured) *Brevibacillus* species from the wall of an indoor environment primarily colonized with moulds. It was reported by Hirvonen *et al*., (2005) and Black *et al.*, (2006) that the dust contained, and air-borne bacterial flora in indoor environment were dominated by gram-positive bacilli *and* actinomycetes, notably *Bacillus cereus, Brevibacillus brevis, B. licheniformis, B. subtilis* and species of *Arthrobacter.* Indoor air and dust contained *Klebsiella oxytoca, Acinetobacter sp., Bacillus cereus* and *Nocardiopsis dassonvillei* come within the status of hazard group.

The PCR products of fungal DNA F1 showed 97% similarily with *Neosartorya fischeri,*

Figure.1 Mixed culture bacterial colonies growing on nutrient agar obtained from sampling of indoor air

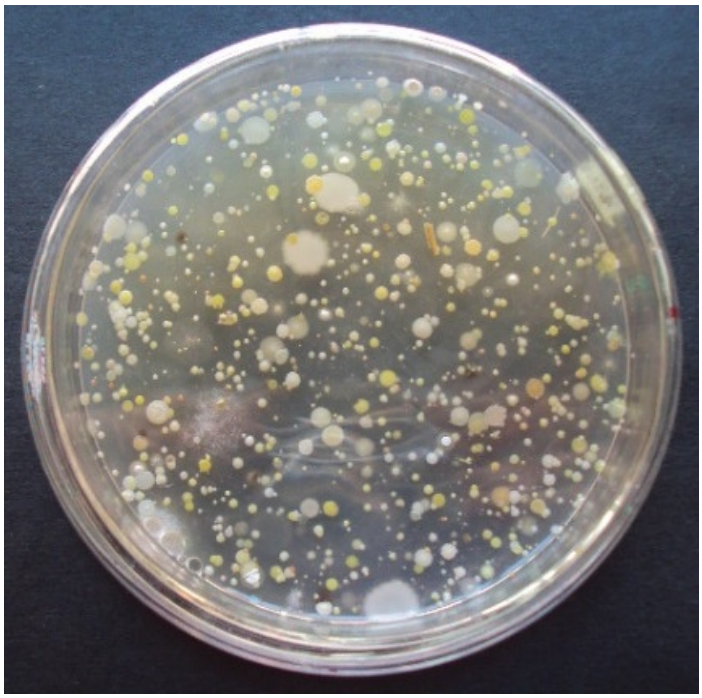

Figure.2 Mixed culture fungal colonies growing in potato dextrose agar obtained from sampling of indoor air

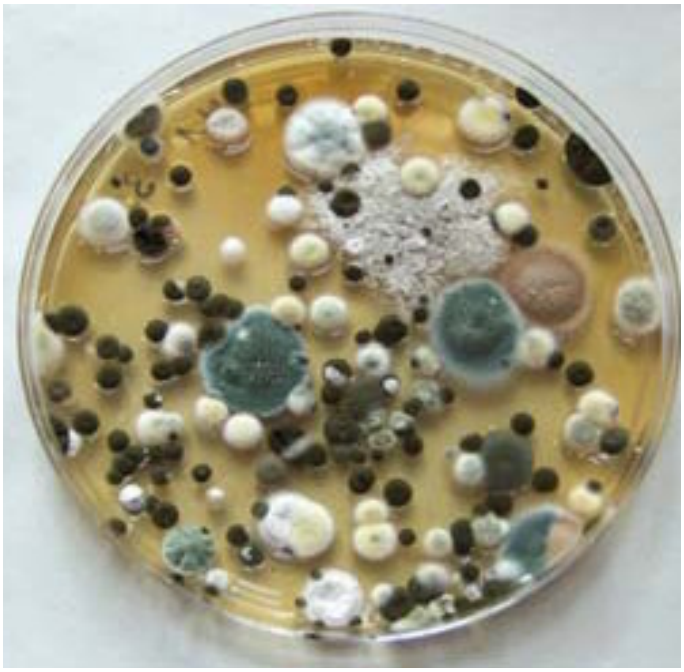

Figure.3 PCR Amplification of Bacterial DNA

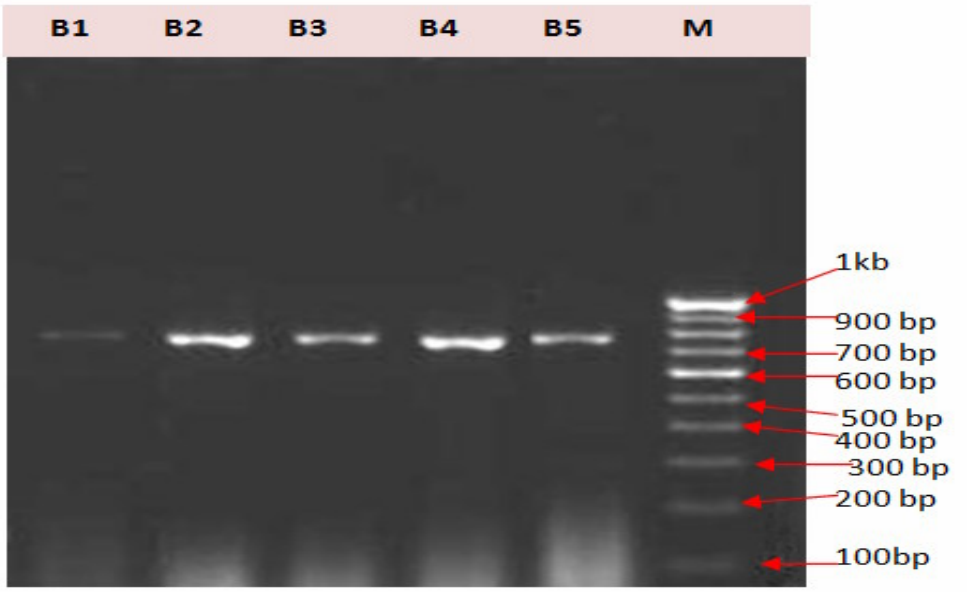

Figure.4 PCR Amplification of Fungal DNA

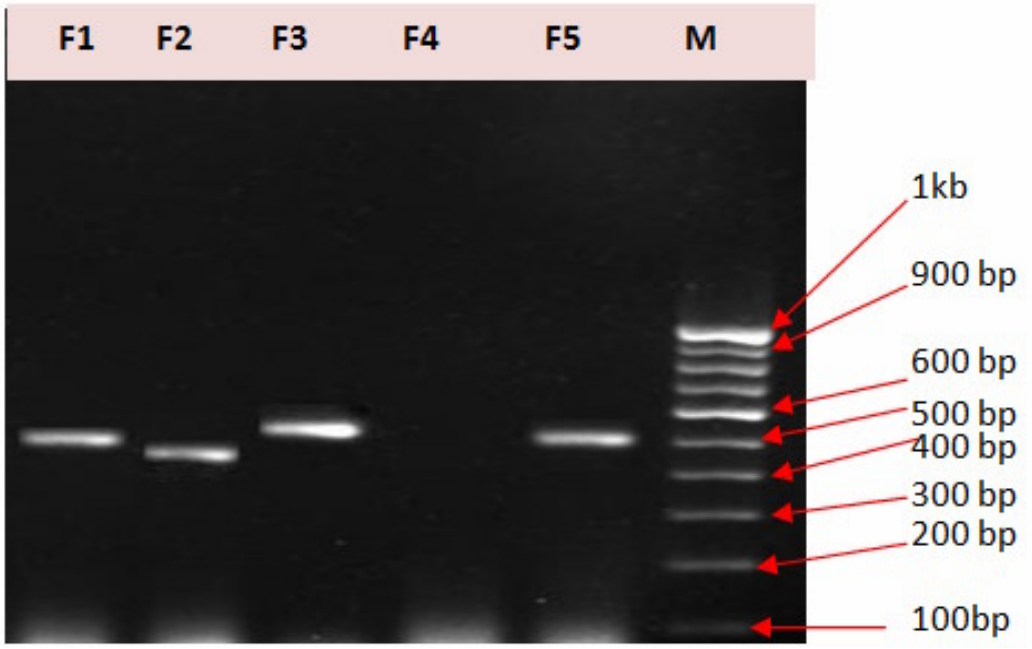

Table.1 Phylogenetic Relationship of Partial 16S rRNA Bacterial and ITS rRNA Fungal Coding Sequences Detected in Indoor Microorganisms**.**

Strain No.	Closest identified phylogenetic relatives	Identity (%)
Bacteria		98
B1	*Brevibacillus brevis*	
B2	*Arthrobacter sp.* FB24	95
B3	*Bacillus cereus*	99
B4	*Brevibacillus brevis*	98
B5	*Brevibacillus brevis*	98
Fungi		97
F1	*Neosartorya fischeri*	
F2	*Aspergillus clavatus*	92
F3	*Trichoderma reesei*	93
F5	*Trichoderma reesei*	94

and F2 found 92% identical to *Aspergillus clavatus* while F3 and F5 showed 93% and 94% identity with *Trichoderma reesei.* The elevated indoor fungal spore levels is indication of indoor pollution and these elevated level of fungal spores were often associated with contamination, and were more frequent when disturbed by activity. Udagava *et al.,* (1996) isolated ascomycetous microfungi from house dust samples from residential dwellings and revealed that species of *Talaromyces, Eurotium* and *Neosartorya* were common ascomycetous propagules in the house dust and reported that it may be a common potential fungal pathogen in the dwelling environment and cause potential hazard to respiratory health of inhabitant. It was reported by Sharma *et al., (*2007) that some species of Aspergillus like *Aspergillus flavus, Aspergillus niger* and *Aspergillus clavatus* cause respiratory allergy, bronchial asthma in allergic rhinitis. Mazur and Kim (2009) reported that the *Aspergillus clavatus* fungus is associated with hypersensitivity pneumonitis. Sen and Asan (2009) found *Trichoderma* and *Aspergillus* spp. in indoor air of different residential houses in Tekirdag City (Turkey) and found that *Trichoderma* spp. actively participate in delignification and biodegradation of cellulose in wooden parts of house. Dampness, especially of carpets, water seepage houses, visible mould and dust rapidly increases the concentration of these fungal spores.

The results of our study showed that the indoor air of rural residential houses of Uttarakhand contained microbes and fungi that could cause important health problems such as allergies, rhinitis and chronic fatigue. The presence of these fungal and

microbial flora in indoor air rural residential houses may be useful both in terms of ecology and allergy prevention and treatment. In the future, further studies on the dependence of indoor fungal and microbial flora on socioeconomic and hygienic factors will be of interest; such studies should clarify the relationship between indoor and outdoor fungal and microbial flora.

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