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Alaa Alyamani, Med Mycol Open Access, 3:2, 2017
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Morphological, physiological and molecular characterization of two fungi isolated from the Yellowstone National Park

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Two fungi were isolated from the Yellowstone national park using Millipore filtration systems equipped with 0.22 µm membrane filters. The filters were transported back to the lab, cut into small pieces, placed on Nutrient Agar plates and incubated at 37°C for 72 hours. Single colonies of YNP6-TSU and YNP7-TSU were selected and inoculated on new PDA media to have a pure culture for characterization. Three approaches were conducted to assist the identification process. The morphological assessment was done by using the agar block technique to examine the structure of the fungi and the spores without disrupting them. It was found that YNP6-TSU has septate hyphae and the spores are inside an ascus, and YNP7-TSU has coenocytic hyphae with spores inside sporangia. To assess the effect of the temperature, two approaches were conducted. To determine the optimal temperature, three agar media PDA, SAM and V8 were used to grow the fungi in 25, 30, 37, 45 and 50°C for 10 days. To test the growth in higher temperature, PDA broth media was used to grow fungi at 55, 60, 65 and 70°C in water bath for 10 days as well. It was concluded that at 37°C and with PDA as a media, the growth of both fungi was at a higher rate, while there was no growth at 50°C and higher. In the molecular process, fungi were grown on PDA for seven days and mycelia were harvested for DNA extraction, PCR and sequencing. The results of sequencing and blasting showed 100% match for YNP6-TSU to *Verruconis calidifluminalis* and for YNP7-TSU to Rhizopus microspores. Future studies will include the determination of the optimal PH for growth of the fungi in three broth media and the extraction and purification of the brown pigment from *Verruconis calidifluminalis* (YNP6-TSU).

Biography

Alaa Alyamani is a graduate student at Tennessee State University getting her Master's degree in Microbiology with a concentration in Mycology. She received her BS in Microbiology from King Abdul Aziz University in 2009. After graduation, she has worked at National Guard Health Affairs Hospital in the bacteriology lab for a year. She then transferred to University of Jeddah and worked as Teaching Assistant for a year and while working there she applied for a scholarship to pursue her graduate studies in the United States.

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Chemical stressors increase polysaccharide secretion in *Lentinus squarrosulus*

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Introduction: *Lentinus squarrosulus* is a seasonal mushroom consumed by millions of people. Beyond nutritive factors, *L. squarrosulus* elicit medicinal benefits through its polysaccharide content. While attempts at domestication and mass production of *L. squarrosulus* (on going) is one way to increase polysaccharide output, mechanisms that increase polysaccharide secretion *in vitro* is an attractive alternative both for process optimization and product recovery. The study evaluated the effectiveness of chemical stressors such as acidity and sodium chloride in increasing polysaccharide secretion in *L. squarrosulus*.

Methodology: *L. squarrosulus* was cultured in submerged fermentation under different concentrations of sodium chloride (0.5, 1, and 2 g/l) and pH levels (3, 4, 5, 6, 7, 8, and 9) including their controls. The basal medium was composed of soluble starch (10 g/L), Dextrose (5 g/L) and yeast extract (2 g/L). Five (5) replicates of each medium were inoculated with 5 mm agar blocks from 3-day old *L. squarrosulus* culture and incubated at 28˚C for 14 days. Mycelia were harvested by filtration and polysaccharide precipitated from supernatant in 3 volume ethanol overnight, centrifuged, dried and weighed.

Result: The two chemical stressors increased secretion of polysaccharides in *L. squarrosulus* tremendously compared to non-stressed cultures. Acidic pH was more favorable to polysaccharide secretion than alkaline pH. Highest polysaccharide (0.026g) was detected in pH 3 and least (0.01g) in pH 6-9. Low sodium chloride concentration (0.5 g/L) resulted in more polysaccharide secretion while higher salt concentrations (2 and 3 g/L) inhibited growth completely. Acidic medium was more effective in inducing polysaccharide than low salt medium (0.26 g vs 0.2 g).

Conclusion: Chemical stress using sodium chloride and acidic pH induced higher polysaccharide secretion in *L. squarrosulus*. The method could be optimized and adapted for commercial production of *L. squarrosulus* polysaccharide.

Biography

Anike F N is an experienced Researcher and Educator with expertise in mushroom and fungal biotechnology. She conducts research and trains students in this field of study, authored and co-authored many peer reviewed journals and publications.

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Breeding new *Cordyceps militaris* strain using mating type molecular markers

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An entomopathogenic *Cordyceps militaris* is being studied and cultivated as a medicinal mushroom having many valuable biological and pharmaceutical activities substituting *C. sinensis* which has been traditionally used as a Chinese herb medicine. *C. militaris* can be cultivated artificially. As a bipolar heterothallic fungus *C. militaris* has two strains of compatible mating types, MAT1 and MAT2 which are determined by the single mating type locus MAT1 consisting of two dissimilar alleles called idiomorphs MAT1-1 and MAT1-2 of MAT1 and MAT2, respectively. They can be differentiated by crossing, fruiting body formation ability and the production of perithecia. Such process is very laborious and time consuming to carry out but molecular markers of these mating types reduces the amount of effort required for the crossing process. In this study, two opposite mating types were assayed using two sets of primers specific for *C. militaries*, which were amplified a 191-bp fragment for MAT1-1 and 233-bp fragment for MAT1-1. After crossing of two compatible mating types F1 hybrids resulted in well-developed perithecial fruiting bodies and their crossings were confirmed by the multiplex PCR assays for the rapid and specific detection of both MAT1-1 and MAT1-2. In the breeding process of new *C. militaris* mushroom, single ascospores were isolated and examined their mating types, mycelial growth, mycelial density, and selected isolates were crossed and hybrids were produced showing high quality fruiting bodies on artificial media. The stromata of new strain 'Chungnam 12' were club-shaped and bright orange-red. Its height was 6.7 cm and the cordycepin content was 0.33% on average. The new strain showed 11% higher yield than 'Yedang 3' with producing firmer fruit bodies. The optimum temperature for mycelial growth was 22~25^{0}C and the optimum temperature for stroma development was 18~22^{0}C. Fruiting bodies were begun to produce 43 days later after inoculation. This new cultivar may serve as a valuable one for artificial cultivation and industrial-scale production of *C. militaris*.

Biography

Miae Lee is an Agricultural Researcher in Crop Research Division, Chuncheongnam-Do Agricultural Research and Extension Services in South Korea for about 10 years and has her expertise in breeding new mushroom cultivars. She is also doing her best in developing various cultivation methods for better quality mushroom production. Recently, she is involved in environmental friendly disease and pest management, particularly against mushroom flies. She investigates the occurrence and development of sciarid flies and the use of plant extracts such as *Ginko biloba* fruits to control mushroom flies in button mushroom cultivation

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Kalsi Avneet Singh et al., Med Mycol Open Access, 3:2, 2017
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Tinea cruris and Tinea genitalis: Clinical manifestations and diagnostic challenges

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The dermatophytes are the causative agent of dermatophytoses. Recently, extensive skin lesions without central clearance and atypical clinical manifestations of dermatophytoses have been seen and reported from different regions of India. Three species of dermatophytes implicated are: Trichophyton, *Microsporum* and *Epidermophyton*. The dermatophyte fungi comprise about 30 species of keratinophilic moulds causing infections of skin, which can manifest in different anatomical regions of the body and have been named accordingly. Thus, tinea capitis affects the scalp, tinea unguium- the nails, tinea cruris- the groin, tinea genitalis- the genitalia. Tinea cruris and Tinea genitalis are the focus of this review. Although dermatophytoses does not cause mortality, it does cause morbidity and poses a major health problem.

Patients and Methods: A study was conducted from January 2016 to August 2016 at Muzaffarnagar Medical College, Muzaffarnagar, Uttar Pradesh, India. A total of 260 patients were enrolled into study and who were not receiving any anti-fungal treatment. When there was involvement of penis and scrotum in males and labia majora and mons pubis in females, the clinical diagnosis of tinea genitalis was made and confirmed by mycology laboratory after preparing Potassium hydroxide (KOH) mount and fungal culture. In the present report, we reviewed a total of 260 cases of Tinea cruris and Tinea genitalis. A total of 128 tinea cruris in males, 72 in females and 40 tinea genitalis with tinea cruris in males and 20 in females were observed. Until now, *Trichophyton rubrum* has been the most frequently isolated species, followed by *Trichophyton interdigitale* (former Tinea mentagrophyte), *Epidermophyton floccosum* and *Trichophyton verrucosum*.

Conclusion: All cases of Tinea cruris, even tinea corporis should be examined for tinea genitalis. The condition is more common than what we have been imagining. Hot and humid climate of the country and promiscuous society are the common contributing factors

Biography

Kalsi Avneet Singh is an eminent Physician who obtained his MBBS degree from Chaudhary Charan Singh University, Meerut, a premier university in India. He obtained his Diploma in Dermatology (Alternative Medicines) degree and Bachelors of Alternative System of Medicines degree from Indian Board of Alternative Medicines. He is an active participant in various CMEs both at National as well as International level. He has been the co-author of manuscript titled *Clinical manifestations of Tinea faciei and Tinea genitalis and their diagnostic challenges*, which has been submitted for publication in *Indian Journal of Dermatology, Venereology, and Leprology*, India. He has been awarded with the prestigious Health Excellence Award by Indian Board of Alternative Medicines, Kolkata, India.

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Saima Liaqat, Med Mycol Open Access, 3:2, 2017
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Prevalence of tinea infections in district swat

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The aim of the study was to find the prevalence of Tinea infections in District Swat, Khyber Pakhtunkhwa. A total of 190 patients were reported from different hospitals and private clinics. 40 samples obtained from different parts of body including infected skin, nails, hairs were cultured of these patients and based on morphological traits, 120 fungal colonies were obtained on PDA medium. In our results 12 different fungus were isolated which were 25 % *Trichophyton rubrum, Candida* species 19.4%, *Pencillium* species 16.6%, *Aspergillus* species 11.1%, *Microsporum canis* and *Alternaria* species 5.5% while *Trichophyton basicola, T. tonsurans, T. violaceum, T. verrucosum, Epidermophyton floccosum and Aureobasidium pullans* have 2.7%. On the basis of geographical condition high prevalence was observed in warm region of Mingora 45.7% followed by Matta 22.6% and minimum of 11k% in colder region of Kalam. The maximum prevalence of Tinea infections was recorded in age group 1-10 years followed by 11-20 years of age with 24.2% and 23.1%, respectively. Males had maximum prevalence 55.2 % and females had 44.7 %. Furthermore, farmers were more susceptible with high number of infection of 32.1 % followed by house wives 24.7 %

Biography

Saima Liaqat has completed her BS (Hons) Microbiology from CBM (Centre for biotechnology and Microbiology) University of Swat, Pakistan. She is the Member of National Academy of Young Scientist, Pakistan which shows her active participation in the field of biological Sciences. She has also participated in different workshops and seminars related to science fields. She has a deep insight into microbiology, bacteriology, immunology, virology, experimental statistics, biochemistry, microbial genetics, genetic engineering and epidemiological concepts.

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Accepted Abstract

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Indian subcontinent – A destination and potential customer for fungal diversity

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Indian motherland has an area of 3287263 km^2 bearing a big population to the tune of 1.3 billion people. Majority of our countrymen totally depend for food and livelihood on agricultural, horticultural, forest and other sectors on their produce. Being an agriculture base country if something goes wrong in any way to the productivity then it becomes a major concerned for all of us. As we all know biodiversity is continuously decreasing day by day because of habitat degradation, various microbial plant diseases, abrupt climate change and many more factors. At this junction, all the national and international organizations are giving a call to conserve the biodiversity for our future existence so much so that this slogan has become a global concern. India lies in between Indo-Himalayan ecozone and contains 3 biodiversity hot spots. About 24.16 % of the total available land is under forest cover. We are the 7th largest and fastest growing economy. In our GDP agriculture and allied sectors impart very important role. However, the yield somehow, sometimes shows a decline and attract the attention of all the concerned. The diseases are caused by a variety of microorganisms of which fungi are the foremost. Therefore, this is high time and there is an urgent need for survey, collections, identification and conservation of these fungal organisms so that possible control measures can be adopted. Fungal organisms are so harmful and dangerous that sometimes due to colossal loss they have caused various famines. I am actively engaged in exploring the fungal diversity from the forest flora of different states of Indian subcontinent from the last 30 years. We have made fungal surveys from possible forest patches of various states such as U.P., M.P., and some parts of Jammu and Kashmir. We have extensively and intensively surveyed the state of Madhya Pradesh being almost in the centre of India. This state (M.P.) is a vast state having a large no. of national parks, wild life sanctuaries and biosphere reserves which provide the additional beauty to the cultural and national heritage. This constitutes a sort of paradise for the growth and development of the fungal diversity. We have high hopes and the scope is limitless for the survey, collection, identification and conservation of the fungal biodiversity as no one knows when and how some of these valuable fungal forms might be lost forever. During the course of survey, we have been able to encounter a large number of powdery mildews, downy mildews, blights, sooty molds, black mildews, tar spots, rusts and many more symptoms. This investigation will certainly enrich the pre-existing treasure of fungi of India in general and different states. This will also directly or indirectly lead to the upliftment of the status/social status (socio economic condition) of the forest dwellers and tribes totally depending on the forests and their produce.

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Mycochemical and proximate composition of selected mushrooms in Lapai, Niger State

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In recent times, mushrooms assume greater importance in the diet of both rural and urban population because they are delicacies. The bulk of mushrooms consumed are hunted from the wild, the practice which is often associated with some degree of negativity and fatality since poisonous ones could be inadvertently picked and eaten. This study therefore investigated myco-chemical, proximate minerals and vitamins present in three selected and identified mushroom: *Macrolepiota procera , Pleurotus roseus* and *Cantherelle cibarius* collected from wild in Lapai Niger State. The samples were sundried and grounded into powder and sieved. Myco-chemical, proximate, minerals and vitamins analyses were done. The results revealed the presence of alkaloid, flavonoid and saponin in all the three samples The results on the proximate composition of the three mushrooms sampled, revealed that carbohydrate content was significantly ($P< 0.05$) the highest food content. It was 30.50% in *M. procera*, 28.8% in *P. roseus* and 29.2% in *C. cibarius*. Crude protein obtained were 9.8%, 11.43% and 10.2% in *M. procera*, *P. roseus* and *C. cibarius* respectively. However, the mineral composition analysis showed that the three samples were very rich in potassium and sodium but poor in cobalt. The samples were also rich in vitamin A. *M. procera* has the highest percentage of moisture content (18.01%) which was significantly different ($P<0.05$) from others. Ash content and crude fibre of the three mushrooms were significantly different ($p<0.05$). The fat content was generally low with *M. procera* having (11.50%), *P. roseus* (13.65%) and *C. cibarius* (12.10%). *M. procera* has the highest content of potassium (6.80 mg/l) while *C. cibarius* was lowest (5.40 mg/l). These mushrooms hold tremendous potentials in contributing to the protein, vitamin and mineral element needs of the people. Therefore, their commercial production and consumption, especially those on low fat dietary food should be encouraged and their use as raw materials to the pharmaceutical industries is recommended.

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Survey bioremediation of chlorophenol by yeast fungi isolated from industrial wastewaters and oil

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Introduction: The potential of various organisms to metabolize organic compounds has been recognized as potentially effective means of disposing of hazardous wastes. Among these compounds, phenolic compounds are toxic pollutants which belong to chlorophenols known as xenobiotic chemicals. They are extensively used as herbicides, insecticides, fungicides, wood preservatives, resins and lubricants. 4-Chlorophenol (4-CP) is one of the chlorophenols with a high solubility in water, so it is most detected in wastewater and can accumulate in their bodies biologically.

Methods: In present study 14 Strains of bacteria and 5 Strains of Yeast and mold phenol degradation was purified from Shahid Tondgooyan petrochemical wastewater treatment unit was first carried out within about 60 days. Then, capability of the isolated microorganisms in biodegradation of 100 ppm 4-chlorophenol in presence of 2 and 5 g/l glucose as a growth substrate was examined. Two microorganisms, selected as superior species. The strains were designated TY1 and TY2 and identification was performed by sequencing of 18S rRNA and confirmed by morphological and biochemical characterization. Various physicochemical parameters are optimized for the maximum biodegradation of 4-CP, i.e. pH, temperature, initial concentration of phenol (100, 200 and 300 ppm) and carbon sources such as glucose (2, 5 g/l) concentrations. The phenol degradation was determined by the spectrophotometric method 4-amino antipyrine.

Results: The results showed that 100% removal of 100 ppm by mixed culture of TY1 TY2:50/50 in presence of 2 gr/l glucose within 15 hours. Percentage of pure cultures in mixed culture had no significant effect on 4-CP removal efficiency however; initial glucose with lower concentration had higher 4-CP removal efficiency. Furthermore, the results of the sequencing showed that the isolates with the genus *Trichosporon*.

Conclusion: The significance and impact of the study is the utilization of native yeast strains isolated from the waste water itself having potential for environmental bioremediation in petroleum refinery and petrochemical industries.

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Evidence that virulent *Cochliobolus lunatus* colonizes potato by down-regulating proteome at late stages of infection

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It is confirmed that *Cochliobolus lunatus* adopt different but highly successful strategies on potato cultivars to incite brown-to-black leaf spot disease. *C. lunatus* abundantly produces four-celled conidia at high temperatures (>30°C) and under suitable conditions; the fungus colonizes potato (*Solanum tuberosum L.*) cultivars by adopting different invasion strategies at the microscopic level. Long-lasting defense during infection requires an upsurge in proteome changes particularly pathogenesis related proteins (PrPs) chiefly under the control of nonexpresser of pathogenesis related proteins. In order to gain molecular insights, we profiled the changes in proteome and potato nonexpresser of pathogenesis related proteins (*StNPR1*) during the infection process. It is found that *C. lunatus* significantly ($P<0.05$) suppressed the host functional proteome by 96 hours after infection (hai), principally, affecting the expression of ribulose bisphosphate carboxylase enzyme, plastidic aldolase enzyme, alcohol dehydrogenase 2 and photosystem II protein prior to the formation of brown-to-black leaf spot disease. Strongest host–response was observed at 24 hai hallmarked by 307 differentially expressed peptide spots concurring with the active phase of production of penetrating hyphae. Additionally, *C. lunatus* differentially down-regulate *StNPR1* transcript by 8.19 fold by 24 hai. This study is the first to elucidate that *C. lunatus* transiently down-regulate the expression of *StNPR1* at the onset of infection, and as a whole, infection negatively affects the expression of proteome components involve in photosynthesis, carbon fixation and light assimilation. This study contributes towards better understanding of the mechanism underlining the invasion strategies of *C. lunatus*.

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Next generation biotherapeutic production system: The filamentous fungus *Trichoderma reesei*

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The filamentous fungus *Trichoderma reesei* is an important production organism used by industrial enzyme companies worldwide. It is a low cost production system that secretes its native enzymes at levels exceeding 100 g/L of culture medium. Several *T. reesei* produced enzymes have obtained the generally recognized safe status by the Food and Drug Administration. *T. reesei* has tremendous prospects to be a cost efficient and high yield system for producing therapeutic proteins. We have adapted the fungus to become more suitable for bio-therapeutic production by reducing secreted protease activity and altering glycosylation pathways needed for adding mammalian glycoforms. Expression strains for monoclonal antibodies, Fab antibody fragments, interferon alpha-2b, insulin-like growth factor 1, and fibroblast growth factor 21 were constructed, cultivated in bioreactors, and expression levels were measured from the culture medium. After deleting 13 of the most critical protease genes, the general secreted protease activity was reduced over 30-fold. Monoclonal antibodies could be produced up to 7.6 g/L, Fab antibody fragments up to 8.2 g/L, interferon alpha-2b at 7.9 g/L, and insulin-like growth factor fusion protein at 8 g/L. With protease inhibitor treatment, interferon alpha-2b could be produced at over 10 g/L, insulin-like growth factor fusion protein at 19 g/L, and full length fibroblast growth factor 21 at 200 mg/L in addition to a shorter form at 3.5 g/L. Human glycoforms such as G0 and FG0 were produced on monoclonal antibodies. Expression levels and product quality improved dramatically after multiple protease deletions and optimization of culture conditions. While the production levels achieved are already relatively high, the strains could be developed further to reach the 100 g/L potential of the organism. This study demonstrates the excellent prospects of *T. reesei* as a host for therapeutic protein production.

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Macrofungal ecology, diversity and ethnomycology in Ethiopia

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Our knowledge of macrofungal diversity in Ethiopia is very limited. The type of vegetation, ecological and habitat variation in an area determines species richness and composition of macrofungi. Our recent mycological study undertaken in three ecologically and geographically different sites, Welmera, Menge and Kaffa in Ethiopia has shown interesting variations in macrofungal flora. Mushroom diversity on the central highlands is dominated by species of *Agaricus*, diverse and common on highland fields, pastures and forested areas. Mushrooms associated with termites, *Termitomyces* spp, are diverse in the low altitude savanna woodland where macrotermitinae termites are common and diverse. Other examples of macrofungal diversity and specificity on the highland and lowland ecosystems have been documented. A high diversity of *Lentinus spp* is found on *Cordia Africana* wood and *Pyrofomes demidoffi* as a specific pathogen of *Juniperus procera* are worth mentioning. Ectomycorrhizal mushrooms found in exotic plantations (Eucalyptus, Pinus and Cupressus) such as species of *Laccaria* and *Suillus* are absent in indigenous forests. Mushrooms are, in general, known to have food and medicine value to local people in many cultures including in Ethiopia. Unlike the central highlands, however, ethnic groups in southwest Ethiopia have a well developed traditional knowledge and habit of using mushrooms for food and medicine. In this region, wild mushrooms are a free source of food during the rainy season, a period of grain scarcity. Deforestation and habitat destruction are the main factors for decreasing variety and abundance of macrofungi in Ethiopia. The need to study macrofungal flora of Ethiopia and conserve the genetic resources is highly recommended.

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Chemistry of fungi: Study of the lipid metabolites of *Hydnellum ferrugineum*

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In this work we explored a chemical study of the lipid fraction of the fungus *Hydnellum ferrugineum* through spectroscopic and chromatographic techniques, in which the following were identified: natural *n*-alkanes (C10–C30); steroids with an ergosterol skeleton (ergosta-7-en-3-one 1, ergosta-7,22-dien-3-one 2, ergosta-7-en-3-β-ol 3 and ergosta-7, 22-dien-3-β-ol 5); two pentacyclic triterpenes (friedeline 7 and taraxerol 8); saturated fatty acids (C11, C12, C13, C14, C15, C16, C17, and C18); ethyl oleate; and ethyl linoleate. This chemical-specific information is expected to provide new data for the chemical-taxonomic classification of this fungus.

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Biodegradation of plasticizers from polypropylene thermoplastic composites by halotolerant fungi

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This study investigated the capacity of two halotolerant fungal strains, which were grown in liquid-surface fermentation, to degrade plasticizers from polypropylene containers. *Paecilomyces variotii* and *Penicillium roqueforti* were grown for 12 days under hypersaline liquid-surface fermentation, with polypropylene commercial storage boxes being used as the fermenters. The biodegradation experiment was conducted in a modified KMV broth. Bis-(2-ethylhexyl)-phthalate (BEHP, 1), a common chemical additive, was identified as the only plasticizer in the culture containers used. It was observed that *P. varioti* was able to transform BEHP into diethyl- and dibutyl- phthalates, while *P. roqueforti* transformed BEHP into diethyl-, bis-(2-methylpropyl)-, dibutyl-, bis-(4-methylpentyl)-, dihexyl-, and dioctyl- phthalates. In this last case, 2-ethylhexyl-adipate (2) also was identified as byproduct. BEHP was not detected in either mycelium after the incubation period. The results suggest that *P. varioti* and *P. roqueforti* are highly efficient in degrading the BEHP plasticizer and can be used for bioremediation of polypropylene wastes. Therefore, efficient biotic degradation of polypropylene by halotolerant fungal strains could provide eco-friendly alternatives for degrading plastic additives, as well as leading to advances in the research and development of bioremediation strategies.

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Genetic diversity of *Aspergillus flavus* and occurrence of aflatoxin contamination in stored maize across three agro-ecological zones in Kenya

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Aflatoxin contamination at post-harvest poses a serious challenge in achieving millennium development goals on food security especially in the developing world. In Kenya, major outbreaks of aflatoxicoses have been attributed to poor post-harvest storage practices. In this study, we conducted a cross-sectional survey within three Agro-ecological zones in Kenya, to determine occurrence and distribution of total aflatoxin in stored maize and the aflatoxigenicity potential of *Aspergillus flavus* in stored maize. The counties selected were; Kitui, Nakuru and Kitale (in Trans-Nzoia County). Sampling sites were selected based on previous aflatoxicoses outbreaks (Kitui) and major maize production areas (Nakuru and Kitale) where little information exists on the occurrence of aflatoxin contamination. A total of one hundred and thirty (130) kernel maize samples were random collected during the period between June and August 2012. Moisture content was determined using the standard oven method and *Aspergillus flavus* was isolated by direct plating technique. Genetic diversity of the isolates was determined by PCR and Single Sequence Repeats (SSR) micro satellites analysis. Positive strains were induced to produce B1 aflatoxins on Yeast Extract Sucrose Agar (YESA) and quantified using competitive ELISA technique. The results indicated mean moisture content of maize ranged between 6% and 34%, although this was found not to be significantly different ($p=0.23>0.05$). However, total aflatoxin contamination of postharvest stored maize samples between sites was significantly different ($p=0.000, <0.05$); with the highest contamination in Kitale at a mean of (9.68 µg/kg). *A. flavus* was isolated in 70% (N= 91) of the maize samples collected at postharvest. *A. flavus* isolates with the highest aflatoxigenicity potential were from Nakuru County with mean aflatoxin level 239.7 µg/kg. Genetic distance based on Neighbor Joining (NJ) clustered the *A. flavus* isolates into five main clusters. Principal coordinate Analysis (PCA) analysis showed five distinct clusters with both axes explaining 60.17% of the variance. This study showed widespread distribution of aflatoxin contamination and a highly toxigenic *A. flavus* in stored maize in three major agro ecological zones in Kenya. These results suggest a potential health risk of aflatoxin outbreaks within these areas, thus call for more investigations.

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Isolation and molecular identification of *Trichophyton mentagrophytes* from patients with dermatophytosis

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Out of 30 clinical specimens isolated from patients with dermatophytosis in the Dermatology unit of the General Hospital in Kalar distric\Sulaimania province\ North region of Iraq, five clinical isolates showed positive results for dermatophytes, four of them were identified as *Trichophyton mentagrophytes* characterized by the production of white colonies at the surface and brown color at the reverse. Primarily, the colonies appeared with cottony texture and after two weeks changed to powdery-granular colonies. Microscopic examination appeared numerous single-celled, spherical shaped microconidia were seen as clustered on both sides of hyphae. Multiseptate cigar shaped macroconidia and spiral hyphae were seen during the formation of granular colonies. *Trichophyton mentagrophytes* was positive for urease and hair perforation testes. Molecular identification according to the conventional PCR by using set primers ITS1 and ITS4 resulted in PCR product about 700bp in all isolates. PCR-RFLP by using *BstN1* digestion enzyme revealed four pattern bands 250,180,150 and 120bp. Sequencing of the ITS region in one of the isolates, *Trichophyton mentagrophytes* revealed similarity of about 86% with *Trichophyton mentagrophytes* isolate ATCC11481 regarding the following parameters: internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence. 2) Sequencing Results: Sequencing of the ITS region in one of the isolated *T.mentagrophytes* revealed similarity about 86% with *Trichophyton mentagrophytes* isolate ATCC11481 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence.

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Probability inspection using solution and ozone gas for lichen removal in historical levels for case study of perspolice pilot

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Lichens consists of algae and mushroom coexistence but it is believed that parasitic life of mushroom on algae in the way that mushroom feed from mineral and organic materials of stone.in result of lichens interactions , stone breaks and convert to soil. Some lichen grow out only 1 mm during one year and this coexistent mushroom live for 4000 years and could resist in -600c.there is lichen hazard in all historical buildings but most risk is in stone inscription in fars province that is observed in stone inscription in perspolice. Many methods are proposed but aren't practical in historical works and results in damages such as mechanical methods or washing by water with high pressure or by bio side which are expensive .in present study , feasibility of ozone is tested and main question is that by what dose and density of ozone solution or gas , lichen could be diminished and also what is destructive effect of ozone on stone and whether it could result in color changes. what is it's effect on utilized bacteria in lichens and what is it's effects on algae chlorophyll? some advantages of ozone in comparison with other methods is it's more safety and it's low-cost also it's high oxidation because of it's ozone structure that include oxygen which affect on microorganism cellular wall very much. Research importance could be inspected from several perspectives ,fist regarding to stone and historical building variety in country and lichen in these works especially in perspolice and present inscription importance in perspolice and high cost of these buildings destruction for country .perspolice inscriptions penetrate some millimeter in stone and in some places ,these lichens could be observed in these inscriptions .lichens could penetrate in stone because of their thallus structure thus it is possible that inscription drawing .mechanical methods for these inscriptions and drawings is disastrous because it is observed that by brushing , thallus would scatter and would grow out more after that. Applying laser and water have it's disadvantages and exhibit stone erosion .thus applying ozone gas and solution in water could have minimum disadvantage and hazard.

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The antifungal effects of alcoholic extract of *Ganoderma lucidum* on candida isolates

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Introduction: Candidiasis is a common fungal infection caused by various species of the *Candida. Candida albicans* is the most important etiologic agent of candidiasis which includes about 60%-75% of this disease. The extent of fungal opportunistic infections in susceptible individuals on one hand and increasing drug resistance on the other hand, leads to the importance of antifungal effects of traditional plant products. One of the edible mushrooms which is named as the best medicinal fungi, having many health benefits and various therapeutic properties is named *Ganoderma lucidum*. In this study, the therapeutic importance of this mushroom, as well as previous studies for antifungal and antibacterial properties of this fungus, was designed.

Materials & Methods: This study was carried out on patients with candidemia admitted to some specialized hospitals in Tehran. To identify the *C.albicans* specie, out of 4850 blood cultures, 43 cases were identified as candidiasis, by using phenotypic and molecular methods, *in vitro*. Then microdilution methods were used to prepare different concentrations of *G. lucidum* ethanol extract to determined MIC (minimum inhibitory concentration) and MFC (minimum fungicidal concentration) for each *C.albicans* isolated species as well.

Findings & Conclusion: The result showed that out of 43 candidiasis, the frequency of candida isolates were as follows: *C. albicans* 22 (52%), *C. parapsilosis* 10 (23%), *C. glabrata* 8 (18%) and *C. tropicalis* 3 (7%) respectively. By microdilution method the concentration of 5.2 mg/ml inhibited most species. The MIC was 3.1 mg/ml and the maximum concentration was 10.4 mg/ml. The MFC was 5.2 mg/ml and the maximum concentration was 20.8 mg/ml. According to the results of this study, the *G. lucidum* ethanol extract can be used as an antifungal product in the future studies to lead for better control and treatment of candidiasis.

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Detection of diverse metabolites in fish feeds from Nigeria

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Fish feed being an indispensable requirement in fish farming is prone to contamination from diverse range of compounds such as pesticides, microorganisms and their metabolites because it is made up of several ingredients from both animal and plant sources. In this study, the contamination level of locally formulated fish feeds from different fish farms in Nigeria was determined. Ninety-four fish feed samples were collected in six states within South-western, Nigeria in 2013 namely; Lagos, Ogun, Oyo, Osun, Ondo and Ekiti states. The spectrum of mold metabolites including mycotoxins in the feeds was assessed using a Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS). Eighty-four metabolites from diverse molds were found in the feeds with co-occurrence of at least 25 per sample. Regulated mycotoxins for animal feeds: Total aflatoxins, fumonisin B1, zearalenone and deoxynivalenol co-occurred in 77.8% of samples, with the main combination being aflatoxins and zearalenone. Deoxynivalenol was detected in 87% of analyzed samples at concentrations ranging between 2–333 µg/kg. Aflatoxins B1 was the most prevalent aflatoxin with 97.9% occurrence and mean 108.17 µg/kg while fumonisin B1 was the highest prevalent fumonisin with occurrence of 88.3% at mean of 6097.90 µg/kg. This is the first report of Aflatoxin M1 in fish feeds and it was detected in 76 samples at mean concentration 4.8 µg/kg. Enniatins {A, A1, B, B1}, beauvericin and moniliformin, are new emerging mycotoxins which were found co-occurring with major mycotoxins at high concentrations. Considering the array and levels of mycotoxins and other mold metabolites detected in the sampled fish feeds, it could be posited that the fish feeds from these warehouses are of poor quality. Consumption of fishes fed these feeds and their products could pose a significant health risk to consumers.

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Mycoflora and aflatoxin levels of edible vegetable oils sold in Nigeria and possible control measures using imarsil and activated charcoal

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Edible oils play vital role in a well-balanced diet. However, the infestation of most edible oils by mycotoxigenic moulds possesses high health risk for humans and animals. It thus necessitates the need to screen the oils and detoxify those using local and inexpensive adsorbents. Ten samples of edible vegetable oils from different plant sources such as canola, palm-kernel, sunflower, olive, groundnut, soya-beans, coconut, cotton seed, palm and corn oils were purchased from Nigerian markets and assessed for fungi and aflatoxins levels using standard microbiological procedures and High-Performance Liquid Chromatography (HPLC) respectively. Adsorption studies of Aflatoxins (AF) were performed on the AF positive oils using *imarsil* and activated charcoal at 2 and 3 % concentrations. Sensory evaluation of treated and untreated oils was also carried out using 10 members panel. Prevalence of isolated fungi were: *Aspergillus fumigatus* (43%), *Mucor sp* (17.9%), Saccharomyces sp (10%), A. niger (7.1%), A. oryzae (7.1%), *A. flavus* (7.1%), Penicillium sp. (7.1%) and Rhizopus sp. (3.6%). Seven samples were positive for AF. Cotton oil, Sun-flower oil and Canola oil had no detectable AF levels while Corn oil, Coconut oil, Olive oil, Soya oil, Palm kernel oil, Palm oil and Groundnut oil had the following aflatoxin concentrations respectively ; 157ng/kg, 49ng/kg, 33ng/kg, 28ng/kg, 9ng/kg, 5ng/kg and 4ng/kg. At ≤ 9 ng/L AF contamination rate, both *imarsil* and activated charchoal exhibited 100 % adsorption efficiency within one hour. At AF contamination rates of 28-157 ng/L, activated charcoal was not effective while *imarsil* had 100 % removal efficiency within 3 hours. Sensory evaluation results showed *imarsil*-treated vegetable oil had good organoleptic properties while activated charcoal –treated vegetable oils had off-flavour. Aflatoxins present in some vegetable oils can be eliminated using *imarsil*.

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Expression of the *Aspergillus fumigatus* α-1, 2-mannosidase gene (*MsdS*) in *Trichoderma reesei* affects cell wall synthesis and polarized growth

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Statement of the problem: α-1, 2-mannosidase is a very important enzyme, essential for N-glycan processing and plays a significant role in the biosynthesis and organization of fungal cell wall. Lack of α-1, 2-mannosidase has been observed to cause cell wall defect in yeast and filamentous fungi. *Trichoderma reesei* is known to be non-toxic to human, and its N-glycan on mature secretory glycoprotein is GlcNAc2Man8, which is different from the GlcNAc2Man6 in the wild-type *Aspergillus fumigatus* and similar to the glycoform of the *MsdS* deletion mutant, which was characterized by cell wall defect and polarized growth.

Methodology & Theoretical Orientation: To gain insight into the physiological function of the N-glycan processing in *T. reesei*, in this study the *A. fumigatus* α-1, 2-mannosidase MsdS was introduced into *T. reesei*.

Findings: The mutant strain expressing MsdS produced a major glycoform of GlcNAc2Man6 on its secretory glycoproteins, instead of GlcNAc2Man8 in the wild-type. Although the cell wall content of the mutant was changed, it appeared that its cell wall integrity was not affected. However, multiple budding and random branching was observed in the mutant. In addition, the mutant showed less dense filamentous material on cell surface under favorable growth condition, while a complete loss of filamentous material accompanied by an increase of cell wall chitin was observed at elevated temperatures.

Significance & Conclusion: Our results, for the first time, indicated that processing of the N-glycan might play a major role in sorting and polarized transportation of certain glycoproteins in *T. reesei*, which is vital for the cell wall synthesis and polarity. Further, our results also indicated that the mechanism of the N-glycan-related sorting of glycoprotein in *T. reesei* is different from that in *A. fumigatus*.

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Discoveries of two new species: *Psathyrella canalus* and *Psathyrella puntini* from low lands of Pakistan by using molecular approach

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Introduction: There are a few mandatory characteristics of mushroom species that need to take into account before title them as a novel one. In this way, morphological features including their habitat are the basic features to be noted critically and extensively. Considering this criterion loads of new ascertains have been made throughout the world. From hilly areas of Pakistan, several gilled and non-gilled macro fungi belonging to different orders have been reported for ages. However, this piece of study dealt with exploration of the lowland species belonged to family Psathyrellaceae. The genus *Psathyrella* has few easily discernible characters which leads it to be a cleaned-up genus to those that has small brown spores. The archetypal little brown mushrooms were collected from plain areas of Pakistan falling in Punjab province.

Methods: Initially both species were examined macroscopically and microscopically. Nonetheless, species identification has confirmed by amplifying their conserve nuclear ribosomal internal transcribed spacer region using model fungal primers.

Results: Final result was inferred when *P. canalus* and *P. puntini* got their separate clades showing 73 and 71 bootstrap values, respectively, when compared among twenty-five other species. Additionally, *P. canale* had bell–shaped to convex, slightly upward pileus margin, adnexed to adnate hymenium and soft, hollow and thick stipe were the key features that made it distinct from other *Psathyrella* species macroscopically. On the other hand, *P. puntini* was convex to umbonate and the presence of small milky–white to lemon chiffon whitish dots all over the pileus surface was the major attribution towards its discovery. Moreover, its hymeniun was dark–brown, adnexed to slightly narrowly adnate while stipe was milky–white to creamy white with an annulus.

Conclusions: New discoveries are vindicated using advanced methods as here a DNA level study has proven the novelty of two new *Psathyrella* species. These findings support in evolutionary history of these particular genera, they may have applications in different industries as well.

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Distribution of amatoxins and phallotoxins in different tissues and development stages of *Amanita subpallidorosea*

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Amanita subpallidorosea is a lethal mushroom discovered recently in China. Due to the morphological similarity to edible mushrooms and the high toxicity, *A. subpallidorosea* had caused severe mushroom poisonings in China. However, the contents and distribution of the major toxins in *A. subpallidorosea* remain poorly studied. In this study, the concentration of the major cyclopeptide toxins, amatoxins and phallotoxins in different tissues and development phases were systematically analyzed for the first time. To find other structure related high risk compounds which are not reported or available as standards to launch toxicological study in *A. subpallidorosea*, a new non-targeted strategy based on liquid chromatography-high resolution mass spectrometer (LC-HRMS) was applied to analyze the toxin profiling of the mushroom and to find new cyclopeptide toxins. The results showed that the concentration of the total amatoxins in *A. subpallidorosea* were remarkably high, which was much higher than the worldly notorious *A. phalloides*, a lethal species from Europe and North America. The distribution of amatoxins and phallotoxins in different tissues showed the highest concentrations of amatoxins and phallotoxins were found in the cap and the lowest concentrations in the volva. Further analysis of mushrooms in different development stages showed that the amatoxin content was relatively high during early development, in which stage the fruit body grew most vigorously and regarded as tasty stage for mushroom picking. With the LC-HRMS based strategy, seven cyclopeptides and two new compounds were found and confirmed by parallel reaction monitoring (PRM) and all ion fragmentation (AIF) mode on a high resolution hybrid quadrupole-orbitrap mass spectrometer.

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