STUDIES ON DEVELOPMENT OF MICROBIAL BASED TECHNOLOGY FOR HOSPITAL WASTE MANAGEMENT

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***Abstract*:**

Background & objectives: The health care organizations generate tremendous hospital waste and existing methods for its management are costlier and liberate toxic byproducts causing ailments in the nearby residents.This study involves the use of fungus *Periconiella sp.* grown on Khillar Deshi Indian cow dung to degrade hospital waste.

Method: Dung from 10 Khillar breed cows was used to grow the fungus *Periconiella* sp which was subcultured on Sabouraud dextrose agar. It was morphologically confirmed on the basis of 10% Potassium Hydroxide (KOH), Lactophenol Cotton Blue(LCB) preparation & slide culture. Its role in degradation of soiled cotton, soiled gauze pieces, and tissue pieces was studied.

Results: The fungus *Periconiella sp.* was found to degrade 5g.of hospital waste containing soiled cotton gauze and tissue pieces in a span of 35 days.

Interpretation & conclusion: Fungus Periconiella sp. is a good degrader of soiled cotton, gauze,bandage; tissue pieces requiring Indian Deshi Khillar breedcow dung, helping to develop cheap microbial based method for hospital waste management.

***Key words*:** Cow dung, hospital waste, low temperature, microbial based, Periconiella, Saprophyte

INTRODUCTION:

Aim: To study the role of fungus Periconiella sp in hospital waste management.

Objectives:

1. To isolate fungus Periconiella sp from Indian Deshi cow (Khillar breed) dung. Sample size –(10 Deshi cows dung)
2. To isolate Periconiella in pure culture on Sabouraud dextrose agar (S.D.A.)
3. To study morphological and biochemical characters of isolated fungus Periconiella sp
4. To study the role of isolated Periconiella sp in degradation of soiled cotton soiled gauze pieces and tissue pieces (hospital waste).

Developing countries with their population explosion are imposing a tremendous stress on the existing health care[1]. To meet the growing need myriads of Govt. and private hospitals and diagnostic centers are increasing in number, that are potentially generating tons of medical waste. It is mandatory for the hospitals to dispose medical waste properly maintaining a clean environment to prevent pollution and spread of infection within and in the community. The effective management of medical waste poses a wide range of unsolved queries. Medical waste disposal is associated with health, environmental and aesthetic hazards. Incineration is an effective method of waste disposal but is associated with environmental hazards, such as generation of dioxins and furans in the smoke as well as flyash[2]. These chemicals can produce neurological disorders in children, reproductive problems in women & increased incidence of skin cancer in the vicinity. Sulfur dioxide generated causes respiratory & cardiac ailments[3]. Plasma pyrolysis is a novel eco – friendly technology, requiring high temperature & tremendous power consumption, thus making it very expensive beyond the reach of poor nations. Hydroclaving & microwaving do not seem financially viable solutions for poor resource countries. This project offers a low cost, safe, environment friendly, low temperature, associated microbial based – Periconiella fungus sp (a saprophyte & plant pathogen) technology that can degrade biomedical (hospital) waste[1].

Literature search:

On morphology, for isolation, cultural characters, characterization, Biochemical reactions, pathogenicity, enzymes synthesized by fungus Periconiella sp & its role in biomedical waste management (BMW). Coprophilous (dung loving) fungi release their spores in the surrounding which is then ingested by herbivorous animals. As the ingested plants are digested, these spores are retained in the intestine of these animals and finally are shed into their faeces.The fungi then grow from the animal feces & reach the new plants. Animal faces are rich in nitrogenous material and various enzymes from the animal's digestive system, supporting the growth of these fungi. The spores are thick walled and thus are protected from digestion and germinate in the dung. The thick wall of spores is loosened by the action of digestive enzymes, making them ready to germinate in the dried dung after hydration[4, 5, 6, 7].

Materials and Methods:

Dung from 10 Indian Deshi cows of Khillar breed was used[1].A culture was prepared by using Indian Deshi Cow dung for fungi. The growth on cow dung appeared in a period of about 2 weeks. The culture was incubated at room temp in dark. - Colonies with entire margin, aerial[1]mycelium compact, raised velvety grayish brown pigment.

Fig. 1; Growth of *Periconiella* on cow dung culture 1

Fig.2: Growth of *Periconiella* on cow dung culture 2.

Smears from the dung culture colony were prepared. Fungal elements tithed with needle. The KOH and LCB preparations were observed under the light compound microscope.

KOH Preparation:

Fungal elements observed. The hyphae were septate with wide angle branching, orthoconidia, blastospores, budding. Double walled hyaline fungal cells, numerous microconidia arranged singly or in short chains or in grape like clusters were also observed. Banana shaped macroconidia were also present.

LCB Preparation:

Revealed hyaline, septate, wide angled branching hyphae. It also revealed monomorphic conidiophores which have branched head with fewer branches. Microconidia arranged singly. Few spores were seen to form short chains and few were arranged in grape like clusters. The creeping hyphae were bearing vertically directed conidiospores. Individual conidiogenous cells were smooth, verrucose and polyblastic. A globus ascus with ascospores was also noted. Occasional chlamydospores were also present

Culture on Sabouraud dextrose agar (SDA):

A culture on SDA incubated at 37^0C in dark. Growth appeared within 48 hours. Mixed growth seen.

Colony No.1 – Colonies with entire margin, aerial mycelium compact, velvety, olivaceous, grayish brown pigment on obverse. On reverse brown pigment. Submerged hyphae verrucose, hyaline, thin walled.

Colony no. 2 – Velvety compact powdery, blackish.

Fungi isolated – Periconiella sp, Aspergillus sp, Mucor sp, Penicillium sp, Fusarium sp, Cladosporium sp

Luxuriant growth on SDA incubated at room temperature in dark within 24 hrs. Brown pigment seen.

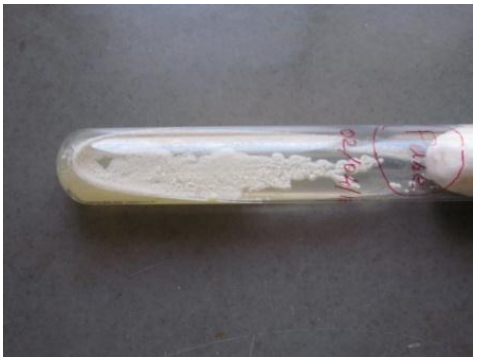

Fig.-3 Pure culture

Slide culture done for both the colonies.

Slide culture 1 – The conidiophores were monomorphic with branched head having fewer branches. Revealed aseptate conidia. Hyphae were septate and branched. Creeping hyphae were bearing vertically arising conidiophores which were straight & thick in upper part, bearing short branches. Polyblastic terminally integrated conidiogenous cells were seen. Fertile part was wide like basal part, proliferating sympodially becoming septate, forming a short straight rachis with brown thickened prominent flat scars. Conidia solitary, occasionally in short chains & grape like clusters. Obvious ascus with ascospores **morphologically confirmed to be Periconiella sp belonging to Ascomycota.**

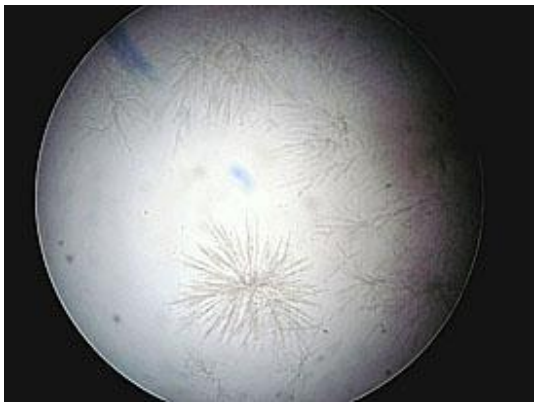

Fig.5 Slide culture Periconiella

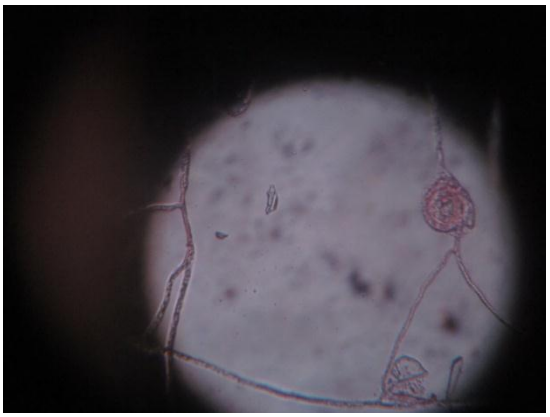

Fig.6 Slide culture Periconiella Ascus.

Slide culture 2 – Reveals basipetal conidia, youngest conidia at base & oldest at the tip of chain. Conidiogenous cell are called phyialidae. Hypha bear swollen vesicle at the apex. Phyialidae uniseriate & biseriate **morphologically confirmed – Aspergillus sp.**

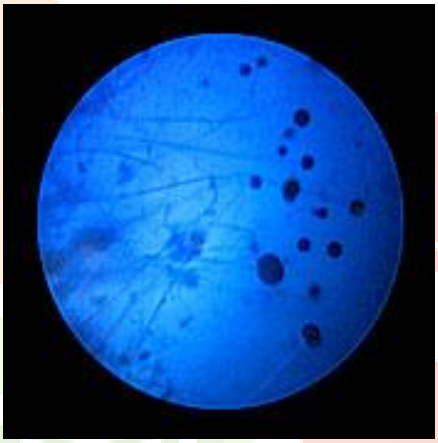

Fig.7 Slide culture Aspergillus

Slide culture 3 – Non septate hyphae with branched sporangiospores with columellae & sporangia. No rhizoids **morphologically confirmed – Mucor.**

Slide culture 4 – Septate hyphae with bean shaped microconidia **morphologically confirmed – Fusarium sp.**

Slide culture 5 – Conidiophores in various forms, Phialides singly or in groups forming branched metullae appearing brush like. Conidia unicellular basipetal **morphologically confirmed – Penicillium sp.**

Slide culture 5 – Non septate hyphae with rhizoids **morphologically confirmed – Rhizopus sp.**

Smears from pure culture – Heat fixed & fixed with spirit spray & stained.

1. Gram stain – Gram positive, wide angled branching septate hyphae. Microconidia in clusters. Banana shaped macroconidia. Budding cells. Ascus with ascospores. Chlamydospores also seen.
2. Neutral Red stain – Viable branched septate hyphae reddish pink.
3. H & E stain – A tuft of wide angled branching septate blue colored hyphae. Microconidia in clusters & banana shaped macroconidia (eosinophilic)
4. Giemsa stain – Branched blue colored septate hyphae. Microconidia, macroconidia & ascus present.
5. PAS stain – Pink colored PAS positive wide angled branching septate hyphae. Grape like clusters of microconidia. Banana shaped macroconidia. Occasional chlamydospores & arthrospores. Ascus with ascospores.
6. Gomori silver methanamine stain – Black colored wide angled branching septate hyphae. Clusters of microconidia. Banana shaped macroconidia.
7. Z.N.stain – Wide angled branching septate hyphae nonacid fast. Conidia also nonacid fast.

Periconiella sp. morphologically confirmed.

5 g of cotton (nonabsorbent soiled) spread on the 2 of the cow dung culture and SDA culture. Cotton invaded by aerial mycelia. At one corner cotton was degraded. **Fungus degrading soiled cotton was confirmed to be Periconiella sp.**

Similarly on remaining cow dung culture plates & SDA culture plates 5 gof soiled gauze pieces, and tissue pieces were kept. The soiled gauze pieces & tissue pieces were invaded by the aerial mycelia & they were degraded – **Degrading fungus confirmed to be Periconiella sp.**

Probable enzymes produced by Periconiella – Cutinases, Pectinases, Polygalacturonases, pectate lysases, cellulase & hemicellulase, lignase & proteases – confirmed biochemically[8,9,10].

Biochemical Reactions – Indol +ve, MR –ve, VP –ve, Citrate –ve, Urease +ve, TSI – K/K no gas.

Sugar Fermentation – Glucose fermented with production of acid but no gas.

Sucrose fermented with production of acid but no gas.

Lactose –ve, Mannitol –ve.

Fig9 Cotton degraded

Fig 10 Gauze degraded

Fig.11 Tissue pieces degraded

Observation and Results–

Table showing Destruction of Biomedical waste in days by fungus *Periconiella sp.* -

Sr. No.	Name of the Fungus	Type of Hospital Waste Degraded in no. of days		
		Soiled Cotton (5g)	Soiled Gauze pieces (5g)	Tissue pieces (5g)
1	Periconiella	28	35	25
2	Aspergillus	-	-	-
3	Fusarium	-	-	-
4	Mucor	-	-	-
5	Rhizopus	-	-	-

Discussion:

Many hospitals are installing incinerators for quick disposal of biomedical waste material. These incinerators are of poor quality and most of which do not comply with the working provisions in the Biomedical Waste (Management and Handling) Rules, thereby nullifying the objective of incineration of biomedical waste as has been observed by Central Pollution Control Board in Delhi as well as reports from other states (Biswas D 2001)[2]. Incinerators are costly. Incineration though an effective method of waste disposal is associated with environmental hazards, such as generation of dioxins and furans in the smoke as well as flyash. These chemicals can produce neurological disorders in children, reproductive problems in women & increased incidence of skin cancer in the vicinity. Sulfur dioxide generated causes respiratory & cardiac ailments. Each hospital should have its own policy depending upon the nature of set up. In hospitals as well as nursing homes, all pathology, microbiology laboratories and blood banks are governed by the rules & regulations of Biomedical Waste Management Rules 1998(Ministry of Environment and Forests, Notification, N.S.O.610E)[2]. One must take into consideration the amount of biomedical waste generated & how it should be disposed of at low cost. Noninfectious & infectious waste must be separated & packed in color coded bags meant for purposes. The next thing is its disposal mechanism which is cheap enough for the hospital set up that does not impose any financial burden. In the present study, fungus Periconiella sp is observed to be a good degrader of the hospital waste like soiled cotton, soiled gauze and tissue pieces. In the present study we also characterized the Periconiella fungus morphologically as well as biochemically through microscopic structure, slide culture and biochemical tests.[9, 10]

Conclusion:

Fungus Periconiella sp. is a good degrader of soiled cotton, soiled gauze pieces, bandage and tissue pieces. The biomedical waste comprised of soiled cotton, soiled gauze pieces and tissue pieces of placenta and operated organ pieces get degraded completely in a span of 5weeks by fungus *Periconiella sp.* It only requires the Indian Deshi cow(Khillar breed) dung to raise the fungus *Periconiella* and it can be used to develop cheap, low cost and cost effective microbial assisted method for biomedical waste management. This is part first of the study. Second part of genetic and phylogenetic characterization of the fungus Periconiella is continued and work is in process.

Acknowledgement: President D.Y.Patil Education Society Kasaba Bavada Kolhapur for permitting to use the resources.

Conflicts of Interest: None

References:

[1] AnupriyaPandey, H.S.Gundevia – Role of fungus Periconiella sp. In destruction of biomedical waste – Journal of Environ. Science &Engg. Vol 50, No.3, P.239 – 240 July 2008.
[2] Biswas D., Restricting the use of incinerator by Individual healthcare facility D.O. letter no. B – 31011/30/93/PCI -2/ 16316 letter Sept.27, 2001, letter written to ShriUpendraTripathi Karnataka Pollution Control Board, Bangalore, Karnataka.
[3] Glasser, H.D.P.Y.Chang, D.K.Hickman, An analysis of Biomedical Waste incineration, J.Air and Waste management Assoc.41, 1180(1991).
[4] Mukerjee D. Health Impacts of Polychlorinated Dibenzo p – dioxins A clinical Review, J.Air and Waste Management Assoc.48, 157(19998).
[5] AntonellaAnastali et all –Isolation and identification of fungal communities in compact and vermicompost – Mycologia,97(1), 2005, PP 33 – 44 @2005 by The Mycological Society of America, Lawrence, KS 660448897.
[6] GurpreetKaurRandhawa, Jagdev Singh Kullar – Bioremediation of Pharmaceuticals, Pesticides and Petrochemicals with Gomeya / Cow Dung – International scholarary Research Network ISRN Pharmacology, Volume 2011, Article ID 362459, 7 pages doi 10.5402/2011/362459
[7] Srivastava S., Mishra A, Pal A. Cow dung: A boon for antimicrobial activity. Life Science 1 Leaflets. 2014; 55; 152
[8] Kartikey Kumar Gupta, Kamal RaiAneja and DeepanshuRana Current status of cow dung as aBioresource for sustainable development -Bioresour. Bioprocess. (2016) 3:28DOI 10.1 /s40643-016-0105-9
[9] M. Charitha Devi, M. Sunil Kumar- Isolation and screening of lignocellulose hydrolytic saprophytic fungi from dairy manure soil - Annals of Biological Research, 2012, 3 (2):1145-1152
[10] Anustrup K (**1979**). Production, isolation and economics of extracellular enzymes. Appl. Biochem. Bioeng. 2: Enzyme Technology, Ed. By Wingard, Jr. L. B. Katchalski – Katzir, Golstein, L. Academic Press, New York, San Francisco, London.