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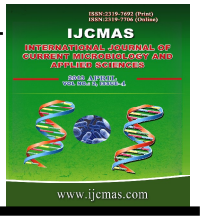


Original Research Article

Isolation, Screening and relative capacity of fungi which causes infestation of finished leather

D.S.Rathore[1]*, Neha Sharma[1] and Shashi Chauhan[2]

[1]Department of Biotechnology, Government K.R.G.P.G. (Aut.) College, Gwalior (M.P.) India
[2]School of Studies in Botany, Jiwaji University, Gwalior (M.P.) India.
**Corresponding author e-mail: rathoredrdevendrasingh@gmail.com*

Keywords

Finished leather; Fungi; Relative capacity; animal skin; bio-deterioration.

A B S T R A C T

Finished leather and animal skin, from which it is made, are very susceptible to fungal attack. The animal skin contains a great variety of fungal forms which are derived from air, water, soil manure and extraneous filth, while the animal is still alive; most of these fungi have little effect on the skin. But after the removal of the skin from the dead animal, these fungi find themselves in a perfect medium for the growth and almost immediately start multiplying at an enormous rate. The present study deals with the collection of various types of finished leather samples. Isolations were made from such samples using standard methods, to know about the qualitative, quantitative spectrum and relative capacity of fungi to deteriorate the collected samples. The numbers of fungi isolated from finished leather samples exposed to tropical chamber were 47. Out of these fungal species, 26 from vegetable tanned leather (Sheep), 25 each from chrome tanned leather (Sheep) and vegetable tanned leather (Goat), 24 and 23, from vegetable tanned sole leather (Buff) and Zuggrain chrome tanned leather (Cow), respectively, 22 from Chrome softy leather (Cow), 20 each from chrome retan leather (Cow) and oil tanned chamois leather (Goat) and minimum 18 each from semi-chrome leather (Buff) and chrome tanned leather (Goat) and relative capacity were recorded for *Aspergillus niger*, *A. flavus*, *A. fumigatus*, *A. amstelodami*, *A.sydowii* and *P.citrinum* etc.

Introduction

Various type of finished leather is very susceptible to fungal attack. The biodeterioration of leather and leather goods includes undesirable and aggressive activities of fungi during leather manufacture, finishing, storage and in use. So, finished leather and leather goods, stored under varying environmental conditions in warehouses, frequently become mouldy. The relative humidity plays an important role. Hence, biodeterioration of finished leather has become a problem of interest in recent years, and it is mainly concerned with fungi, which are present in the form of

spores and hyphae. Availability of a suitable nutrient source stimulates the spores and hyphae to germinate and then in turn support their growth. The observations of many types of samples will give a large number of fungi, which grow and infest the various types of finished leather and cause deterioration.

The present paper deals with the collection of various types of finished leather samples from different places and belongs to different animals. Isolations were made from such samples to know about the qualitative, quantitative spectrum and relative capacity of fungi to deteriorate the collected samples.

Materials and Methods

Collection of samples

The survey of the different leather factories and tanneries were made in Gwalior, Kanpur and Chennai followed by the collection of different qualities of finished leather belonging to different animals, tanning and also the leather from different stages of tanning process. The various types of semi finished and finished leather i.e. Vegetable tanned sole (buff), Semi chrome (buff), Chrome retan (cow), Zuggrain chrome (cow), Chrome softy (cow), Chrome tanned (Sheep), Vegetable tanned (Sheep), Full chrome (goat), Vegetable tanned (goat) and Oil tanned chamois (goat) leather. The sampling of these types was made from various factories.

Method of Sampling

Collection of leather samples was made following IS: 5868-1969 methods. Samples were placed into sterilized polythene bags and brought to the laboratory.

Tropical chamber

The tropical chamber fabricated for these studies measured 54X25.5X26.5 inches with double walls (wooden). The inner surface was coated with white enamel paint with 0.2 – 0.3 percent w/v β-naphthol and ρ- nitrophenol to check the fungal growth on inner side of chamber. A layer of good sandy loam soil (4'' thick) was spread over the floor. The soil surface was covered with jute cloth to avoid the displacement of soil during spraying process. The humidity within the cabinet was maintained by sand saturation of alternative days and a carbon filament bulb (25 W) was used for maintaining temperature inside the chamber during winter season. The observations of R.H. inside the chamber were made regularly each day at 18.0 and 6.0 hour. The values of R.H. and temperature were maintained throughout the experimental period between 85-90 percent and 28-30°C, respectively. The assessment of R.H. was made using dial hygrometer.

Fungal growth on leather

The sample was exposed to air and dust for 7 days to get them charged with micro-organisms in open air. The exposed leather were then hanged on wire strings in tropical chamber at 28± 1°C temperature and approximately 90 ± 5 percent R.H. The observations were taken after 7 days interval regularly for fungal growth on the leather samples.

Isolation of fungi

Fungi were isolated from various

deteriorated finished leather samples, footwear and other articles following methods out line by CMI, Kew, England (1960) as followed by smith (1969) and Orlita (1975).

Direct agar inoculation method

The samples were observed under binocular microscope and spores were picked up by inoculating needle from distinct colonies developing on leather surface and were directly inoculated into agar plates under aseptic conditions. The plates were incubated at 28 ± 1°C for fungal growth upto 7 days. The pure fungal isolates were maintained on Czapek's dox agar slants.

Serial dilution method

The fungi from deteriorated surface were isolated by swabbing the sample with sterilized moist cotton which was shaken in 10.0 ml sterilized distilled water in a culture tube. Dilution (1:10, 1:100, 1:1000, 1:10000) were made and 1.0 ml of each of these dilutions was asceptically tranfered to sterilized petridishes. 20.0 ml of sterilized medium was added to each dish. The media used were potato- dextrose agar and Czapek's dox agar. The petridishes were incubated for 7 days at 28± 1°C temperature. The isolates were transferred into Czapek's dox agar slants.

Purification, maintenance and identification of cultures

The fungal isolates thus obtained in various isolations were purified by spore suspension streak method. The pure culture were maintained on Czapek's dox agar and potato- dextrose- agar slants and were kept at 5 ± 1°C in refrigerator. Identification of fungi was made following Raper and Thom (1949), Gilman (1957), Barnett (1960), Raper and Fennell (1965), Smith (1969), Subramanian (1971), Mukerjee and Juneja (1974), Ellis (1971) and Singh *et at.,* (1991). Finally the identification was confirmed through the curtsey of director, Commonwealth Mycology Institute, Kew, England.

Determination of the relative capacity of fungi for attacking variously tanned leather

The isolated fungi were screened to select the active (profusely growing) leather deteriogens and to study their relative capacity for attacking different types of leather following the method (ALCA procedure) developed by physical testing committee of the American Leather Chemist's Association (1945) with slight modifications.

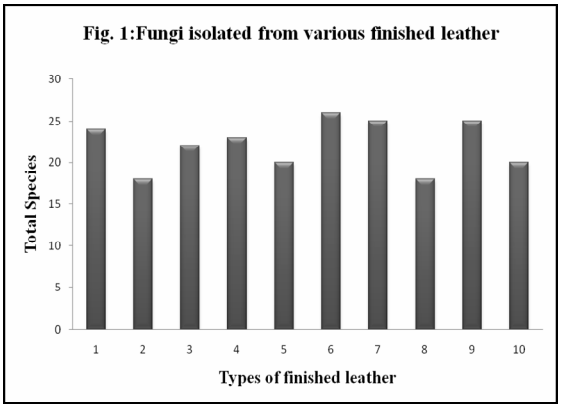
The strips of leather samples under test were cut to 5X 5 cm in size and sterilized in a desiccators of adequate size (8" dimeter), containing 100 ml of methanol at the bottom and the test material was kept over a wire gauze about 2 " away from the surface of alcohol. The desiccators were kept at room temperature for 24 hours. During this period, methyl alcohol vapours were able to sterilize the test material completely.

Further, the leathers were aseptically transferred to other desiccators having a stop cork arrangement to evacuate the alcoholic vapours and then these were again transferred into sterilized glass jars with adequate moisture. The strips were charged with 1.0 ml spore suspension of each fungus to be tested and were incubated at 28 ± 1°C temperature for 30

days. On completion of incubation period, the test materials were visually examined to the extent of fungal growth.

Results and Discussion

The numbers of fungi isolated from finished leather samples exposed in tropical chamber were 47. Out of these fungal species, 26 from vegetable tanned leather (sheep), 25 each from chrome tanned leather (sheep) and vegetable tanned leather (goat) 24 and 23, from vegetable tanned sole leather (buff) and zuggrain chrome tanned leather (cow) respectively, 22 from Chrome softy leather (Cow), 20 each from chrome retan (cow) oil tanned chamois leather (goat) and minimum 18 each semi chrome leather (buff) and chrome tanned leather (goat), as shown in table.1 and Fig.1.



An isolated fungus was screened for the assessment of relative capacity for attacking different types of leather as shown in Table.2. A perusal of the results indicated that on the basis of visual examination for relative amount and severity of growth on leather samples, the fungi were grouped into 3 categories viz. (I) Active, (ii) Moderate and (iii) slow deteriogens. In all, 12 fungi were placed in first category (Active deteriogens) i.e. *Aspergillus niger*, *A. fumigatus, A. flavus, A. sydowii*, *Penicillium stipitatum, P. purpurogenum, P. citrinum, Trichoderma koningi, T. lignorum, Botrytis cinerea*, *Chaetomium globosum and Paecilomyces varioti,* profusely developed on all type of leather samples. A few fungi *i.e., A. terreus, A. amstelodami, A. sulphureus, P. asperum, P. oxalicum, P. funiculosum, A. humicola* and *Botryoderma* sp., were found as a moderate deteriogens, which exhibited normal growth. Some fungi were grown very poorly *i.e., Alternaria geophila* and *A. alternata,* placed into third category (Slow deteriogens). Rest of the fungi exhibited different growth on different leather samples i.e. Active, Moderate and Slow. Mycelia sterile could not grow except on oil tanned chamois leather. No growth was observed on the samples in the control set.

In all the cases the luxuriant growth of mycelium was observed towards grain side of the leather samples and comparatively less growth was recorded on the flesh side. Vegetable sole leather samples showed uniform profuse pattern of growth.

A perusal of results reveal that variable number of fungal species were reported from various types of finished leather collected from different places belonging to different animals. Susceptibility of leather depends on the animals, sex of animals (The wealth of India, 1970) breed (Cundiff, 1987), hair (Khachatryan, 1990), dye(Bragulat *et al*.,1991), age of animal (Boncio *et al*.,1991), castration and season (Adersen *et al*., 1991),hence the difference of the number in fungal species in all samples.Hutton *et al*., (1967) showed that the number of both fungi and bacteria in the air and deposited on the surface varied substantially from month to month, hour to hour, place to place, and especially with

Table.1 Fungi isolated from various finished leather in tropical chamber test.

S,.No. Fungi	Leather samples									
	1	2	3	4	5	6	7	8	9	10
1.*Aspergillus niger*	+	+	+	+	+	+	+	+	+	+
2.*A.chevalieri*	+	+	-	+	-	+	-	-	+	+
3.A.*nidulans*	-	-	-	-	-	+	-	-	-	+
4.*A.fumigatus*	+	+	+	+	+	+	+	+	+	+
5.*A.conicus*	-	-	+	-	-	-	-	-	-	-
6.*A.humicola*	-	-	-	-	-	-	+	-	+	+
7.*A.flavus*	+	+	+	+	+	+	+	+	+	+
8.*A.terreus*	+	-	-	+	-	+	-	+	-	-
9.*A.repens*	-	-	-	+	-	-	-	-	-	-
10.*A.sulphureus*	-	-	-	+	-	+	-	-	+	-
11.A.*tamari*	+	-	-	-	+	+	+	-	-	-
12.*A.luchuensis*	-	+	+	-	+	+	+	+	+	-
13.*A.amstelodami*	+	+	+	+	+	+	+	+	+	+
14.*A.sydowii*	+	+	+	+	+	+	+	+	+	+
15.*A.candidus*	-	-	-	-	-	-	+	-	+	+
16.*A.ochraceous*	-	-	-	-	-	-	+	-	+	+
17.*Penicillium stipitatum*	-	-	+	-	-	-	-	-	-	+
18.*P.camemberti*	-	-	+	-	-	-	-	-	-	-
19.*P.purpurogenum*	+	-	-	+	+	+	+	-	+	-
20.*P.asperum*	-	-	-	+	-	-	-	-	-	-
21.*P.oxalicum*	+	+	+	+	+	+	+	+	+	+
22.*P.funiculosum*	+	+	+	+	+	+	+	+	+	+
23.*P.citrinum*	+	+	+	+	+	+	+	+	+	+
24.*Alternaria geophila*	+	-	+	-	-	-	+	-	-	-
25.*A.humicola*	-	-	-	-	-	+	+	-	-	-
26.*A.alternata*	+	-	-	-	-	-	-	-	-	-
27.*Curvularia lunata*	+	-	-	+	+	-	-	-	-	-
28.*C.pallescens*	-	+	-	-	-	-	-	+	+	-
29.*Fusarium neoceras*	+	+	+	-	+	+	+	+	+	-
30.*F.solani*	+	-	-	-	-	-	-	-	-	-
31.*Fusarium* sp.	-	+	-	+	-	+	-	+	-	+
32.*Rhizopus nigricans*	-	-	+	-	+	+	+	-	+	-
33.*R. oryzae*	-	-	+	-	+	-	+	-	-	-
34.*Trichoderma koningi*	-	-	-	-	-	-	+	-	+	-
35.*T. lignorum*	-	+	+	+	-	-	+	+	+	-
36.*Botrytis cinarea*	-	+	+	+	+	-	+	+	-	-
37.*Cunninghamella* sp.	+	-	-	-	-	+	-	-	-	-
38.*Cladosporium herbarum*	+	-	+	+	+	+	-	-	-	+
39.*Chaetomium globosum*	+	+	-	+	+	+	+	+	+	+
40.*Drechslera papendorfii*	+	+	+	+	-	+	+	+	+	-
41.*Helminthosporium* sp.	-	-	-	+	-	-	-	-	-	-
42.*Botryoderma* sp.	+	-	-	-	-	+	-	-	-	-
43.*Mucor ambiguus*	+	-	-	-	+	+	-	-	+	-
44.*M. mucedo*	-	-	-	-	-	-	-	-	+	-
45.*Torula lucifuga*	-	-	-	-	-	-	-	-	-	+
46.*Paecilomyces varioti*	+	+	+	+	+	+	+	+	+	+
47.Mycelia sterile	-	-	-	-	-	-	-	-	-	+
Total species	**24**	**18**	**22**	**23**	**20**	**26**	**25**	**18**	**25**	**20**

1 = Vegetable sole leather (Buff), 2 = Semi-chrome leather (Buff), 3 = Chrome softy (Cow), 4 = Zuggrain chrome (Cow), 5 = Chrome ratan (Cow), 6 = Vegetable tanned leather (Sheep), 7 = Chrome tanned leather (Sheep), 8 = Chrome tanned leather (Goat), 9 = Vegetable tanned leather (Goat), 10 = Oil tanned chamois leather (Goat). + = Present, - = Absent.

Table.2 Screening of relative capacity of isolated fungi for attacking different types of leather.

Name of fungi	1	2	3	4	5	6	7	8	9	10
Aspergillus niger	3	3	3	3	3	3	3	3	3	3
A. chevalieri	2	2	1	2	1	2	2	1	2	2
A. nidulans	2	2	1	2	1	2	2	2	3	2
A. fumigatus	3	3	3	3	3	3	3	3	3	3
A. conicus	2	2	2	2	3	3	3	3	3	2
A. humicola	3	2	2	2	3	3	2	2	3	2
A. flavus	3	3	3	3	3	3	3	3	3	8
A. terreus	2	2	2	2	2	2	2	2	2	2
A. repens	1	3	2	2	1	2	2	2	2	1
A. sulphureus	2	2	2	2	2	2	3	2	3	2
A. tamarii	3	2	2	2	2	3	3	3	3	3
A. luchuensis	3	2	2	2	2	3	3	3	3	3
A. amstelodami	2	2	2	2	2	2	2	2	2	2
A. sydowii	3	3	3	3	3	3	3	3	3	3
A. candidus	1	1	1	1	1	1	2	1	2	1
A. ochraceous	1	1	1	1	1	2	2	2	2	1
P. stipitatum	3	3	3	3	3	3	3	3	3	3
P. camemberti	3	3	3	3	3	2	3	2	3	2
P. purpurogenum	3	3	3	3	3	3	3	3	3	3
P. asperum	2	2	2	2	2	2	2	2	2	1
P. oxalicum	2	2	2	2	2	2	2	2	2	1
P. funiculosum	2	2	2	2	2	2	2	2	2	2
P. citrinum	3	3	3	3	3	3	3	3	3	3
Al. geophila	1	1	1	1	1	1	1	1	1	1
A. humicola	2	2	2	2	2	2	2	2	2	2
A. alterneta	1	1	1	1	1	1	1	1	1	1
Cur. lunata	2	2	1	1	1	1	2	1	2	1
C. pallescens	2	1	1	1	1	1	2	2	2	1
F. neoceras	2	2	1	3	1	2	2	1	2	1
F. solani	1	1	1	1	1	2	2	2	2	1
Fusarium sp.	2	2	1	3	1	2	2	1	2	1
R. nigricans	3	3	2	2	2	3	3	3	3	2
R. oryzae	3	3	2	2	2	3	3	3	3	2
T. koningi	3	3	3	3	3	3	3	3	3	3
T. lignorum	3	3	3	3	3	3	3	3	3	3
B.cinarea	3	3	3	3	3	3	3	3	3	3
Cunninghamella sp.	2	1	1	1	1	2	2	1	2	1
C. herbarum	2	1	1	2	1	2	2	1	2	1
Chae.globosum	3	3	3	3	3	3	3	3	3	3
D. papendorfii	3	3	2	2	2	2	3	2	3	2
Helminthosporium sp	3	3	2	2	2	2	3	2	3	2
Botryoderma sp.	2	2	2	2	2	2	2	2	2	2
Mucor ambiguus	3	3	1	1	1	3	3	3	3	1
M. mucedo	3	2	1	1	1	3	3	3	3	1
Torula lucifuga	2	2	3	3	3	3	2	2	2	2
Pae. varioti	3	3	3	3	3	3	3	3	3	3
Mycelia sterile	0	0	0	0	0	0	0	0	0	2

3=Active, 2=Moderate, 1= Slow, 0= No growth

1= Vegetable tanned sole (buff), 2= Semi chrome (buff), 3= Chrome retan (cow), 4= Zuggrain chrome (cow), 5 = Chrome softy (cow), 6 = Chrome tanned (Sheep), 7= Vegetable tanned (Sheep), 8= Full chrome (goat), 9= Vegetable tanned (goat), 10= Oil tanned chamois (goat) leather.

difference in height above ground. These results are significant in spite of variation between parallel samples. The data strongly suggest that the basic variables which influenced the presence of microbial forms in the environment were not adequately defined in simple terms of site, time, elevation and season. Instead, data tend to confirm Gregory's (1952) contention that specific climate elements of the microenvironment such as temperature, wind speed, relative and absolute humidity, as well as light may in combination exert primary influences. Furthermore, there is some evidence, in the form of the variation between calculated relative rates of deposition of airborne forms on surfaces, that rate of deposition are related to all of the environmental factors measured.

According to Orlita (1968, 2004) leather is a biological product and very suitable medium for the growth of microorganism due to presence of protein and lipids in the form of glycerides. Similarly, in tanneries microorganisms are provided with environments suitable for growth. The proteins and fats in the hide represent an ideal source of nutrients with a pH of about 4, for fungal growth. In particular, picked pelts, wet-blues and vegetable tanned moist leathers are prone to mould growth when stored or shipped. *Aspergillus* sp., *Mucor* sp., *Paecilomyces varioti*, *Penicillium* sp., *Rhizopus nigricans* and *Trichoderma viride* are mainly responsible for leather damage. *Trichoderma* species is the most frequently found. (Linder and Neuber, 1990).

In all type leather it was also observed that grain side was very much susceptible for fungal growth than flesh side. This may be due to the incorporation of leather finishes i.e. pigments, protein binders, casein, gelatin, egg and blood albumins, waxes and blood albumins, waxes and mucilaginous substances on this side during tanning process. During liming after soaking process, ca++ are provided to the hide and it has been appreciated by Pitt and Kaile (1989) that certain activities of fungi may also be regulated by the cation. Geoffery et al (1991) demonstrated that the phialide production phase is *Fusarium graminearum* may also be triggered by a rise in intracellular ca ++. The fungi preferred acidic environment, fungi are usually encountered in the tannery after the pickling. All these substances serve as the best source in primary colonization of the microorganisms (Nandy, 1975; Montanari *et al*., 2012).

However, chrome tanned leather is assumed highly resistant to moulds because of the presence of chromic oxide as reported by Zemanova (1984) However, their high content of fat somewhat decrease their resistant to mould growth. The present results were supported by Sharma and Sharma (1978) who recorded 14 species on chrome tanned leather. Further the chrome retain and oil tanned chamois leather are usually heavily oiled and consequently difficult to wet, hence moulding were reported less rapidly.

The type of fungi on most of the leather appeared to be *Aspergillus niger* and various species of *Penicillium*. Vegetable tanned sole leather had the heaviest growth of mildew in comparison to chrome tanned leather (Kanagy *et al*., 1949). Many other workers like Orlita (1968), Sharma and Sharma (1978) and Stakishatite and Lugauskas (1981) isolated a number of fungi from different leather and tan liquor stated that the most

frequently occurring fungi were the species of *Aspergilli* and *Penicillia* while some other i.e. *Curvularia, Fusarium, Cladosporium, Verticillium, Cephalosporium* and *Scopulariopsis* sp. were not found commonly on all types of leather samples.

Krishnamurti *et al*., (1968) recorded a good growth of *A. niger* at high R.H. on leather samples. He found that vegetable tanned sole leather during drying was covered with a heavy mat of moulds and pink to deep red spots were produced by the fungus. This was reported as due to increased atmospheric humidity and delay in the drying of the leather. The leather was with wattle extract and myrobalan and oiled with admixture of groundnut and pungan oil. The fungus was isolated and identified as *P. purpurogenum*. It was also reproducible on similar vegetable tanned leather. *A. nidulans* and *A. niger* were isolated from violet spots on E.T. Kips. Mc Ginnis *et al.*,(1975) isolated 27 species of fungi representing 19 genera. Strzelczyk *et al*.,(1989) found *Chaetomium globosum* the most active organism in the decay process of leather and leather goods.

It was also noted during present work that the vegetable tanned leather absorbed maximum moisture in comparison to the chrome tanned leather in the same conditions. This capacity is related to the compactness of leather fibres and presence of relative amount of fat and water soluble. All these observations support emphatically the results obtained in the present study.

It has been concluded by this study that the finished leather, used to prepare a number of commodities of daily use are highly susceptible for fungal attack. No leather type is completely resistant. These are subjected to heavy microbial infection during storage and user conditions. The present investigation provides impetus to develop certain preventive measures to make these articles free from infestation of microfungi under the conditions of high R.H. and optimum temperature, the knowledge of specific microflora and their relative capacity to infest leather is necessary for solving this problem.

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