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Investigation on the fungal spoilage of crude drugs (1)**

Crude drugs, as other plant materials, are the subject of microbial spoilage under certain conditions and this is a problem for the dealers for a long time. In ordinary storage conditions, the water content of crude drugs is too low to allow the growth of bacteria, then only fungal spoilages will arise. The mycoflora of crude drugs is made up of a wide variety of fungi, especially the common saprophytic genera, such as *Aspergillus*, *Penicillium*, and other air, water, dust, and soil borne fungi are quite universal by its very nature.

The factors which determine the range of the mycoflora will be: (1) the pre-harvested position of the crude drug in the plant; (2) the geographical location, climatic, and environmental conditions where the plant was growing; and (3) the treatments and storage conditions after harvest.

The experience of the microbial spoilage of foods and similar materials shows that, in a particular situation, the deterioration is actually caused by only a small proportion of the micro-organisms initially present, so that a specific type of spoilage arises in the given variety of materials under normal conditions of storage. This guiding principle will be applicable to the fungal deterioration of the crude drugs.

Under practical conditions, the spoilage type of a crude drug is determined by groups of factors: (1) the initial infection of the substrate; (2) factors depending on the properties of the substrate (‘intrinsic’ factors), i.e., the nutrient value, the biological structures, and the specific effects of some of the ingredients for certain fungi; (3) the conditions of storage (‘extrinsic’ factors), i.e., the moisture content of the substrate, oxygen availability, and temperature; (4) the properties of the dominant fungi, for whose influence the term ‘implicit’ factors has been coined, i.e., differences in the rate of development of the fungi, synergism, and antagonism. The way in which these influence the development of the fungal association is the subject of this paper.

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Material and method

All crude drug samples were kindly supplied by Takashimado Pharmacy, Hongo, Tokyo. These were in storage just as ordinary dealers do. For this investigation, three experimental conditions were made:

- temperature 25°C. and relative humidity (R.H.) 75.4%
- temperature 25°C. and relative humidity 84.3%
- temperature 25°C. and relative humidity 100.0%

R.H. 75.4% and 84.3% were made by putting Petri plates in desiccators containing the saturated salt solutions of KCl and NaCl, respectively. In R.H. 100%, distilled water was used. For each experiment, one 20 cm-desiccator was used and two Petri plates with a thin layer of a sample were placed in it and incubated at 25°C.

The observation and isolation schedule was as follow:

R.H. 75.4% series

1st. observation .............................................after 7 days
2nd. observation .............................................after 14 days
3rd. observation .............................................after 21 days
4th. observation and isolation .............................after 28 days

R.H. 84.3% series

1st. observation .............................................after 4 days
2nd. observation .............................................after 7 days
3rd. observation and isolation .............................after 10 days
4th. observation .............................................after 14 days
5th. observation and isolation .............................after 21 days
6th. observation (and isolation, if necessary) .............after 28 days

R.H. 100% series

1st. observation and isolation .............................after 4 days
2nd. observation and isolation .............................after 7 days
3rd. observation and isolation .............................after 10 days
4th. observation and isolation .............................after 14 days
5th. observation (and isolation, if necessary) .............after 21 days
6th. observation (and isolation, if necessary) .............after 28 days

In each observation, dominant species, subdominant species, minor species, and very minor species were determined by naked eyes, and portions of these were taken to microscopes and genus identifications, and in some cases species identifications...
were done. In the isolation, each fungal species which developing on the sample was transferred to Petri plates containing acidified potato-dextrose-agar (pH 4.0) or 10% NaCl added potato-dextrose-agar depending on the species, taking precaution to prevent contaminations by unobjective fungi. After appropriate growth, *Aspergillus glaucus* group, other *Aspergillus* and *Penicillium* species, and other genera of fungi were transferred to test tubes containing 20% sucrose Czapek's solution agar, and potato dextrose agar, respectively. In many cases, direct transfers of the developing fungi on the samples in the Petri plates to test tubes were done successfully. These test tube cultures were served for species identifications. In some cases, special media were used for certain fungi.

**Experimental result**

Ôren, *coptis* subterranean stem of *Coptis japonica* Makino

R. H. 75.4%: In 28 days, *Aspergillus mangini* (Mangin) Thom and Raper was growing very poorly.

R. H. 84.3%: In about 14 days, *Aspergillus mangini* (Mangin) Thom and Raper began to develop. The growth in 28 days was poor.

R. H. 100%: Until 7 days, only *Aspergillus glaucus* group was growing, but later *Aspergillus wentii* developed rapidly. In 14 days, *Aspergillus wentii* dominated over other fungi, and the development was very good.

Dominant species: *Aspergillus wentii* Wehmer
Subdominant species: *Aspergillus ruber, Aspergillus mangini* (Mangin) Thom and Raper
Minor species: *Aspergillus awamori* Nakazawa

Onji Polygala root of *Polygala tenuifolia* Willdenow

R. H. 75.4%: In 28 days, no fungal development was observed.

R. H. 84.3: In 28 days, no fungal development was observed.

R. H. 100%: Until 7 days *Aspergillus glaucus* group was predominant, but later *Aspergillus sydowi* and *Mucor silvaticus* developed rapidly. 7 days later, *Aspergillus sydowi* showed very good growth.

Dominant species: *Aspergillus sydowi* (Bain. and Sart.) Thom and Church
Subdominant species: *Aspergillus ruber, Aspergillus amstelodami, Aspergillus restrictus, Mucor silvaticus* Hagen
Minor species: Aspergillus niger van Tieghem, Penicillium frequentas Westling, Aspergillus ochraceus Wilhelm

Chimo, Anemarrhena subterranean stem of Anemarrhena asphodeloides Bunge

R. H. 75.4%: In about 14 days, Aspergillus umbosus Bain. and Sart. began to develop. In 28 days, it showed fairly good growth.

R. H. 84.3%: In 6 days, the growth of Aspergillus glaucus group became visible. 5 days later, its development was fairly good.

Dominant species Aspergillus ruber, Aspergillus umbrosus Bain. and Sart.

R. H. 100%: In the early stage, Aspergillus glaucus group was predominant. In 14 days, Aspergillus glaucus group and Penicillium wortmanii were about equal in their development. 14 days later, Penicillium wortmanii dominated and showed very good growth.

Dominant species: Penicillium wortmanii Klöcker

Subdominant species Aspergillus ruber, Aspergillus umbrosus Bain. and Sart.

Minor species: Aspergillus wentii Wehmer, Penicillium implicatum Biourge, Aspergillus niger van Tieghem, Unidentified Dematiaceae (Fumicola grisea?)

Very minor species: Aspergillus ochraceus Wilhelm, Rhizopus nigricans Ehrenberg

Ôgi, Astragalus root of Astragalus Hoantchy Franchet or related species

R. H. 75.4%: In 28 days, no fungal development was observed.

R. H. 84.3%: In 4 days, Torula saccharii (Syn. Catenularia fuliginosa Saito) became visible. 3 days later, Aspergillus ruber appeared. The fungal growth in 28 days was poor and inconspicuous.

Dominant species: Torula saccharii

Very minor species Aspergillus ruber

R. H. 100%: At the early stage, Rhizopus nigricans and Torula saccharii were predominantly growing. In 7 days, Torula saccharii was inconspicuous according with the development of Rhizopus nigricans and Scopulariopsis species. In 14 days, Scopulariopsis species predominated and its growth was very good.

Dominant species: Scopulariopsis brevicalis (Sacc.) Bainier, Scopulariopsis brevicaulis var. glabrum Thom, Rhizopus nigricans Ehrenberg

Minor species: Aspergillus ruber, Aspergillus restrictus, Penicillium sp. (asymmetric)

Very minor species: Aspergillus wentii Wehmer, Chaetomium sp. Unidentified fungus (Fumicola grisea?), Trichothecium roseum Link
Biyakushi, Radix Angelicae root of Angelica glabra
R. H. 75.4%: Torula saccharii and Aspergillus ruber became visible in 10 and 14 days, respectively. The fungal growth in 28 days was poor.
Dominant species: Torula saccharii, Aspergillus ruber
R. H. 84.3%: In 6 days, Torula saccharii appeared. Being retarded 1 day, Aspergillus ruber became visible. The fungal growth in 28 days was poor.
Dominant species: Aspergillus ruber
Minor species: Torula saccharii
R. H. 100%: At the early stage, Torula saccharii and Rhizopus nigricans predominated. In 7 days, Torula saccharii was inconspicuous with the development of Rhizopus nigricans and Aspergillus ruber. In 14 days, Scopulariopsis species appeared and grew rapidly. In 28 days, Scopulariopsis species and Rhizopus nigricans were predominating, but their growth was relative retarded and not so good comparing to other crude drugs.
Dominant species: Scopulariopsis brevicaulis (Sacc.) Bainier, Scopulariopsis brevicaulis var. glabrum Thom. Rhizopus nigricans Ehrenberg
Minor species: Aspergillus ruber
Very minor species: Aspergillus ochraceus Wilhelm, Penicillium sp. (asymmetrica)

Dockatsu, Radix Angelicae polycladae root of Angelica polyclada
R. H. 75.4%: In 7 days Aspergillus gracilis began to develop. The growth in 28 days was poor.
Dominant species: Aspergillus gracilis Bainier
R. H. 84.3%: In 3 days, Aspergillus ruber began to develop. In 28 days, it showed fairly good growth.
Dominant species: Aspergillus ruber
R. H. 100%: Until 7 days, Aspergillus glaucus group was predominating, but in 14 days Scopulariopsis species appeared and developed rapidly. The fungal growth in 28 days was very good.
Dominant species: Scopulariopsis brevicaulis (Sacc.) Bainier, Scopulariopsis brevicaulis var. glabrum Thom, Aspergillus ruber, Aspergillus repens
Minor species: Aspergillus elegans Gasperini, Penicillium sp. (asymmetrica)
Very minor species: Aspergillus niger van Tieghem, Aspergillus flavus Link, Aspergillus wentii Wehmer, Rhizopus nigricans Ehrenberg, Trichothecium roseum Link
Uikyo, *Foeniculum* seed of *Foeniculum vulgare* Miller

R. H. 75.4%: In 28 days, the fungal growth was very poor.
Growing species: *Aspergillus ruber*, *Aspergillus mangini* (Mangin) Thom and Raper

R. H. 84.3%: In 7 days, the growth of *Aspergillus glaucus* group became visible. The growth in 28 days was poor.
Dominant species: *Aspergillus ruber*, *Aspergillus mangini* (Mangin) Thom and Raper

Very minor species: *Scopulariopsis brevicaulis* (Sacc.) Bainier

R. H. 100%: In the early stage, *Aspergillus ruber* was predominant. In middle stage *Aspergillus versicolor* and *Aspergillus ruber* showed good growth. After 14 days *Scopulariopsis* species predominated. The fungal growth in 28 days was very good.
Dominant species: *Scopulariopsis brevicaulis* (Sacc.) Bainier, *Scopulariopsis brevicaulis* var. glabrum Thom
Minor species: *Chaetomium globosum* Kunze
Very minor species: *Alternaria* sp.

Chikusetsu-ninjin, *Panasis Rhizoma* subterranean stem of *Panax japonicus* C. A. Meyer

R. H. 75.4%: In 14 days, a trace of fungal growth was observed. In 28 days the fungal growth was very poor.
Growing species: *Aspergillus ruber*

R. H. 84.3%: In 7 days, *Aspergillus glaucus* group showed a poor development. The growth in 28 days was relatively good.
Dominant species: *Aspergillus ruber*, *Aspergillus repens*
Minor species: *Penicillium fellutanum* Biourge

R. H. 100%: Until about 10 days, *Aspergillus glaucus* group was predominant. Later, *Aspergillus versicolor* and *Scopulariopsis brevicaulis* began to develop. In 21 days, *Aspergillus versicolor* and *Scopulariopsis brevicaulis* dominated and their growth was very good.
Dominant species: *Aspergillus versicolor* (Vuill.) Tiraboschi
Subdominant species: *Scopulariopsis brevicaulis* (Sacc.) Bainier

Very minor species: Aspergillus ochraceus Wilhelm, Rhizopus nigricans Ehrenberg, Scopulariopsis brevicaulis var. glabrum Thom, Verticillium sp. (colonies brick red), Unidentified (yellow mycelia)

Kanzo, Glycyrrhiza root and subterranean stem of Glycyrrhiza uralensis Fischer et De Candolle and related species

R. H. 75.4% No fungal development was observed in 28 days.

R. H. 84.3%: In 12 days, Aspergillus mangini began to appear. The growth in 28 days was relatively good.

Dominant species: Aspergillus mangini (Mangin) Thom and Raper

R. H. 100%: In 3 days, Aspergillus umbrosus appeared, and predominated until about 10 days. Later, Penicillium variabile began to develop rapidly and predominated in 14 days. The fungal growth in 20 days was very good.

Dominant species: Penicillium variabile Sopp

Subdominant species: Aspergillus umbrosus Bain. and Sart

Minor species: Aspergillus niger van Tieghem


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References


