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SOME MOLDS ASSOCIATED WITH MEAT IN COLD STORAGE LOCKERS IN IOWA

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In the past few years throughout the United States cold storage locker plants have been introduced as a new industry, offering their services to the public as storage depots for perishable foods, particularly meats. The wide-spread popularity of this industry is attested by the existence of approximately two thousand cold storage locker plants in the United States of which two hundred are located in Iowa. Over one hundred of these plants in Iowa were introduced during the past year, and there is every indication that their number will continue to increase.

In any new industry such as this there inevitably arise many problems. The problem of molds on meat has already reached considerable importance. The occurrence of these molds is not unexpected in view of the experiences of the meat shipping industry of other countries and the variable conditions encountered in the use of these cold storage locker plants. Fluctuations in temperature and humidity in these lockers, and the extent of contamination of the meat by micro-organisms subsequent to slaughter are factors of some importance. In view of the possible seriousness of this problem, many of its aspects require investigation, such as the kinds of molds occurring on such meat, the lowest temperatures permitting their development, their effect on palatability of the meat, the suitability of meat as a substrate for their growth, and possible means for their control. It is the object of this present paper to record the species of molds isolated from such meat in local storage plants and to point out those recorded by investigators elsewhere.

MOLDS ISOLATED FROM MEAT IN LOCAL COLD STORAGE LOCKER PLANTS

The following molds have been isolated and identified: *Thamnidium elegans* Link, *Chaetostylum fresenii* Van Tieghem et Le Monnier (syn. *Thamnidium chaetocladioides* Brefeld). The nomenclature followed is that used by Zycha (14). These two molds

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form growths on meat generally referred to as "whiskers." It was from such a growth that they were obtained.

Cladosporium herbarum Persoon. This mold has been fairly well established as being responsible for the formation of "black spot" on meat. Such a spot yielded the culture noted here.

ASPERGILLUS SPP. Four different cultures of this genus were obtained, all of which fell in the *Aspergillus glaucus* group of Thom and Church (11). Two cultures, differing only in the amount of pigment production in the medium, were identified as *A. repens* (Corda) Sacc. The third culture was identified as *A. ruber* (Spieckermann and Bremer) Thom and Church. The fourth culture did not correspond with any of the species described by Thom and Church in this group, but it could be included in the same sub-group with *A. chevalieri* (Mangin) Thom and Church. The following is a description of the fourth culture: Colonies similar in appearance on potato dextrose agar and Czapek's solution agar; very much restricted in growth, developing about 1 cm. in diameter in two weeks; surface of colony dense, of a buff brown color (Ridgeway) with a central raised area of Dresden brown; margin of colony of narrow white aerial mycelium passing to a thin area of young conidia initially tea green or glaucus; reverse of colony plane, not convoluted or ridged, van Dyke brown in color; agar not colored; mycelium coarsely granular, approximately 2.8μ in width; conidiophores smooth with globose head 18μ - 21μ in diameter and covered with sterigmata from one-half to two-thirds of its surface; sterigmata flask-shaped, 17μ-21μ x 4.2μ; conidia globose to elliptical, varying from 4.2μ - 7.2μ in diameter and faintly verrucose; Perithecia were observed in the upper drier part of potato dextrose agar slants, yellow, 54μ - 85μ in diameter; asci hyaline, eight spored, varying from globose of 8.4μ in diameter to elliptical of 8.4μ x 13.6μ; ascospores hyaline, elliptical, 4.2μ x 5.6μ, deeply ridged with a distinct furrow. This culture probably differs in the main from *Aspergillus chevalieri* in the type of colony growth. The latter is recorded (11) as more or less floccose, being quite similar to that of *A. repens*.

PENICILLIUM SPP. Seven cultures, visibly different, were obtained belonging to the genus *Penicillium*. They were all similar in producing velvety colonies on potato dextrose agar and on Czapek's solution agar. They did not exhibit fasciculations nor were there any detectable funiculose hyphae. The colors of these colonies varied, being green, blue green, deep grey green and deep olive green (Ridgeway). The penicilli were all asymmetrical. On the basis of

these characters, therefore, the asymmetrica-velutina group of Thom (10) would include all seven cultures.

One culture (No. 7) from the group was identified as being *P. oxalicum* Thom and Currie. It differed from the others in being wide-spreading and having spores that were much larger and decidedly elliptical. The spores broke off from the colony very easily.

The remaining cultures could not be identified definitely with any of the species described by Thom in the asymmetrica-velutina group. However, it is believed that they could be included in the following series designated by him.

PENICILLIUM CHRYSOGENOSUM SERIES. *Culture No. 2.* On potato dextrose agar, at first blue green then becoming deep grape green and finally reddish brown in age; fairly broadly spreading; odor mushroom-like; reverse bright yellow in color, somewhat zonate, with a tendency to orange in the center; agar not colored at first but becoming reddish brown as evidenced in test-tube slant cultures; on Czapek's solution agar similar, except for presence of colorless water drops and calcium oxalate crystals in the medium; conidia globose, smooth, 2.4μ - 3.8μ in diameter; sterigmata approximately 7μ in length; metulae approximately 8.4μ in length and in verticils of two and three; conidiophores slightly pitted; conidial chains up to 135μ in length. This culture approaches the description of *P. chrysogenum* Thom.

Culture No. 5. On potato dextrose agar fairly broadly spreading, approximately 270μ deep, deep greyish green; edge of colony with area of submerged mycelium 2 mm. wide; odor none; calcium oxalate crystals present in the medium; reverse reddish brown; agar colored brown; on Czapek's solution agar similar, but with margin of white aerial mycelium 1.5 mm. wide and submerged mycelium 1 mm. wide; colorless drops abundant; conidia smooth, globose, 2.0μ - 2.8μ in diameter; conidiophores granular; sterigmata approximately 9.4μ long and in groups of three; metulae in verticils of two, three and four, approximately 14μ in length. This culture approaches the description of *P. notatum* Westling.

PENICILLIUM PUBERULUM SERIES. *Culture No. 3.* On potato dextrose agar fairly broadly spreading, dark green with a white border 1 mm. wide; zonate with age; reverse yellowish; agar uncolored; odor of rotten apples; on Czapek's solution agar similar, colorless water drops present; reverse white to cream color; calcium oxalate crystals present in the medium; medium not colored; conidia markedly echinulate, globose, 3.5μ to 4.2μ in diameter; conidiophores

granular; sterigmata 7μ - 8.4μ in length, in groups of three; metulae in verticils of three, 9.2μ - 14μ in in length; conidial chains 144μ - 270μ in length.

Culture Nos. 1, 4 and 6. On potato dextrose agar fairly broadly spreading, bluish green, with very narrow white margin; zonate with age; conidia breaking off in flakes with age; reverse yellowish, the intensity differing with the culture; agar not colored; odor of potato cellar; calcium oxalate crystals present in the medium; on Czapek's solution agar similar; reverse of colony differing in color from that on potato dextrose agar, varying from cream to purplish; conidia from apparently smooth to faintly echinulate, globose, 3.5μ - 4.2μ in diameter; conidiophores apparently smooth to faintly granular to definitely granular; sterigmata 7μ - 15μ in length, in verticils of two and three. It was found difficult to identify these cultures with any species included in this series. However, they may be considered as strains of *P. melinii* Thom indicated by Thom (10) as forming one of the series of strains with "velvety colonies, rough conidiophores, verticils of metulae and spinulose conidia."

To compare these seven cultures with those obtained from meat by others is difficult since descriptions of their cultures are lacking. As indicated by the list below, a number of species of *Penicillium* have been obtained. Here, as often elsewhere, species names are not of much value in identification of cultures.

YEASTS. Two different cultures of yeasts were obtained. These, however, have not been further identified.

MOLDS ISOLATED BY OTHERS FROM MEAT HELD IN COLD STORAGE

The following is a list of these molds:

Botrytis elegans Link (1)
B. pennicula Sacc. (1)
B. rosea Link (1)
Botrytis, form *Isaria* (1)
Cephalothecium roseum Corda (1)
Chaetostylum fresenii Van Tieghem et Le Monnier (syn. *Thamnidium chaetocladioides* Brefeld) (1, 3, 7, 8)
Cladosporium herbarum Persoon (1, 2, 3, 5, 7, 8, 12, 13)
Dematium spp. (3, 9)
Hormodendron cladosporioides (syn. *Cladosporium herbarum*) (1, 3)
Monilia candida (1)
Mucor spp. (9)
M. lusitanicus (3)
M. mucedo L. (1, 3, 5, 6, 7, 12)
M. pusillus (1)
M. racemosus (1, 3, 5)
M. spinosus Van Tieghem (1)
Oidium carnis (3)
Oospora carneola Sacc. (5)
Penicillium spp. (9)
P. anomalum (3)
P. anormalium (?) (7)
P. asperulum (3)
P. candidum Link (5)
P. crustaceum Link (5)
P. expansum (3, 4, 7)

P. glaucum Link (5, 6)
P. herbarum (12)
Phycomyces nitens Kunze (5)
Rhizopus sp. (6, 12)
Rhizopus nigricans Ehrenberg (1)
Sporotrichium sp. (9)
Sporotrichium carnis Brooks and Hansford (3, 4, 7)
Stysanus stemonitis Persoon (1)
Thamnidium elegans Link (1, 3, 6, 7)
Torula spp. (1, 3)
T. botryoides Brooks and Hansford (3)
Verticillium lateritium Berk. (5)
Wardomyces anomala Brooks and Hansford (3)
Yeasts (not classified) (1, 9)

Discussion

Of the large number of molds known to be capable of saprophytic existence on many substrata it is indeed surprising that so few of them occur on meat under conditions of cold storage, and that a still more limited number are recorded as reaching any importance in frequency and extent of development. These latter are *Thamnidium elegans, Chaetostylum fresenii, Mucor* spp., *Cladosporium herbarum, Sporotrichium carnis, Penicillium* spp. and certain yeasts. The others have been recorded only occasionally and usually occur when the meat is exposed to temperatures above 32°F.

The various factors contributing to the presence and development of these molds on meat held in cold storage are, in large part, those accompanying the exposure of the meat subsequent to slaughter and during storage.

The carcass is constantly subject to surface contamination by micro-organisms. The contamination in the period previous to freezing is undoubtedly of great concern. In slaughter houses this is perhaps reduced to a minimum, but under the conditions usually prevalent on farms this factor of contamination is given little consideration. The practice amongst farmers of killing and dressing the animal in or near the barn affords ample opportunity for an even distribution of molds over the surface of the carcass. This is particularly true inasmuch as the atmosphere of the barn or any similar building is heavily laden with mold spores or fragments of mold mycelium carried on particles of soil or plant and animal debris. It is therefore not unexpected to find that molds occurring on meat are usually those found in soil and in similar places.

The development of molds on meat is influenced to a great extent by the temperatures maintained in the lockers. It has been demonstrated repeatedly that most molds developing on meat are capable of extensive growth at temperatures above 32°F. However, there are only a few known that develop at temperatures much below

32°F. With the onset of fluctuating temperatures, however, conditions are produced that are more favorable for the development of these organisms.

The frequency and stage of development of these molds on meat is influenced by the length of time that the meat is exposed to outside atmospheric conditions previous to cold storage. This in turn influences the readiness with which they appear when placed in cold storage and also the extent to which they develop. With some molds a few days of development at room temperature is equivalent to several months of development at a temperature of 32°F. or lower. Several days of development of molds on meat previous to cold storage decreases the storage value of the meat. If the temperatures maintained in the cold storage lockers permit mold growth then placing the meat in these lockers as soon after slaughter as possible is of advantage to retard the first appearance of visible molds.

The factor of length of time the meat is stored in these lockers determines the occurrence of molds, providing other conditions are favorable. In practice very little trouble is encountered by the retail butcher shops with mold growth even though the temperatures of their coolers are maintained above freezing. This difficulty is avoided primarily by the rapid turn-over in marketing the meat. In the system of cold storage locker plants, however, where the meat may be stored for a period of from six to twelve months, considerable opportunity is given for mold development, particularly when the temperatures fluctuate over a wide range and at times reach 32°F. and above.

The additional factor of humidity is one of some concern. Molds usually develop to the greatest extent in humidities near saturation. The moisture present on the surface of the carcass is sufficient for the particular requirements of the molds. Freezing the meat usually removes the moisture but with alternate freezing and thawing frequently introduced by fluctuating temperatures, sufficient moisture is usually formed on the meat to exert a favorable influence on mold development.

There are indeed many aspects to this problem of molds on meat. The possibility of preventing or retarding mold development on meat by various agents toxic to molds is of especial concern, particularly when the meat is exposed to temperatures above 32°F. in the process of ageing. The suitability of meat as a substrate for mold growth is little known. The effect of association of molds with one another and with other micro-organisms on

their growth on meat needs special study. It is sufficient for the present, however, to assert that considerable development of molds on meat is usually objectionable. Their occurrence on meat in cold storage locker plants requires control if these plants are to maintain their public service.

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