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Non-lactose fermenting yeasts and yeast-like fungi from cream and butter

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NON-LACTOSE FERMENTING YEASTS AND YEAST-LIKE FUNGI FROM CREAM AND BUTTER

by

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INTRODUCTION

The yeasts and yeast-like organisms, often grouped under the name Blastomycetes or budding fungi, are of considerable importance in milk and milk products. Some of these organisms are able to cause defects, such as the foaming of cream and the development of bitterness in cheese and rancidity in butter. Certain of these organisms contribute to the character of some fermented drinks and of some types of cheese. The number of yeasts and yeast-like fungi may be used as an index of sanitation of processing conditions.

In studying the changes caused by these organisms, dairy bacteriologists have given most of their attention to the lactose fermenting group, while the non-lactose fermenting group has been relegated to a more or less secondary position. This viewpoint is understandable since the former group usually is considered to be the cause of most undesirable changes, while the latter group is thought of as being rather inert and therefore of little importance.

When reporting the yeasts in samples of dairy products it is customary to mention the numbers only, giving no attention to the types. Consequently, the number of yeast forms, whether or not they ferment lactose, is the usual basis for judgement. If only for this alone, the non-lactose fermenting yeast forms should be of enough significance to warrant further investigations.

In the medical world certain of the yeast-like fungi, commonly known as Monilia or Candida, have been studied intensively, particularly as

dermal parasites. These fungi, which form true-or pseudomycelium with blastospores, have been isolated, in most instances, from human sources, and some of them are considered pathogenic. In the dairy world non-lactose fermenting yeast-like fungi, which develop "radial thread-like hyphae" or "long filament-like cells", have been isolated repeatedly. These structural characteristics pointed to some possible relationships between these fungi from two widely separate sources. The non-lactose fermenting yeasts and yeast-like fungi, therefore, deserve serious consideration by the dairy bacteriologists.

This study of non-lactose fermenting yeasts and yeast-like fungi from cream and butter was undertaken with two objectives in mind: the identification of representative cultures by use of modern procedures and the determination of the ability of this group of organisms to produce defects in cream and butter.

REVIEW OF LITERATURE

General Classification of Yeasts and Yeast-Like Fungi

The study of any micro-organism necessitates a systematic classification. The budding fungi usually are separated into two rather unequal divisions, the spore-forming (ascosporogenous) or true yeasts represented by the family Endomycetaceae and the nonsporing (asporogenous) or false yeasts which are represented by the families Torulopsidaceae, Rhodotorulaceae, and Nectaromycetaceae. Some feel that separating the sporogenous and asporogenous may be artificial but most taxonomists agree on these primary divisions. However, no unanimity with respect to further subdivision into genera and species has been reached, although in the last decade some unification of opinions has been attained.

Ascosporogenous yeasts

According to Guilliermond (1920) the study of yeasts was first undertaken by Leeuwenhoek in 1680. Numerous investigators studied these organisms but satisfactory progress in taxonomy was not made until Hansen (1904) introduced adequate methods for systematic study. He first distinguished between spore-forming and non-spore-forming yeasts, giving the family name Saccharomycetaceae to the spore-formers that reproduce vegetatively by budding and Schizosaccharomycetaceae to those that reproduce vegetatively by transverse fission. To the non-spore-forming group he gave the family name Torulaceae. The family Saccharomycetaceae was

divided into three groups. The first group included yeasts which are able to form a scum only at the end of fermentation. This scum is mucous and is without occluded air bubbles. In this group are the genera Saccharomyces, Zygosaccharomyces, Saccharomycodes, and Saccharomycopsis. The second group includes the yeasts which form a scum at the beginning with bubbles of air in it. In this group are the genera Willia and Pichia. A third group is represented by the genera Nematospora and Monospora. These are grouped under the name of doubtful Saccharomycetes. The family, Schizosaccharomycetaceae, is represented by one genus, Schizosaccharomyces.

Anderson (1917) proposed a scheme for the classification of those yeasts which reproduce by budding. His primary division also was based on the ability of the organisms to form spores. He retained many of the names proposed by Hansen. However, he recognized the genus Endomyces, the members of which form mycelium under certain conditions and whose vegetative cells are predominately of the budding type. He did not include the genera Schizosaccharomyces, Monospora and Nematospora.

Guilliermond (1920) devised a key for the identification of yeasts. His primary division on the basis of sporulation is similar to that of Hansen. However, Guilliermond included all spore-forming yeasts, whether they reproduce vegetatively by fission or by budding, in the family Saccharomycetaceae but excluded the fungi belonging to the family Endomyces. The family Saccharomycetaceae is divided into five groups. The first group, represented by the genus Schizosaccharomyces, is characterized by transverse partition. The second group, represented by the genera Nadsonia,

Zygosaccharomyces, Debaromyces, Schwannimyces, and Torulaspora, is characterized by vegetative reproduction by budding and is subdivided according to the mode of origin of the ascus, particularly on the degree of sexuality.

The third group, which is represented by the genera Saccharomycodes, Saccharomycopsis, Saccharomyces and Hansenia, forms spores without any traces of sexuality and the cells develop in the beginning at the bottom of liquid culture and can later produce a scum at the surface. Organisms of the fourth group, which is represented by two genera Pichia and Willia, also form spores without any traces of sexuality. However, yeasts from this group rapidly produce a pellicle on liquid medium. In the fifth group are the genera, Monospora, Nemotospora, and Coccidiascus whose characters were doubtful to Hansen. They are differentiated from the other groups and themselves by the shape and/or number of their ascospores.

Henrici (1930) formulated another classification of the spore-forming yeasts based upon the arrangement and description of Guilliermond. He included the genus Zygosaccharomycodes which forms spores by isogamic conjugation but which reproduces vegetatively by a process intermediate between budding and fission. This genus Zygosaccharomycodes was described by Nishiwaki (1929).

The more recent and most complete classification of the sporogenous yeasts was made by Stelling-Dekker (1931). She includes all of the yeasts and yeast-like fungi which form ascospores in a single family, the Endomycetaceae, which is subdivided into four subfamilies, the Eremascoideae, the Endomycoideae, the Saccharomycoideae, and the Nematosporoideae. They

are differentiated on the basis of growth form, whether mycelial, pseudomycelial, yeast cells or oidia, singly or in combination; vegetative reproduction, whether by transverse fission or budding; spore production, whether by isogamous or heterogamous copulation or parthenogensis; and shape of ascospore, whether spherical, hemispherical, angular, sickle- or spindle-shaped, smooth, warty, or with an encircling rim. The common spore-forming yeasts are mostly included in the Saccharomycoideae which is further subdivided into three tribes, the Endomycopseae, the Nadsonieae, and the Saccharomyceteae. The most important genus of the sporogenous yeasts, the Saccharomyces, is included in the tribe Saccharomyceteae.

Henrici (1941) in his comprehensive review on yeasts adopted Stelling-Dekker's classification of the ascosporogenous yeasts.

Asporogenous yeasts

Lodder (1934) divided the asporogenous yeasts into three families; the Rhodotorulaceae, the members of which form carotinoid pigments, the Nectaromycetaceae, those types which develop conidia, and the Torulopsidaceae for the remaining asporogenous yeasts.

Rhodotorulaceae. Harrison (1928) suggested the genus Rhodotorula for all yeasts producing pink or red pigment in the family Torulaceae. Lodder (1934) justified the elevation of the genus Rhodotorula to the family Rhodotorulaceae on the grounds that all pink or red yeasts form a homogenous group since they are all non-fermentatives, produce carotinoid pigment, and apparently are imperfect forms of the family Sporobolomycetaceae. Only one genus, Rhodotorula, is recognized in the family.

Nectaromycetaceae. Ciferri and Redaelli (1929, 1935) placed the asporogenous yeasts, which develop a true conidial form, in the family Nectaromycetaceae. Two genera, Sporobolomyces and Nectaromyces, are included in the family. Lodder (1934) retained the single genus Nectaromyces in the family Nectaromycetaceae but rejected the genus Sporobolomyces on the ground that members of the latter form basidiospores and not conidia.

Torulopsidaceae. In Lodder's classification the family Torulopsidaceae contains all the remaining species of asporogenous yeasts. Further division of the family into subfamilies or of subfamilies into genera is still a matter of opinion.

According to Guilliermond (1920), Hansen in 1888 first distinguished between the ascosporogenous and asporogenous yeasts. The name Torula was given to the latter group. However, because of the meager details and poor description, it is not possible at this time to distinguish between species studied by Hansen to re-identify them.

Wills (1916) grouped all the non-sporulating yeasts in the family Torulaceae. He subdivided this family into three genera; the Eutorula with round to elliptical cells and with fat droplets present, the Torula with round to elliptical cells but without fat droplets present, and the Mycotorula which form pseudomycelium. He studied the first two genera thoroughly but his treatment of the third genus was only superficial.

In an attempt to classify the asporogenous yeasts, Anderson (1917) based his division on pellicle formation, vegetative reproduction, and presence or absence of mycelial growth. In his systematic classification,

he used the genus Cryptococcus (Kützing) for typically yeast cells which were probably all from human or animal sources.

Guilliermond (1920) placed the non-sporulating yeasts in the family Non-Saccharomycetes. The family was divided into five genera. The genus Torula included those species whose cells are spherical and which do not form a pellicle in the medium immediately; pellicles when formed are always slimy without the presence of air bubbles. The genus Pseudo-saccharomyces included those species whose cells are apiculate. The genus Mycoderma forms a slimy scum with air bubbles and the cells are elongate. The genus Medusomyces is characterized by a thick, stratified, gelatinous scum. The genus Cryptococcus includes all the pathogenic yeasts. Guilliermond also recognized the genera Monilia and Pseudomonilia, which are characterized by mycelium formation, as members of the asporogenous yeasts but set them apart in a doubtful position because of their complexity.

Berkhout (1923) established the generic name Candida for a group of yeasts of the family Torulopsidaceae which has been confused with fungi belonging to the genus Monilia as understood by mycologists. In the genus Candida she placed all those species which are often pathogenic to men and animals, excluding those forms which produce ascospores. In the Monilia she placed all those species which are generally pathogenic to plants and are related to ascosporogenous forms. She also proposed the generic name Pullularia for a group of dark-pigmented yeasts which formed mycelium but whose conidial forms are produced directly on the mycelium, rather than on conidiophores.

Ota (1924a), working with saprophytic and parasitic yeasts and yeast-like organisms, proposed three groups, Saccharomyces, Cryptococcus, and

Monilia, for the so-called blastomycetes. Again in the same year, Ota (1924b) rearranged this brief classification to include the two groups, Cryptococcus, which possesses no mycelium, and Myceloblastanon, which forms true mycelium or outlines of mycelium. The Myceloblastanon is divided into the Blastodendrion, which is characterized by pseudomycelium, the Mycelorrhyzodes, which is characterized by the presence of true mycelium, and Monilia, which is characterized by species budding in chains and ramifying at the extremeties of the mycelial hyphae.

Ciferri and Redaelli (1925, 1929) proposed a classification which divided the asporogenous yeasts and yeast-like organisms into two families, the Nectaromycetaceae, with conidial form, and the Torulopsidaceae, without conidial form. The name Torulopsidaceae was created as a substitute for the family Torulaceae of Will and was divided into two subfamilies, the Torulopsideae (Torulopsidoideae), which is characterized by saccharomycetiform cells only and the Mycotoruleae (Mycotoruloideae), which is characterized by the saccharomycetiform cells mixed with true-or pseudomycelium. The Torulopsideae included five genera; Asporomyces, Kloeckera, Pityrosporum, Eutorulopsis, and Torulopsis.

The genus Asporomyces is identical to Torulaspora and Kloeckera to Pseudosaccharomyces as suggested by Guilliermond. The genus Pityrosporum is frequently referred to as the "bottle bacillus" and the authors questioned it as a yeast. The other two genera, Eutorulopsis and Torulopsis, are similar to those of Will and are differentiated similarly by the presence of fat globules in the former. The Mycotoruleae includes seven genera which are distinguished on the basis of morphology and biochemical and pathogenic activities.

Harrison (1928) divided the family Torulaceae of Will into three genera: the Rhodotorula, with red pigment, the Chromotorula, with pigment other than red, and the Torula, without any pigment. He further divided the genus Torula into nine groups based on sugar fermentation. He retained the genus Mycotorula for the asporogenous yeasts which form pseudomycelium.

Benham (1931) studying only those asporogenous yeasts which form true mycelium and/or pseudomycelium, was among the first to recognize that other characteristics beside morphology is necessary for clearer identity of these micro-organisms. She used a combination of morphology, which correlated closely with results obtained by agglutination technics, fermentation and cultural characteristics to clearly define Monilia albicans, Monilia parapsilosis, Monilia krusei, and Monilia candida.

However, Langeron and Talice (1932) published a classification of the pathogenic fungi in which they emphasized only the morphology of the cells and the mycelia. Six genera were recognized, namely, Mycotorula, Candida, Mycotoruloides, Mycocandida, Blastodendrion, and Geotrichoides.

Lodder (1934) presented a classification of the Torulopsidoideae, a subfamily of the Torulopsidaceae. This subfamily, the members of which do not form any mycelium, was divided into seven genera. She retained the genera Kleockera, Pityrosporum, Asporomyces, and Torulopsis of Cifferi and Redaelli (1929). She combined the Eutorulopsis and Torulopsis of the aforementioned authors into the single genus Torulopsis. She recognized the genus Trigonopsis, which is characterized by yeasts which bud at three angles. She also included two genera, the Mycoderma and the Schizoblastosporium, both of which form a dry, matte pellicle in wort culture from the beginning. The latter genus reproduces by transverse fission whereas the former does not.

Ciferri and Radaelli (1935) contributed further to the classification of the asporogenous yeasts, which they referred to as superfamily Adelosaccharomycetaceae Guilliermond. This superfamily is divided into the families Histoplamaceae, Nectaromycetaceae, and Torulopsidaceae.

The Histoplamaceae is recognized by budding cells enclosed in endothelial sac (in vivo), the Nectaromycetaceae possesses conidial form, and the Torulopsidaceae is composed of the saccharomycetiform types with or without mycelium formation and without the forms of multiplication characteristic of the two preceding genera. The family Torulopsidaceae is subdivided into the following subfamilies Torulopsideae, which is characterized by absence of mycelial growth, Mycotoruleae, which is characterized by blastospores and mycelial growth, and Trichosporeae, which is characterized by blastospores, mycelial growth and arthrospores. The subfamily Torulopsideae is divided into seven genera among which is included the new genus Schizotorulopsis, characterized by vegetative reproduction by fission. The genus Mycoderma of Lodder's classification is not included.

Martin, et al (1937) basing their classifications of the yeast-like fungi on morphological, cultural, and biochemical characters, placed them all in one genus, Monilia. With their methods, they were able to identify 169 of the 172 cultures studied as members of six species, Monilia albicans, M. parapsilosis, M. candida, M. krusei, M. mortifera, and the new species M. stellaloides.

A classification of the genus Trichosporon was prepared by Puntoni (1938). He divided the genus into six species mainly on the basis of morophological and to a lesser extent on the basis of cultural characteristics. Fermentation tests, growth in milk, and gelatin liquefaction were observed but

none of these tests were used for identification. Puntoni recognized that the differences in characteristics used for classification were very slight, especially when the medium was not of the right composition.

Langeron and Guerra (1938) adopted more complex methods for differentiation in accordance with the procedures of Stelling-Dekker (1931) and Lodder (1934) for study of the genus Candida. Seven groups obtained on the basis of sugar fermentation were further divided into 16 species on their ability to utilize sugars and nitrogen sources.

In a study of over 300 strains of Mycotoruloideae, Diddens and Lodder (1940) used morphology as the basis for division into two genera, Trichosporon (Behrend) and Candida(Berkhout). The genus Trichosporon is characterized by pseudo-and true mycelium with blastospores as well as arthrospores. The genus Candida develops a pseudomycelium (also to a smaller extent often a true mycelium) with blastospores in arrangement which may be typical of the species; chlamydospores may also be present. They admitted 23 species in their classification of the latter genus but their descriptions are not published in detail.

Conant (1940) summarized the methods and classifications of a number of investigators of the yeast-like fungi.

Mackinnon and Artagaveytia-Allende (1945) studied 14 of the 16 Candida species of Langeron and Guerra and admitted all the species except C. triadis and C. aldoi. These two species were considered as identical with C. albicans in agreement with a view expressed earlier by Conant (1940). They added to their list the species C. stellatoidea and C. macedoniensis.

Occurrence and Activities of Non-Lactose Fermenting Yeasts and Yeast-Like Fungi in Dairy Products

The study of non-lactose fermenting yeasts and yeast-like fungi has received limited attention because, in general, they are thought of as an inert group of organisms. Nevertheless, they occur frequently in dairy products and some of them are able to cause certain defects.

In their studies on the influence of rennin upon the ripening of Swiss cheese, von Freudenreich and Orla-Jensen (1897) isolated two yeasts, one a mycoderma and the other a true yeast, which they could not identify with any of the species of yeasts known to them. The yeast formed three to four spores on gypsum block after 24 hours at 25° C.

In an investigation on rancid butter, Orla-Jensen (1902) showed that a mycoderma type possessed no fat-splitting ability. It fermented maltose and developed an esterlike odor on the surface of the butter. He also mentioned a fungus, intermediate between yeast and mold and isolated by Reinmann, which when inoculated into sterile cream, increased the acid number of the fat from 3.1 to 24.1 in one month and developed an unpleasant odor.

Rogers (1904) demonstrated that a torula, which he isolated from canned butter, possessed to a limited degree the ability to split glycerides with the separation of free fatty acids. The acid number of the fat increased from .92 to 57.56 after 71 days incubation at 23° C. This torula fermented maltose slowly at 37° C. but not levulose, lactose, sucrose or mannose. Milk at 30° C. was digested very slowly by this yeast without previous curdling. It developed readily under both aerobic and anaerobic conditions.

Sayer, Rahn, and Farrand (1908), working on the keeping qualities of butter, found pink, white liquefying, and white non-liquefying yeasts. The latter included several types fermenting maltose, some of which do not ferment lactose, and a small "irregular"yeast, which frequently was found in exceptionally large numbers. This type was seldom found in fresh butter, but developed rapidly in storage in spite of the salt and low temperature. It was found in 127 out of 168 tubs of storage butter.

Rahn, Brown, and Smith (1909) made further investigations on the keeping quality of butter and found two torulae which occurred with remarkable frequency in storage butter. Two types, the "rapid liquefying" and the "small irregular" yeasts, were the same as those found in the preliminary examination. The non-liquefying torulae, which included the "small irregular" type, were found to increase in all of the samples of butter examined, but the numbers were never high. These non-liquefying types were quite inert, producing no apparent change except slight alkalinity in sterile milk.

Burri and Staub (1909) investigated the development of black spots in Emmenthaler cheese. They isolated a filamentous yeast, Monilia nigra, which they incriminated as responsible for the defect.

Dombrowski (1910) made a very comprehensive study of yeasts occurring in dairy products. Of 13 cultures of torula with which he worked, 7 did not ferment lactose. These torula forms were isolated from mazum, kefir, kefir grain, milk, and butter. He divided the non-lactose fermenting organisms according to cultural characterization and morphology into two groups, Torula lactis delta and Torula lactis epsilon. Dombrowski also described the red torula, Torula lactis No. 15, which he isolated from milk. This yeast produced no fermentation in sugar bouillon, but developed

well, when grown in milk at 25° C. It produced an intensely red membrane.

Three strains of the mycoderma type also were isolated from "natural" rennin, Finland butter and a butter culture. Strains from the first two isolations were named Mycoderma lactis alpha while that from the butter culture was named Mycoderma lactis beta. The two types differed slightly in character, Mycoderma lactis beta being the smaller organism of the two and possessing a weaker fermentative ability. Both species produced a "true mold" membrane on the surface of sugar bouillon and both fermented dextrose but not galactose, lactose, maltose or sucrose.

Edwards (1913) studied the effect of 12 strains of yeasts and yeast-like organisms which were isolated from cheese on the flavors of milk and Cheddar cheese. Nine of the strains were non-lactose fermenters. In general, all cultures caused deterioration of milk and cheese. The defects which developed in milk were yeasty, putrefactive, rotten, rancid and stagnant water flavors; those in cheese were bitter, fruity, dirty and stinking. He also showed that all the cultures were destroyed between 65° to 70° C. with an exposure of 10 minutes.

Sandelin (1920) isolated various yeast forms from 30 samples of butter and studied their characteristics. They were quite similar to the yeasts studied by Orla-Jensen. A limited number of the cultures fermented lactose. He also verified Orla-Jensen's conclusion that some of the yeasts can hydrolyze fat, especially in butter made from sour cream. He further showed that the group as a whole can develop in butter serum containing 10 percent salt but not 15 or 20 percent when held at 22° C. for 14 days.

Guilliermond (1920) referred to a strain of yeasts, isolated by Marpmann in 1891, which produced black colonies on gelatin and other substrates. The strain, regarded as related to Pichia membranaefaciens,

produced no mycelium in sugar solution. This yeast did not seem to utilize saccharose or lactose but it used a small quantity of dextrose. Guilliermond declared this species possessed characteristics which class it in the genus Dematium.

Cordes (1920) made a study of 202 cultures of yeasts isolated from different quality creams, milk, butter, butter and cheese cultures, silage, whey, and matzum. Some of these materials were secured from Armenia, Denmark, and Norway. Of the total cultures studied, 150 did not ferment lactose.

Baker (1923) isolated 68 cultures of mycodermata from a variety of dairy products. These organisms grew poorly alone, but in the presence of S. lactis, growth was increased. All the cultures fermented glucose and levulose but not lactose, maltose or galactose.

Nelson (1923) isolated 160 cultures of "common white" yeasts from dairy products. None of the cultures fermented lactose. They were relatively inactive in dairy products but were very resistant as shown by the fact that all of these cultures were still alive after being stored for 10 months and 20 days at a temperature near the freezing point.

In a study of the action of various types of yeasts commonly found in dairy products, Grimes (1923) found them not a significant factor in the deterioration of butter held at -6° F.

Cordes and Hammer (1927b) isolated 90 cultures of pink yeasts from milk, cream, soft cheese, and butter obtained near Ames. Most of the cultures conformed closely to Torula glutinis except for a slightly smaller dimension than commonly given. The two other types encountered were tentatively named Torula rubicinda and Torula paraglutinus. They

did not mention the effect of these organisms on the flavor and aroma of milk or butter.

Maurizio and Staub (1928), in another study of development of black spots in Emmenthaler cheese, verified the conclusion of Burri and Staub that Monilia nigra was the species of fungus responsible for the defect.

Since it was thought that in a majority of instances, fruity, sweet, bitter, whey tank, and "not clean" flavors in cheeses were caused by the presence of yeasts, Harrison (1927) studied the yeast flora of about 100 samples of cheese. With two exceptions, all samples contained yeasts. However, cheese graded as good contained yeasts in much smaller numbers than did cheese with definite flavor defects. Of 27 species isolated, 18 of them did not ferment lactose.

Glüthe (1935) investigated a creamery near Leipzig in which a foamy scum appeared during the churning process, resulting in considerable losses of butterfat. Yeasts were responsible for the trouble, but he did not incriminate any particular one of the 9 strains isolated. Six did not ferment lactose.

In an investigation of lipolytic micro-organism in dairy products, Long (1936) studied a number of cultures of liquefying yeasts. Although not all the cultures were tested for hydrolysis of fat, they were thought to be related to Mycotorula lipolytica because of their general cultural reactions. M. lipolytica is characterized by its ability to attack fat readily. Some cultures produced rancidity in butter, while others brought about cheesiness. All the cultures developed putrefaction in cream. All strains were inert in galactose, lactose, and maltose but produced acid in levulose, while only a few strains were able to form gas from dextrose.

Stacey (1939) reported that the filamentous yeast, Monilia, is one of the main causes of decomposition of soft cheese, especially when the cheese contains a large proportion of whey. Production of acid by bacteria favors the development of monilia and yeasts which are able to cause an alcoholic taste in the cheese.

Garrison (1943) found there was no correlation between the total yeast count and the type. The yeast flora of some samples of cream was predominatively non-lactose fermenting. Some samples containing over 500,000 yeasts per ml. had less than 10 lactose-fermenting types per ml.

Classification of Yeast Forms in Dairy Products

Although the occurrence of budding fungi is quite common in dairy product, the position of these micro-organisms in a generally recognized scheme of classification has received relatively little attention. Some attempts have been made, but most of these investigations were carried out before more recent refined methods were developed. It should be noted here that with yeasts occurring in dairy products, the ability to ferment lactose stands out as a natural division.

Sayer, Rahn, and Farrand (1908) outlined a scheme for the identification of yeasts isolated from butter in their investigation on the keeping qualities of butter. No nomenclature was involved; rather, it was a division into types. The bases for division were chromogenicity, gelatin liquefaction, colony character, and ability to ferment maltose.

Dombrowski (1910) prepared a classification of yeast forms with cultures mainly isolated by others from milk beverages. He considered the ability to form spores, mode of vegetative reproduction (whether by fission or budding), fermentation properties, morphology, and formation of membrane

on liquid media important for taxonomic purposes.

Basing the division on morphological, cultural, and physiological differences, Cordes (1923) proposed 9 types for various yeast forms isolated from milk and milk products. He also considered that allowance should be made for expansion. In this classification, the primary division was on the basis of lactose fermentation. Among the organisms not fermenting lactose, the mycoderma group was well-defined and readily separated from the rest of the group. The organisms not possessing mycoderma characteristics were divided into the "common white" type, "rapidly liquefying" type, "dull white" type, and red yeasts. The "common white" type included both ascosporogenous and asporogenous yeasts. Ability to form spores was considered of relatively minor importance on account of marked similarity in cultural characteristics of both sporulating and non-sporulating types in this group. The advisability of determining spore formation was questioned because of the time and technique involved and the variability in results often secured by different investigators working with the same yeasts.

Grimes (1923) studied the action of certain yeasts on the keeping quality of butter in cold storage. Recognizing that the usual scheme followed for classification of dairy yeasts was unsatisfactory, he proposed a means of differentiation based on chromogenicity and cultural and physiological characteristics. Spore formation was included but was relegated also to a relatively minor position.

Nelson (1923) proposed a classification for the "common white" yeasts. The divisions were made chiefly on morophology, growth temperature, and action in litmus milk. Spore production was not considered constant and was not used for identification purposes.

In a further study and rearrangement of the classification of the yeast forms present in milk, Cordes and Hammer (1927a) suggested a tentative grouping. In group I were placed the chromogenic yeasts. This group included not only the pink yeasts but others which can produce a yellow color when grown in association with Aspergilus niger. In group II were placed the yeasts which produce dull, spreading colonies in whey agar. Group III included the lactose fermenting organisms Torula sphaerica and Torula cremoris, which have been studied extensively. In group IV were placed the many divergent types which generally are known as the "common white" yeasts. No mention was made of spore formation.

Harrison (1927) studied 27 species of saccharomycetiform yeast cells isolated from over 100 samples of cheese. His first division was on the basis of spore formation; he placed the ascosporogenous yeasts under Guilliermond's (1920) second sub-group of the genus Saccharomyces, and the asporogenous yeasts, apparently including those that form mycelium, under the family which he designated as Torulaceae (Wills). The family, separated on the basis of chromogenicity, was divided into three genera, Rhodotorula, Chromotorula, and Torula. He subdivided the Torula into the nine subgroups on the basis of fermentation of various sugars. All those that do not ferment lactose were placed in the first seven groups.

Laffar (1936) studied 95 strains of yeast forms freshly isolated from dairy products. Although all the organisms fermented lactose, his scheme may provide suggestions for identification of non-lactose

fermenters. His divisions are based on morphological, physiological, and cultural characteristics. Sporulating ability of the yeast forms is important in his scheme of identification. Fermentation of some of the rarer carbohydrates, such as raffinose and inulin, are considered significant.

METHODS

Sources of Samples

A total of 136 cream and 203 butter samples were used for the experiment. Samples were obtained from producer's cream delivered during winter, spring, summer, and fall of 1945 to the Iowa State College creamery and from 22 samples obtained in northwestern Iowa during the summer season. Those samples obtained at the College included cream of various grades produced in Story and Hamilton counties. Platings usually were made soon after the samples were taken at the receiving dock. The set of cream samples from northwestern Iowa included cream of various grades and was held overnight at room temperature while in transit. A few of these samples were "foamy" when plated.

The butter represented supplies from various parts of Iowa and the immediately surrounding states throughout the year of 1945. Many were samples sent to the Iowa State College Dairy Chemistry Section for a project pertaining to Vitamin A analyses and to the Dairy Industry Service Laboratory for contest butter judging and other analyses. Some butter samples were secured from the college butter plant, while a number were from the Iowa State Brand Creameries at Mason City.

Detection of Non-Lactose Fermenting Yeasts and Yeast-Like Fungi

Yeasts and yeast-like fungi were enumerated on plates poured with potato dextrose agar, using the methods described in Standard Methods for

the Examination of Dairy Products, eighth edition (1941). However, in the winter, spring, and a portion of the summer series, the plates were incubated at 30° C. for 3 days, because at this temperature growth of yeast forms possibly was enhanced while that of molds was retarded. Later, 21° C. for 4 days was used, after isolation from a plate held at 4° C. of one strain whose growth range did not extend to 30° C. Dilutions of 1/10, 1/100, and 1/1,000 were used on all the cream samples from the College butter plant while 4 dilutions ranging from 1/100 and 1/100,000 were used on the cream samples from northwestern Iowa. Dilutions of 1/5 and 1/100 were used on all butter samples.

Representative colonies with characteristics usually attributed to yeasts and yeast-like fungi were selected from each sample and streaked on potato dextrose agar slants. After incubating at 25° C. for 24 hours to 36 hours, morphological characters were determined by staining. Only those which reproduced vegetatively by budding or transverse fission were retained for this study. Purification was accomplished by replating materials from the slants. All stock cultures were maintained on potato dextrose agar slants and stored at 4° C.

The lactose and non-lactose fermenting yeast forms were differentiated by growing the cultures in fermentation tubes containing nutrient broth plus 2 percent lactose with bromcresol purple as an indicator and incubating at 25° C. for 12 days. All those cultures which did not ferment lactose were retained for further study.

Culture Designation

Cultures were designated by numbers with the prefix B added for those cultures isolated from butter and the prefix C added for those cultures isolated from cream. Cultures with numbers from 1 to 146 were from winter samples, from 147 to 242 from spring samples, from 243 to 360 from summer samples and from 361 to 504 from fall samples.

Procedures for Identification and Characterization

The inoculum

In a number of tests performed, suspension of cells was used for inoculation. This inoculum was obtained by suspending in sterile water, growth from a 24 to 36 hour wort agar slant.

Optimum temperature and growth range

The optimum temperature and growth range were determined by inoculating 5 wort agar slants with single drops of suspension of the cultures and incubating at 10°, 21°, 30°, 37°, and 45° C. Growth was recorded in 1, 2, 4, and 7 days for all tubes. The tubes incubated at 10° C. were observed at intervals up to 6 weeks. All subsequent tests, except when otherwise indicated and except for one culture which would not grow at 30° C., were carried on at 30° C., which was within the optimum growth temperature range for all but the one culture.

Morphology

Gram stains were made from 24-hour-old potato dextrose agar slants with all the strains isolated. Additional stains were made from 24-hour-old wort agar slants with all yeast forms which do not form mycelium. The size, shape, arrangement, staining reaction, and mode of vegetative reproduction were recorded.

Mycelium development was determined by slide cultures. The medium was made up according to the formula of Langeron and Guerra(1938), as follows:

Pulp of potatoes	- 20 grams
Pulp of carrots	- 20 "
Agar	- 20 "
Water (tap)	- 1,000 ml.

The potato and carrot were ground finely and boiled for 1 hour in a liter of water. They were strained through a heavy thickness of cotton, filtered through filter paper and then restored to original volume. The agar was added and the medium was autoclaved at 120° C. for 5 minutes, filtered, distributed into 2 ounce jars with depth approximately that of microscopic slide, and sterilized.

The procedure for the slide culture was a slight modification of that of the aforementioned authors. Microscope slides and cover slips were kept in 95 percent alcohol. Prior to the preparation of the slide, the agar was melted and held at about 60° C. The slide was flamed to remove any alcohol adhering, dipped into the agar, and then placed in a sterile petri dish. The slide was supported on a U-shaped glass rod under which was a piece of blotting paper. When the agar congealed, the

inoculum was streaked lightly across the middle of the slide. The length of the streak was about an inch longer than the cover slip, thus giving both aerobic and partially anaerobic environment. The cover slip was flamed, cooled, and placed across the line of inoculation. A humid condition was maintained by the addition of a few ml. of sterile water on the absorbent paper. The cultures were incubated at 25° C. and examined on the fifth or sixth days, using a magnification of approximately 450x. Before examination, the agar was wiped off the back of the slide. When photomicrographs were taken, they were of the unstained slide preparations.

Spore formation

Ability to form ascospores was determined by inoculating a suspension of fresh organisms on carrot infusion-calcium sulphate agar slants as described in Levine (1938), and incubating at 21° C.; stains for spores were made at 7 days, one month, and 2 months. The staining was accomplished with a yeast spore stain suggested by Langwill by smearing the yeasts from the sporulating medium into a loopful of sterile milk previously placed on a clean slide. A drop of water was added to dilute the mixture. After drying, the smear was fixed with glacial acetic acid for 20 minutes, rinsed with water, and a carbol fuchsin preparation of the following composition was added:

Solution A -	Basic Fuchsin	1 gram
	95% ethyl alcohol	10 ml.
Solution B -	Phenol	5 grams
	Water	100 ml.

One ml. of "A" was mixed with 100 ml. of "B"

After steaming the slide for 2 to 3 minutes, it was rinsed, decolorized with 95 percent ethyl alcohol for 30 seconds, and counterstained with Loeffler's methylene blue diluted to 1/3 strength with distilled water. The slide was then rinsed with water, dried, and examined.

An agar medium composed of infusion from carrots, beets, cucumbers and potatoes as devised by Mrak, Phaff, and Douglas (1942) also was employed in sporulation studies in conjunction with the Langwill staining procedures.

Growth on agar slants

The growth on wort and potato agar slants was observed after incubating for 3 days at 30° C. Then the slants were stored at 4° C. and any subsequent changes were recorded.

Growth on agar plates

The appearance of colony types on poured agar plates, using potato dextrose agar, was observed after incubating for 5 days at 30° C.

Carbohydrate fermentation

Fermentation of carbohydrates was observed throughout a period of 12 days. The medium was composed of nutrient broth plus two percent of the test carbohydrates. One ml. of a 1.6 percent bromcresol purple was added per liter of medium. The medium was sterilized at 15 pounds pressure for 15 minutes. The sugars used were glucose, fructose, mannose, galactose, sucrose, and maltose for all the cultures. Raffinose and melibiose also were used for the ascosporogenous yeast forms.

Henrici (1941) said of the use of melibiose:

> The fermentation of raffinose occupies a special place in the Stelling-Dekker's system. Some yeasts split this trisaccharide to levulose and the dissacharide melibiose, fermenting the levulose but not attacking the melibiose; such yeasts are described as fermenting '1/3 of raffinose'. Other yeasts completely hydrolyze raffinose to its component monosaccharides, and are said to ferment raffinose completely. This procedure is, therefore, a method of separating yeasts upon the basis of their ability to ferment melibiose.

In the study of fermentation of carbohydrate by yeast forms both anaerobic and aerobic techniques have been used. Stelling-Dekker (1931) and Lodder (1934) employed the fermentation flask according to Einhorn. With doubtful cases the results were verified by the use of a quantitative apparatus of van Iterson-Kluyver. Martin, et al (1937), Langeron and Guerra (1938) and MacKinnon and Artagaveytia-Allende (1945) also used anaerobic techniques. The first group of authors used sterile vaseline to seal the tubes while the latter two groups of investigators employed paraffin. On the other hand, Wickerham and Rettger (1939) found that the use of paraffin oil was not an important factor in the fermentation tests, providing a deep column of medium is used. Henrici (1941) concurred and used large fermentation tubes containing about 20 ml. of medium with no seal. In the present study deep column of media in unsealed tubes were used. Later in the experiment, tubes sealed with sterile vaseline was compared with the unsealed tubes on about 60 representative strains with results which agreed with those obtained by the aerobic technic.

Henrici (1941) claimed that the criterion for fermentation is the production of gas and that development of acid alone has no place in the systematics of yeasts. In this study the fermentation is not recorded as positive unless gas is formed.

Identical results were obtained with glucose, fructose, and mannose. Consequently, only the results with glucose are recorded in the summaries of data.

Carbohydrate utilization

The ability to utilize various sugars as the sole source of carbon was determined by plating a heavy emulsion of a fresh culture in a medium of the following composition, as described by Lodder (1934):

Ammonium sulphate	0.5 grams
Potassium dihydrogen phosphate	0.1 "
Magnesium sulphate	0.05 "
Washed agar	2.0 "
Distilled water	100 ml.

After plating, the surface of the agar was dried by placing it in an incubator at 30° C. for a number of hours. Minute amounts of the various sugars, previously sterilized by immersion in ethyl ether for 48 hours and dried, were then spotted at determined positions on the surface. Sucrose, maltose, lactose, glucose, fructose, mannose and galactose, as recommended by Lodder, were used for the tests on the yeast forms which neither develop mycelium nor ferment any sugars. Six sugars, sucrose, maltose, lactose, glucose, raffinose, and galactose, as recommended by Mackinnon and Artagaveytia-Allende (1945) were used for the tests on all the yeast forms which develop mycelium. These authors employed inulin to test only 4 of the 14 species in their study. Furthermore, the auxanogram with inulin is only of a substantiating nature; therefore, this sugar was not employed as a test substance in the present study. The plates were incubated at 25° C. and observations were

made in 24, 48, and, if necessary, in 72 hours. Definite growth surrounding the location where the sugar was spotted was an indication of utilization. Figure 1a shows diagrametically where the different sugars were spotted and Figure 1b illustrates a representative auxanogram with these sugars.

In a few cases, this auxanographic procedure did not give clear or favorable results. Then the following accessory factors as used by Rugosa (1944) were added per liter of basal medium:

Biotin	2 gamma
Inositol	10 mg.
d-Ca Panthothenate	5 mg.
Thiamin Hydrochloride	1,000 gamma

Identical results were obtained with glucose, fructose, and mannose. Consequently, only the results with glucose were recorded.

Nitrogen utilization

The auxanographic procedure for nitrogen assimilation is similar to that employed for sugar assimilation. Instead of using ammonium sulphate in the basal medium, this is replaced by glucose according to Lodder (1934), as follows:

Glucose	2.0 grams
Potassium Dihydrogen Phosphate	0.1 "
Magnesium Sulphate	0.5 "
Washed Agar	2.0 "
Distilled Water	100.0 ml.

The nitrogen sources used for all the cultures were urea, peptone, asparagine, ammonium sulphate, and potassium nitrate, and these were

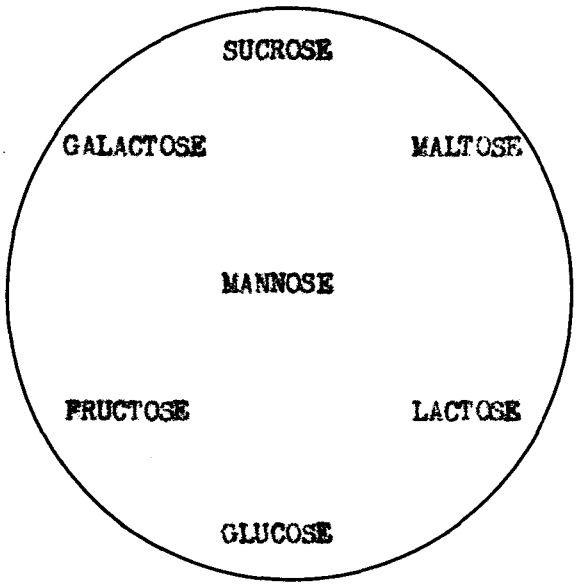

Fig. 1a. Locations where the different sugars were spotted.

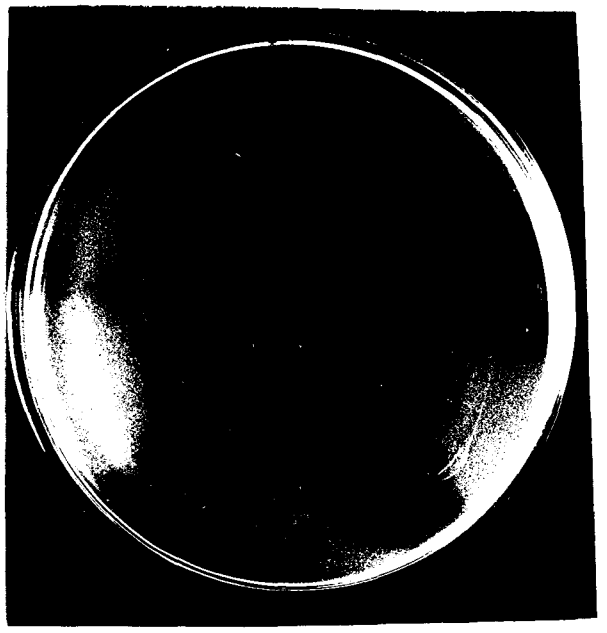

Fig. 1b. Representative auxanogram illustrating utilization of sugars.

sterilized by immersion in ethyl ether for 48 hours and then dried. In some cases, where the result was not clear or the organism did not grow on the supplemented basal medium, accessory factors as outlined in the section on carbohydrate utilization were added to the basal medium. The plates were incubated at 25° C. and observations were made in 18 and 48 hours. Observation in 18 hours was necessary because in some instances the growth at the different nitrogen sources was so rapid that the areas no longer were well-defined, making the interpretation of results difficult. Figure 2a shows diagrametically where the different nitrogen sources were spotted and Figure 2b illustrates a typical auxanogram with these nitrogen sources.

Growth in ethyl alcohol

The ability to develop in ethyl alcohol was determined by inoculating single drops of suspension of the culture into two tubes of a medium described by Lodder (1934) and of the following composition:

Magnesium sulphate	0.05 grams
Potassium dihydrogen phosphate	0.1 "
Ammonium sulphate	0.1 "
Distilled water	100.0 ml.

Three percent of ethyl alcohol was added to one tube and nothing to the other. The tubes were incubated for 4 to 5 days and growth compared. Growth was determined by clouding of the medium. If a membrane was formed, this was recorded.

Growth in malt extract broth

Growth in malt extract broth (Levine, 1938) was observed at 24 hours and at 12 days.

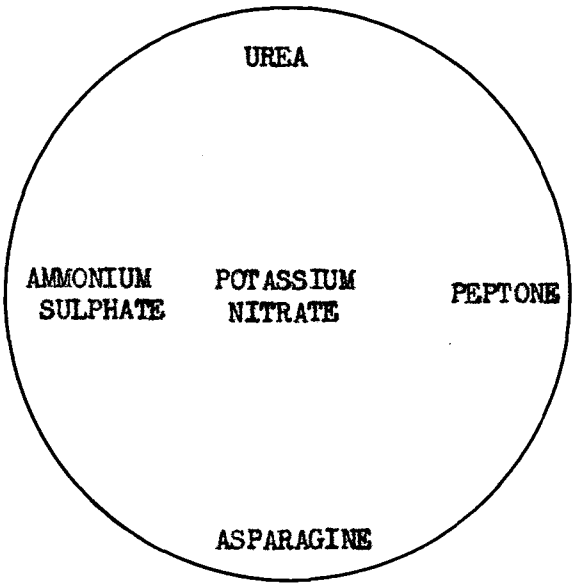

Fig. 2a. Locations where the different nitrogen sources were spotted.

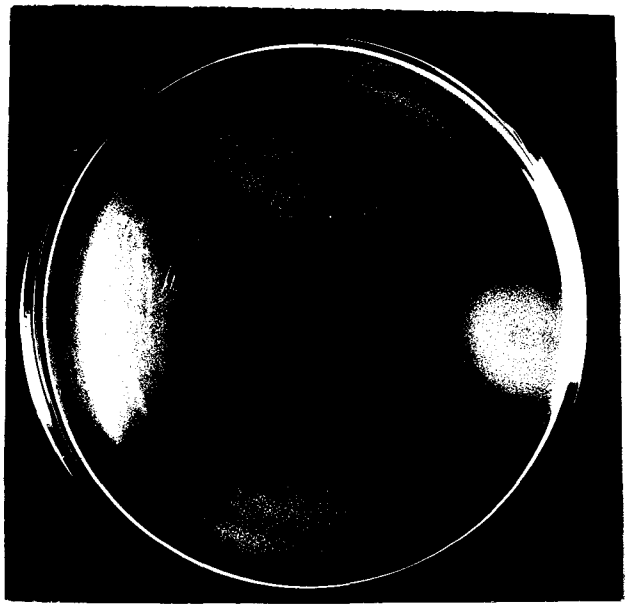

Fig. 2b. Auxanogram indicating the utilization of urea, peptone, asparagine, and ammonium sulphate but not potassium nitrate.

Action in litmus milk

Action in litmus milk was observed at intervals for 60 days.

Gelatin liquefaction

Gelatin liquefaction and growth in line of puncture were observed on stab cultures incubated at 21° C. for 30 days in a medium of the following composition:

Maltose	12.75 grams
Malt Extract	15.00 "
Dextrin	2.75 "
Glycerin	2.35 "
Potassium Monohydrogen Phosphate	1.00 "
Ammonium Chloride	1.00 "
Peptone	.78 "
Gelatin	12.00 "
Distilled Water	100.00 ml.

Lipolysis

Lipolysis was determined by the modified nile blue sulphate and natural fat techniques as suggested by Long (1936). The basal medium used was:

Yeast Extract	1.5 grams
Beef Extract	1.5 "
Peptone	5.0 "
Agar	15.0 "
Distilled Water	1,000.0 ml.

Both the fat emulsion and nile blue sulphate were added to the medium before pouring. Results were recorded in 4 days.

Proteolysis

Proteolysis was detected on agar plates by the addition of 5 ml. of sterile skimmilk to 100 ml. of the basal medium as used for lipolysis detection. Observations were made in 4 days.

Heat resistance

Heat resistance was determined by transferring ½ ml. portions of an actively growing milk culture into 3 tubes of sterile milk. Ability to survive was tested after heating them at 61.7° C. (143° F.) for 5, 10, and 30 minutes by incubating the heated and cooled tubes for 2 days and then streaking the cultures on the surface of poured plates.

Oxygen relationship

Oxygen relationship was studied by inoculating single drop of suspension of a culture into a tube of melted agar medium of the following composition and observing the area of growth after incubating for 1 week:

Yeast Extract	3.0 grams
Peptone	5.0 "
Glucose	10.0 "
Agar	15.0 "
Distilled Water	1,000 ml.

General Action of Non-Lactose Fermenting Yeasts and Yeast-Like Fungi in Cream and Unsalted Butter

Cream

Sweet cream of good quality was pasteurized in 2-ounce jars in flowing steam for 20 minutes on each of 3 successive days. Representative strains of organism, 47 in all, were inoculated into 2 jars of cream. One jar of each group was further inoculated with a milk culture of *Streptococcus lactis* to study the effect of simultaneous acid production, such as would occur in usual producer cream. The cream was then incubated at 21° C. and examined organoleptically at 2 and 4 days by two judges and at 7 days by three judges.

Unsalted butter

Sweet cream of good quality was pasteurized in flowing steam for 30 minutes and then held overnight at 4° C. to solidify the fat globules. Then 500 ml. portions of cool cream were poured into sterile quart jars. Each jar was inoculated with 2 ml. of 24 to 36-hour milk culture of each of 47 representative test organisms. The cream was churned, buttermilk poured off, the butter washed twice with sterile water, and worked thoroughly. The butter was then divided into 2 lots; one lot was incubated at 21° C. and examined for development of off-flavor at 2, 4, and 7 days, while the second lot was incubated at 4° C. and examined for development of off-flavor at the end of one month. Examination at 2 and 4 days was by two judges and at 7 days and one month by three judges.

RESULTS

Determination of Yeasts and Yeast-Like Fungi in Cream and Butter

The yeast counts obtained from 124 samples of cream secured at different seasons are given in Table 1. Fifty-five samples (44.3%) had yeast counts less than 1,000 per ml.; 50 samples (40.3%) had yeast counts between 1,100 and 50,000 per ml.; while 19 samples (15.3%) had yeast counts from 51,000 to over 1,000,000 per ml.

A relationship between yeast count and season of the year was apparent. Twenty-two (70.9%) of the 31 winter samples and 16 (59.3%) of the 27 fall samples had yeast counts less than 1,000 per ml.; 9 (29.1%) of the winter samples and 10 (37.0%) of the fall samples had yeast counts between 1,100 to 50,000 per ml.; while none of the winter samples and only 1 (3.7%) of the fall samples had yeast counts higher than 50,000 per ml. The yeast counts on cream samples obtained during the spring were somewhat higher than for the fall and winter collections. Nine (34.6%) of the 26 spring samples had yeast counts less than 1,000 per ml.; while 16 (61.5%) had yeast counts between 1,100 to 50,000 per ml.; and 1 (3.8%) had a yeast count of over 1,000,000 per ml. The yeast counts on cream samples collected during the summer were considerably higher than those of the other three seasons. Only 8 (20.0%) of the 40 samples had yeast counts lower than 1,000 per ml.; 15 (37.5%) had yeast counts between 1,100 and 50,000 per ml.; 13 (32.5%) had yeast counts from 51,000 to 1,000,000 per ml.; and 4 (10.0%) had yeast counts exceeding 1,000,000 per ml.

Table 1

Distribution of Cream Samples According to Yeast Count and Season of the Year

Counts per ml.	No. of Samples Examined				Totals
	Winter	Spring	Summer*	Fall	
Less than 100	9	7	-	2	
100 - 500	9	1	2	8	55
510 - 1,000	4	1	6	6	
1,100 - 5,000	4	8	6	8	
5,100 - 10,000	3	3	4	1	50
11,000 - 50,000	2	5	5	1	
51,000 - 100,000	-	-	2	1	
110,000 - 1,000,000	-	-	11	-	19
Greater than - 1,000,000	-	1	4	-	
Totals	31	26	40	27	124

*Predominance of Geotrichum (Oospora) prevented the counting of plates on 12 additional samples.

Yeasts counts obtained on 203 samples of butter secured at different seasons are given in Table 2. Eighty-four samples (42.3%) had yeast counts of less than 50 per ml.; 81 (39.9%) had yeast counts between 51 and 500 per ml.; 24 (14.3%) had yeast counts between 510 and 5,000 per ml.; while 14 (6.9%) had yeast counts exceeding 5,000 per ml. Although a somewhat greater percentage of the fall and winter butter samples had low yeast counts, in general little relationship between distribution of yeast counts and season of the year was apparent.

In the isolation of yeast strains from samples of cream and butter, usually one strain was selected from each sample since macroscopically most plates showed a predominance of one colony type and frequently only one type of colony appeared on a plate. When the yeast flora was varied, as happened occasionally, then representative strains were picked of the different colony types. Sometimes two colony types were seen but very seldom were three or four different colony types encountered from one sample.

A total of 369 strains of lactose and non-lactose fermenting yeasts and yeast-like fungi was isolated from the 136 samples of cream and 203 samples of butter. A summary of the numbers of lactose and non-lactose fermenting yeast forms isolated from cream and butter at different seasons of the year is presented in Table 3. Of the 139 cultures isolated from 124 samples of cream, 120 (86.3%) did not ferment lactose. Of the 19 fermenting lactose, 12 (63.2%) were isolated during the summer months. These 12 cultures represented 32.4 percent of the 37 cultures isolated from cream for that season. Of the 230 cultures isolated from 203 samples of butter, 222 (96.5%) did not ferment lactose. The 8 cultures fermenting lactose were isolated during the winter and spring seasons.

Table 2

Distribution of Butter Samples According to Yeast Count and Season of the Year

Counts per ml.	No. of Samples Examined				Totals
	Winter	Spring	Summer	Fall	
Less than - 10	18	12	4	6	84
10 - 50	15	18	3	8	
51 - 100	5	9	3	4	81
110 - 500	18	17	10	15	
510 - 1,000	2	4	2	1	24
1,100 - 5,000	9	3	2	1	
Greater than - 5,000	2	7	2	3	14
Totals	69	70	26	38	203

Table 3

Numbers of Lactose Fermenting and Non-Lactose Fermenting Yeast Forms Isolated From Cream and Butter During the Various Seasons

Seasons	Cream			Butter		
	No. of Samples	No. of Cultures		No. of Samples	No. of Cultures	
		Lactose Fermenting	Non-Lactose Fermenting		Lactose Fermenting	Non-Lactose Fermenting
Winter	31	5	40	69	4	69
Spring	26	0	18	70	4	68
Summer	40	12	25	26	0	31
Fall	27	2	37	38	0	54
Totals	124	19	120	203	8	222

Description of the Non-Lactose Fermenting Yeasts and Yeast-Like Fungi

An attempt was made to separate the 342 cultures of non-lactose fermenting yeasts and yeast-like fungi isolated from cream and butter on the bases of morphological, cultural, and physiological characteristics. Twenty-eight types were obtained on these bases.

Description of Type 1

One strain, B166, was encountered in this type.

Morphology

Form and size

Potato agar slant: oval to slightly ellipsoidal; 1.6 to 1.8 x 2.5 to 2.8 microns

Wort agar slant: oval; 1.9 to 2.5 x 2.5 to 3.5 microns

Arrangement: singly, single bud attached, or in short chains

Staining reaction: gram positive

Spore: 1 to 4 spores per ascus; almost spherical; 1.8 to 2 microns

Slide culture: no mycelium observed

Cultural characteristics

Potato agar slant: filiform with slightly papillate edge; raised, slightly verrucose, glistening, whitish and butyrous growth

Potato agar colony: circular with lobate margin, pulvinate, contoured to smooth, somewhat glistening, whitish grey; 2.0 to 2.5 mm. in diameter

Gelatin stab

Growth in line of puncture: filiform to beaded

Liquefaction: none

Malt extract broth

24 hours: no surface growth; granular sediment

10 days: ring; slightly granular to slightly flocculent sediment

Biochemical features

Carbohydrate fermentation: gas production from glucose

Nitrogen utilization: only peptone utilized

Hydrolysis of fat: negative

Proteolysis: negative

Ethyl alcohol utilization: negative

Litmus milk: slight reduction in 2 weeks, becoming slightly alkaline in 2 months

Growth conditions

Oxygen relationship: aerobic

Growth temperature: optimum 30° to 37° C.; growth at 10° C. but not at 45° C.

Heat resistance: no survival at 61.7° C. for 5 minutes

Identity of Type 1. The characteristics of this organism place it in the genus Saccharomyces as defined by Stelling-Dekker (1931). This organism could not be identified with any species previously described. Stelling-Dekker provided no place for species of Saccharomyces which ferment glucose, fructose and mannose only.

Description of Type 2

Two strains, C26 and C314, were encountered in this type.

Morphology

Form and size

Potato agar slant: oval and sausage-shaped; oval 2.0 to 3.0 x 2.8 to 5.0 microns, sausage-shaped - 1.8 to 2.0 x 6.0 to 8.0 microns

Wort agar slant: oval and round; oval - 2.0 to 3.5 x 3.0 to 7.0 microns, round - 3.5 to 6.0 microns

Arrangement: singly, single bud attached or in short chains

Staining reaction: gram positive

Spores: 1 to 4 spores per ascus; almost spherical; 1.8 to 2.0 microns

Slide cultures: no mycelium observed

Cultural Characteristics

Potato agar slant: filiform, convex, smooth to slightly contoured, glistening, cream-colored and butyrous growth

Potato agar colony: circular, entire, convex, smooth, glistening, cream-colored; 1 to 1½ mm. in diameter

Gelatin stab

Growth in line of puncture: papillate

Liquefaction: none

Malt extract broth

24 hours: no surface growth; slightly viscid sediment

10 days: very thin film; slightly viscid to slightly granular sediment

Biochemical features

Carbohydrate fermentation: gas production from glucose, galactose, sucrose, maltose, and "1/3 raffinose"

Nitrogen utilization: only peptone utilized

Hydrolysis of fat: negative

Proteolysis: negative

Ethyl alcohol utilization: negative

Litmus milk: slight reduction in 2 weeks; becoming slightly alkaline in about 6 weeks

Growth conditions

Oxygen relationship: aerobic

Growth temperature: optimum 21° to 30° C.; growth at 10° and 37° C. but not at 45° C.

Heat resistance: no survival at 61.7° C. for 5 minutes

Action in cream. Strain C26 required 7 days at 21° C. to develop slightly yeasty and slightly rancid flavors. In the presence of S. lactis, a slightly yeasty flavor was produced in 2 or 4 days while yeasty and slightly bitter flavors with slight astringency were produced in 7 days.

Action in butter. Strain C26 caused an unclean flavor in 7 days at 21° C. At the end of one month at 4° C. it caused an unnatural flavor.

Identity of Type 2. Organisms of this type resemble Saccharomyces cerevisiae (Hansen) "Presshefe a Delft", as described by Stelling-Dekker (1931) in nearly all characteristics studied although the cells were slightly smaller. She mentioned weak utilization of alcohol; no utilization was encountered with organisms of Type 2. The spore-forming Type E yeast of Cordes (1920) corresponded closely to the cultures isolated also.

Gas was produced from the same sugars. He mentioned only oval cells varying from 2.8 to 3.5 microns in width and from 3.7 to 5.3 microns in length. This type may be considered as similar to Type E of Cordes and may be regarded as S. cerevisiae (Hansen).

Description of Type 3

Thirty-one strains, C19, C22, C27, C31, C45, C52, C53, C58, C62, C68, B81, B101, B125, B132, B190, B246, C279, C281, C294, B340, B343, B347, B419, C433, C436, C454, C460, C469, C472, C476, and C479, were encountered in this type.

Morphology

Form and size

Potato agar slant: oval; 1.4 to 2.0 x 2.4 to 3.3 microns

Wort agar slant: oval; 1.6 to 2.5 x 2.3 to 4.0 microns

Arrangement: singly, single bud attached

Staining reaction: gram positive

Spores: none

Slide cultures: no mycelium observed (Figure 3)

Cultural characteristics

Potato agar slant: filiform, convex, smooth, glistening, pink, slimy and fluid growth; due to the consistency of growth, the material flowed to the bottom of the tube leaving a nearly translucent and flat area of growth on the surface of the slant

Potato agar colony: circular, entire, convex, smooth, glistening, pink; 1 to 2½ mm. in diameter

Gelatin stab

Growth in line of puncture: beaded to papillate

Liquefaction: none

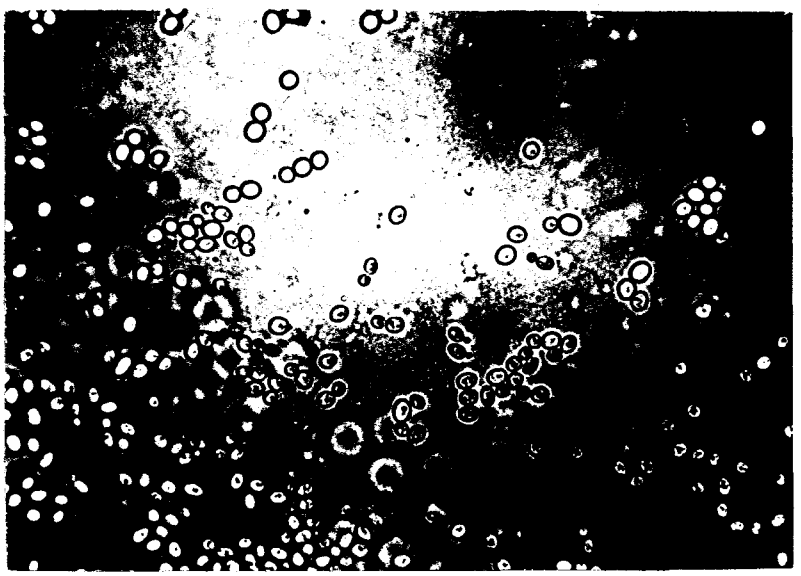

Fig. 3. Strain B340 of Type 3.
Budding cells only. 555x.

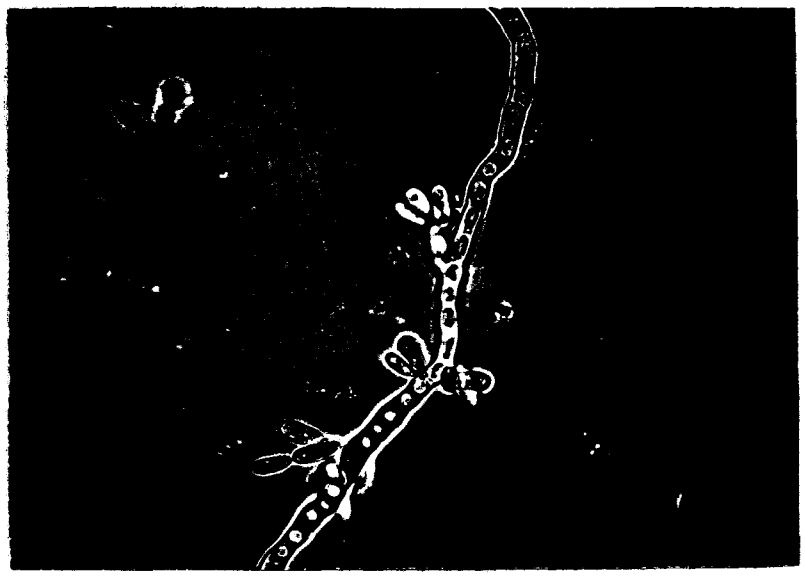

Fig. 4. Strain C462 of Type 5. Elongated-oval blastospores on septate true mycelium. 555x.

Malt extract broth

24 hours: no surface growth; slightly viscid sediment

10 days: pink ring; viscid sediment

Biochemical features

Carbohydrate fermentation: no gas production from any sugars

Carbohydrate utilization: glucose, galactose, sucrose, and maltose utilized

Nitrogen utilization: peptone, asparagine, ammonium sulphate, and urea utilized

Hydrolysis of fat: negative

Proteolysis: negative

Ethyl alcohol utilization: negative

Litmus milk: pink ring and pink sediment in about 4 days; reduction at bottom of tube; alkaline reaction in about 3 weeks; coagulation and wheying-off in about 6 weeks by 4 strains

Growth conditions

Oxygen relationship: aerobic

Growth temperature: optimum 21° to 30° C.; growth at 10° C. and 37° C. but not at 45° C.

Heat resistance: no survival at 61.7° C. for 5 minutes

Action in cream. Strains B132 and C454 were studied. Both strains required 7 days at 21° C. to develop slightly unclean and very slightly bitter flavors. In the presence of S. lactis, strain B132 developed a slightly bitter flavor in 2 days and unclean and bitter flavors in 7 days while strain C454 developed rancid and moderately bitter flavors in 7 days.

Action in butter. Strains B132 and C454 did not develop any defect in 7 days at 21° C. but developed a very slightly unclean flavor in one month at 4° C.

Identity of Type 3. Organisms of this type closely resemble Rhodotorula mucilaginosa (Jörgensen) as described by Lodder (1934). She mentioned slightly larger sizes. These organisms also resemble the pink yeasts isolated from dairy sources and identified as Torula glutinis by Cordes and Hammer (1927b). All strains studied by the latter workers were able to coagulate milk in old cultures; only 4 strains of Type 3 were able to do so in 60 days. The organisms isolated may be considered as similar to Torula glutinus of Cordes and Hammer, but in view of the more recent methods adopted for identification of yeasts by Lodder (1934), Type 3 should be considered as R. mucilaginosa (Jörgensen)

Description of Type 4

Ten strains, C28, B213, C224, C239, C288, C292, C434, C447, C450, and C458, were encountered in this type.

Morphology

Form and size: oval; 2.0 to 2.5 x 2.4 to 4.5 microns

Arrangement: singly, single bud attached

Staining reaction: gram positive

Spores: none

Slide cultures: no mycelium observed

Cultural characteristics

Potato agar slant: filiform, convex, smooth, glistening, deep pink and butyrous to slightly slimy growth

Potato agar colony: circular, entire, pulvinate, smooth, glistening, deep pink; 1½ to 3 mm. in diameter

Gelatin stab

Growth in line of puncture: beaded to papillate

Liquefaction: none

Malt extract broth:

24 hours: no surface growth; slightly viscid sediment

10 days: pink ring; viscid sediment

Biochemical features

Carbohydrate fermentation: no gas production from any sugars

Carbohydrate utilization: glucose, galactose, sucrose, and maltose utilized

Nitrogen utilization: peptone, asparagine, ammonium sulphate, and urea utilized

Hydrolysis of fat: negative

Proteolysis: negative

Ethyl alcohol utilization: negative

Litmus milk: reduction in 2 weeks; slight pellicle formation by some strains; digestion of the casein in about 6 weeks by some strains

Growth conditions

Oxygen relationship: aerobic

Growth temperature: optimum 21° to 30° C.; growth at 10° and 37° C. but not at 45° C.

Heat resistance: no survival at 61.7° C. for 5 minutes

Action in cream. Strain C28 required 7 days at 21° C. to develop a slightly yeasty flavor. In the presence of S. lactis, a slight astringency was produced.

Action in butter. Strain C28 did not develop any defect in 7 days at 21° C. or in one month at 4° C.

Identity of Type 4. Organisms of this type resemble Rhodotorula mucilaginosa var. Carbonei as described by Lodder (1934). R. mucilaginosa var Carbonei is described as having slightly larger cells and liquefying gelatin in 130 days. Organisms of Type 4 differ only in minor details from yeasts isolated from dairy sources and identified as Type A or Torula rubicunda by Cordes and Hammer (1927b). Their cultures of this type digested milk; only 3 of the 10 strains isolated in this study had this ability. Their cultures had slightly larger dimensions. Type 4 may be regarded as R. mucilaginosa var. Carbonei.

Description of Type 5

Four strains, B327, C445, C462, and C467, were encountered in this type.

Morphology

Form and size: oval, sausage-shaped, pleomorphic; oval - 2.3 to 7.0 x 5.0 to 14.0 microns, sausage-shaped - 2.2 to 2.5 x 4.0 to 10.0 microns

Arrangement: singly; mutipolar budding. Two strains apparently reproduced vegetatively by fission on early transfer but this characteristic was lost on further transfer.

Staining reaction: gram positive

Spores: none

Slide cultures: elongate-oval blastospores in small clusters on septate true mycelium; cross-walls very close together in some mycelia (Figure 4)

Cultural characteristics

Potato agar slant: spreading with ridge in center, flat to slightly convex, smooth, glistening, and ropy growth; cream-colored at first then turning pink and then black; one culture, after a number of transfers, lost its ability to turn black

Potato agar colony: circular, entire, convex, smooth, glistening, ropy, white to creamy but turning black in about 7 days; 2 to 3 mm. in diameter

Gelatin stab

Liquefaction: completely liquefied in 2 weeks

Malt extract broth

24 hours: no surface growth; slight flocculent sediment

10 days: smoky ring; flocculent sediment

Biochemical features

Carbohydrate fermentation: no gas production from any sugars

Carbohydrate utilization: glucose, sucrose, maltose and raffinose utilized

Nitrogen utilization: all nitrogen sources utilized

Hydrolysis of fat: negative

Proteolysis: positive

Ethyl alcohol utilization: negative

Litmus milk: soft coagulum and slight digestion noted in 5 days; complete digestion in 3 weeks

Growth conditions

Oxygen relationship: aerobic

Growth temperature: optimum 21° C. to 30° C.; growth at 10° C. but not 37° C.

Heat resistance: no survival at 61.7° C. for 5 minutes

Action in cream. At 21° C. strain C462 required only 4 days to develop a putrid flavor and 7 days to develop yeasty, rancid and cheesy flavors. In the presence of S. lactis, a very pronounced bitterness was produced in 7 days in addition to the above defects.

Action in butter. Strain C462 developed a slightly unclean flavor in 2 or 4 days and a rancid flavor in 7 days. It produced a definite rancid flavor in one month at 4° C.

Identity of Type 5. Strains of this type resemble organisms which have been described by several authors. Guilliermond (1920) stated that Marpmann described under the name of Schizosaccharomyces niger a black yeast which possessed a complex mycelium. The genus name manifesting the mode of vegetative reproduction indicates a further common characteristic.

Three black yeast forms isolated by Will also were mentioned by Guilliermond. There were a number of characteristics in common with the organisms of Type 5, such as development of a black ring on the side of container and inability to grow at 35° C. or ferment any sugars. The mention of ellipsoidal or spherical conidia in Will's strains brings out a difference; single blastospores or pseudo-conidia were observed on the mycelium in this study. However, it is not entirely impossible that the term conidia was used by Will to designate blastospores or pseudo-conidia as used at present.

Organisms of this type also resemble Monilia fusca, a black-pigmented fungus described by Berkhout (1923) as possessing mycelial and yeast forms. She observed blastospore formation on the mycelium, multipolar budding on large vegetative cells, liquefaction of gelatin, and the inability to

ferment sugars. Berkhout proposed a change of the name Monilia fusca to Pullularia pullulans var. fusca because of absence of conidiophores.

Although the published descriptions of black yeasts are not sufficiently complete to identify definitely the strains in the present study, there is no doubt that relationships do exist between Type 5 and previously described forms, especially the organisms described by Berkhout.

Description of Type 6

Five strains, B14, B114, B121, B140, and B191, were encountered in this type.

Morphology

Form and size: oval, pointed at one pole; 1.6 to 2.5 x 2.4 to 4.0 microns

Arrangement: singly, single bud attached

Staining reaction: gram negative

Spores: none

Slide cultures: oval blastospores (scarce), singly on branching septate true mycelium

Cultural characteristics

Potato agar slant: filiform; convex, smooth, glistening, dirty greyish, changing to very dark olive green, viscid and ropy growth. On prolonged incubation at room temperature, 2 strains developed sooty green aerial hyphae.

Gelatin stab

Growth in line of puncture: arborescent to plumose

Liquefaction: none

Malt extract broth

24 hours: no surface growth; slight flocculent sediment

10 days: no surface growth; viscid sediment

Biochemical features

Carbohydrate fermentation: no gas production from any sugars

Carbohydrate utilization: glucose, sucrose, and maltose utilized

Nitrogen utilization: only peptone utilized

Hydrolysis of fat: negative

Proteolysis: negative

Ethyl alcohol utilization: negative

Litmus milk: partial reduction; blackish green ring formed in one week; black wax-like pellicle formed in 3 weeks completely sealing the surface

Growth conditions

Oxygen relationship: aerobic

Growth temperature: optimum 30° to 37° C.; growth at 10° C. but not at 45° C.

Heat resistance: no survival at 61.7° C. for 5 minutes

Action in cream. Strain B121, with or without the addition of S. lactis, did not develop any defect in 7 days at 21° C.

Action in butter. Strain B121 did not develop any defect in 7 days at 21° C. or in one month at 4° C.

Identity of Type 6. The description of the organisms in this type agrees rather closely with the Dematium nigrum which also developed both mycelial and yeast forms. Berkhout, (1923) observed glistening black growth, inability to liquefy gelatin or ferment sugars, and cell dimensions of

2.5 by 5.0 microns, characteristics which are common to the organism she studied and to the organisms in Type 6. Since the conidia were not borne on conidiophores, she suggested the genus name Dematium be changed to Pullularia. It seems that the blastospores formed on the mycelium with organisms of Type 6 corresponds to the conidia she observed. Organisms of Type 6 were the only ones in this study which were gram negative but Berkhout did not mention staining reaction of her organism. A correlation in this respect would be additional evidence of relationship of the two types. The reported characteristics in which the organisms agree are too few to identify the organisms definitely, but they do suggest some relationship, especially since no significant differences between these organisms are apparent.

Description of Type 7

Eighteen strains, B5, B10, B15, C43, B83, B99, B107, B136, B138, B399, B147, B151, B155, B270, B335, B392, B404, and B425, were encountered in this type.

Morphology

Form and size

Potato agar slant: oval to sausage-shaped; 1.7 to 2.3 x 2.5 to 7.0 microns

Wort agar slant: oval; 1.7 to 2.3 x 2.5 to 4.0 microns

Arrangement: singly; single bud attached

Staining reaction: gram positive

Spores: none

Slide cultures: no mycelium observed

Cultural characteristics

Potato agar slant: filiform, convex, smooth, glistening, and creamy

Potato agar colony: circular, entire, convex, smooth, glistening, and whitish; 1.5 to 2 mm. in diameter

Gelatin stab

Growth in line of puncture: papillate

Liquefaction: none

Malt extract broth

24 hours: no surface growth; slightly viscid sediment

10 days: thin veil; slightly viscid sediment

Biochemical features

Carbohydrate fermentation: gas production from glucose

Nitrogen utilization: only peptone utilized

Hydrolysis of fat: negative

Proteolysis: negative

Ethyl alcohol utilization: negative

Litmus milk: growth without change

Growth conditions

Oxygen relationship: aerobic

Growth temperature: optimum 21° to 45° C.; growth at 10° C.

Heat resistance: one strain survived 61.7° C. for 5 and 10 but not 30 minutes; 2 strains survived 61.7° C. for 5 but not 10 minutes; only 15 strains did not survive 61.7° C. for 5 minutes

Action in cream. Strain B335 did not develop any defect in 7 days at 21° C. In the presence of S. lactis, slightly yeasty and slightly bitter flavors were produced in 7 days.

Action in butter. Strain B335 did not develop any defect in 7 days at 21° C. or in one month at 4° C.

Identity of Type 7. Organisms of this type conform to Torulopsis Molischiana (Zikes) Lodder (1934) in all important characteristics. The main difference lies in the degree of sliminess on wort agar slant. She described slimy growth while the cultures isolated were slightly slimy to butyrous. Other than this, even the high optimum growth temperature related these organisms to T. Molischiana. Type 7 may be regarded as T. Molischiana.

Description of Type 8

Two strains, C374 and C473, were encountered in this type.

Morphology

Form and size

Potato agar slant: oval to round; 2.0 to 3.2 x 2.5 to 3.5 microns

Wort agar slant: oval and round; oval - 2.0 to 2.8 x 2.8 to 3.5 microns, round - 2.5 to 4.0 microns

Arrangement: singly, single bud attached

Staining reaction: gram positive

Spores: none

Slide cultures: no mycelium observed

Cultural characteristics

Potato agar slant: filiform, convex, smooth, glistening, and creamy

Potato agar colony: circular, entire, convex, smooth, glistening, and cream-colored; 1 to 1½ mm. in diameter

Gelatin stab

Growth in line of puncture: beaded

Liquefaction: none

Malt extract broth

24 hours: no surface growth; slightly viscid sediment

10 days: no surface growth; slightly viscid to slightly flocculent sediment

Biochemical features

Carbohydrate fermentation: gas production from glucose, galactose, and sucrose

Nitrogen utilization: peptone, asparagine, ammonium sulphate, and urea utilized

Hydrolysis of fat: negative

Proteolysis: negative

Ethyl alcohol utilization: negative

Litmus milk: growth without change

Growth conditions

Oxygen relationship: aerobic

Growth temperature: optimum 21° to 30° C.; growth at 10° and 37° C. but not at 45° C.

Heat resistance: no survival at 61.7° C. for 5 minutes

Identity of Type 8. Organisms of this type conform in nearly all details to Torulopsis Holmii (Jorgensen) as described by Lodder (1934). She mentioned slightly larger dimensions of the cells and observed ring formation on wort and growth in ethyl alcohol. These differences in characteristics are not considered sufficient justification for creation of a new species. Type 8 may be considered as Torulopsis Holmii or as a variety of this species.

Description of Type 9

One strain, C432, was encountered in this type.

Morphology

Form and size

Potato agar slant: oval; 2.3 to 3.5 x 3.0 to 5.0 microns

Wort agar slant: oval; 2.2 to 4.5 x 3.5 to 5.0 microns

Arrangement: singly, single bud attached

Staining reaction: gram positive

Spores: none

Slide cultures: no mycelium observed

Cultural characteristics

Potato agar slant: filiform, convex, smooth, glistening, and white

Potato agar colony: circular, entire, convex, smooth, glistening, and white; 1 to 1½ mm. in diameter

Gelatin stab

Growth in line of puncture: beaded

Liquefaction: none

Malt extract broth

24 hours: no surface growth; slight sediment

10 days: no surface growth; slightly viscid to compact sediment

Biochemical features

Carbohydrate fermentation: gas production from glucose, galactose, sucrose, and maltose

Nitrogen utilization: peptone, asparagine, ammonium sulphate, and urea utilized

Hydrolysis of fat: negative

Proteolysis: negative

Ethyl alcohol utilization: negative

Litmus milk: slight reduction

Growth conditions

Oxygen relationship: aerobic

Growth temperature: optimum 21° to 37° C.; growth at 10° C. but not at 45° C.

Heat resistance: no survival at 61.7° C. for 5 minutes

Action in cream. Strain C432 developed a very slight bitter flavor in 7 days at 21° C. In the presence of S. lactis, it produced a yeasty flavor in 4 days and yeasty and slightly bitter flavors in 7 days.

Action in butter. Strain C432 required 7 days at 21° C. to develop a slightly acid flavor. It caused astringency in one month at 4° C.

Identity of Type 9. Organisms of this type resemble in most respects to Torulopsis colliculosa (Hartmann) Saccardo as described by Lodder (1934). T. colliculosa utilizes ethyl alcohol, forms a ring on wort, and does not

form gas from galactose. These differences in characteristics are not considered adequate for creation of a new species. Consequently, Type 9 may be considered either as T. colliculosa or as a variety of that species.

Description of Type 10

Ten strains, C25, C32, B345, B409, B410, C437, C453, C465, C475, and C481, were encountered in this type.

Morphology

Form and size

Potato agar slant: oval; 1.7 to 3.0 x 2.8 to 5.0 microns

Wort agar slant: oval to slightly ellipsoidal; 2.0 to 3.0 x 3.0 to 5.0 microns

Arrangement: singly, single bud attached

Staining reaction: gram positive

Spores: none

Slide cultures: no mycelium observed (Figure 5)

Cultural characteristics

Potato agar slant: filiform, pulvinate, smooth, very glistening, dirty cream-colored at start but turning brownish in 2 weeks, fairly transulcent, slightly ropy and very slimy

Potato agar colony: circular, entire, pulvinate, smooth, very glistening, and creamy; 1½ to 4 mm. in diameter

Gelatin stab

Growth in line of puncture: papillate

Liquefaction: a slight napiform liquefaction in the first week followed by drying of the surface without further liquefaction

Malt extract broth

24 hours: no surface growth; slight sediment

10 days: thin film; viscid sediment

Biochemical features

Carbohydrate fermentation: no gas production in any sugars

Carbohydrate utilization: all sugars utilized

Nitrogen utilization: peptone, asparagine, ammonium sulphate, and urea utilized; potassium nitrate utilized weakly by one strain

Hydrolysis of fat: negative

Proteolysis: negative

Ethyl alcohol utilization: negative

Litmus milk: reduction; coagulation in about 2 weeks followed by partial or complete digestion; one strain did not coagulate but became alkaline in 6 weeks

Growth conditions

Oxygen relationship: aerobic

Growth temperature: optimum 21° C. to 30° C.; growth at 10° C. but not at 37° C.

Heat resistance: no survival at 61.7° C. for 5 minutes

Action in cream. Strains B345 and C475 required 7 days at 21° C. to develop a very slightly bitter flavor. In the presence of S. lactis, they produced moderately bitter and slightly yeasty flavors

Action in butter. Neither strain B345 nor C475 developed any defect in 7 days at 21° C. but both caused a slightly acid flavor in one month at 4° C.

Identity of Type 10. Organisms of this type conform in nearly all details to Torulopsis Laurentii (Kufferath) Lodder (1934). She described very weak utilization of potassium nitrate; the auxanograph by the cultures studied was negative with 9 strains and weakly positive with one strain. In view of such close similarities of characteristics, Type 10 is regarded as Torulopsis Laurentii.

Description of Type 11

Nine strains, C61, B135, C225, C232, C313, B338, C446, C455, and B493 were encountered in this type.

Morphology

Form and size

Potato agar slant: oval to slightly ellipsoidal; 2.5 to 4.0 x 3.5 to 6.0 microns

Wort agar slant: oval; 2.2 to 4.0 x 3.5 to 7.0 microns

Arrangement: singly, single bud attached

Staining reaction: gram positive

Spores: none

Slide cultures: no mycelium observed

Cultural characteristics

Potato agar slant: filiform, convex, smooth, glistening, and cream-colored turning to a very light pink in about 10 days; 3 strains showed very slight rugosity and slightly glistening lustre

Potato agar colony: circular, entire, convex, smooth, glistening, and greyish white; 1½ to 2 mm. in diameter

Gelatin stab

Growth in line of puncture: papillate

Liquefaction: none

Malt extract broth

24 hours: no surface growth; slightly viscid sediment

10 days: ring; slightly viscid sediment

Biochemical features

Carbohydrate fermentation: no gas production in any sugars

Carbohydrate utilization: glucose, sucrose, and maltose utilized

Nitrogen utilization: all nitrogen sources utilized

Hydrolysis of fat: positive except with one strain

Proteolysis: negative

Ethyl alcohol utilization: negative

Litmus milk: reduction followed by coagulation with slight digestion; some strains only caused reduction

Growth conditions

Oxygen relationship: aerobic

Growth temperature: optimum 21° to 30° C.; growth at 10° C. but not at 37° C.

Heat resistance: no survival at 61.7° C. for 5 minutes

Action in cream. Five strains, B135, C225, C232, B338, and B493, were studied. All, except B338, were lipolytic. These cultures did not develop any defect in 2 days, but developed a slightly unclean flavor in 4 days and slightly yeasty, slightly unclean and bitter flavors in 7 days

at 21° C. In the presence of S. lactis, the development of defects was similar but slightly enhanced.

Action in butter. Three strains, C225, B338, and B493, did not develop any defects in 7 days at 21° C. nor in one month at 4° C. while 2 strains, B135, and C232, did not develop any defects in 2 or 4 days but developed a slightly unclean flavor in 7 days at 21° C. One strain, B135, caused a slightly unclean flavor in one month at 4° C.; the others were negative under these conditions.

Identity of Type 11. Organisms of this type closely conform to Torulopsis rotundata (Redaelli) as described by Lodder (1934) in all reported characteristics. Type 11 developed a very light pink pigmentation on the agar slant whereas Lodder recorded a shade of red pigmentation.

Description of Type 12

One strain, B485, was encountered in this type.

Morphology

Form and size

Potato agar slant: oval to slightly ellipsoidal; 2.3 to 3.5 x 3.4 to 5.5 microns

Wort agar slant: oval; 1.8 to 3.0 x 3.5 to 6.5 microns

Arrangement: singly, single bud attached

Staining reaction: gram positive

Spores: none

Slide cultures: no mycelium observed

Cultural characteristics

Potato agar slant: filiform, convex, finely verrucose, slightly glistening, and light tan; after a number of transfers, the growth was smooth, glistening, and light pink in color

Potato agar colony: circular, entire, convex, smooth, glistening, and greyish white; 1½ to 2 mm. in diameter

Gelatin stab

Growth in line of puncture: papillate

Liquefaction: none

Malt extract broth

24 hours: no surface; slightly viscid sediment

10 days: ring; slightly viscid sediment

Biochemical features

Carbohydrate fermentation: no gas production from any sugars

Carbohydrate utilization: auxanogram very unsatisfactory, possibly due to slow development even at optimum temperature

Nitrogen utilization: only peptone utilized

Hydrolysis of fat: negative

Proteolysis: negative

Ethyly alcohol utilization: negative

Litmus milk: slight reduction only

Growth conditions

Oxygen relationship: aerobic

Growth temperature: optimum at 21° C. but requiring a relatively long period, about one week, to establish a reasonably heavy streak on agar slant; growth at 10° C. but not at 30° C.

Heat resistance: no survival at 61.7° C. for 5 minutes

Identity of Type 12. Organisms of this type conform to Torulopsis rotundata in form and size of cells. The light pinkish color on wort agar slant adds to the resemblance. Lodder (1934) stated that the auxanogram by T. rotundata with all nitrogen sources, with the possible exception of potassium nitrate, was positive; organisms of Type 12 utilized only peptone. The auxanogram by organism of Type 12 was very unsatisfactory due to the slowness of development. Of all the types studied, this is the only one which did not establish growth on agar slants at 30° C. This strain was isolated from colonies which developed on an agar plate after being kept in a cooler for about 2 months at 4° C. In a recent transfer of this strain, the growth seemed to develop more rapidly. This organism may be a form of T. rotundata which grows only at comparatively low temperatures.

Description of Type 13

Twenty-one strains, B12, B13, B16, C33, C49, C90, B148, B164, B250, B253, C283, C301, C330, B337, B381, B383, B384, B411, B420, C435, and C468, were encountered in this type.

Morphology

Form and size

Potato agar slant: round; 2.0 to 3.5 microns

Wort agar slant: oval to round; 1.8 to 2.8 x 2.0 to 3.0 microns

Arrangement: singly, single bud attached

Staining reaction: gram positive

Spores: none

Slide cultures: no mycelium observed

Cultural characteristics

Potato agar slant: filiform, convex, smooth, glistening, and white

Potato agar colony: circular, entire, pulvinate, smooth, glistening, and white; up to 1 mm. in diameter

Gelatin stab

Growth in line of puncture: papillate

Liquefaction: none

Malt extract broth

24 hours: no surface growth; slightly viscid sediment

10 days: no surface growth; slightly viscid compact sediment

Biochemical features

Carbohydrate fermentation: no gas production from any sugars

Carbohydrate utilization: glucose, galactose, sucrose, and maltose utilized

Nitrogen utilization: peptone, asparagine, and ammonium sulphate utilized; urea utilized in presence of accessory growth factors

Hydrolysis of fat; negative

Proteolysis: negative

Ethyl alcohol utilization: negative

Litmus milk: growth with either no change or very slight reduction

Growth conditions

Oxygen relationship: aerobic

Growth temperature: optimum 21° to 30° C.; growth at 10° C. but not at 37° C.

Heat resistance: no survival at 61.7° C. for 5 minutes

Action in cream. Two strains, B384 and C468, were studied. In 7 days at 21° C., B384 developed a very slightly bitter flavor while C468 caused yeasty and cheesy flavors. In the presence of S. lactis, slight rancidity and slight bitterness were produced by B384 while yeasty and cheesy flavors were produced by C468.

Action in butter. Strain B384 developed an astringency in 7 days at 21° C. or in one month at 4° C. Strain C468 required 7 days at 21° C. to develop slightly acid and unclean flavors. It caused a slightly acid flavor in one month at 4° C.

Identity of Type 13. Organisms of this type have many characteristics of Torulopsis candida (Saito) as described by Lodder (1934). Only a few differences in characteristics exist. She stated that the cell dimensions were between 3 to 5 microns; the dimensions of the cultures studied were slightly smaller. She noted utilization of alcohol; all the cultures studied were negative in this respect. Her auxanogram with all sugars was positive; the cultures in this study did not utilize lactose. However, she cited Saito who found that lactose was very weakly utilized. The organisms may be considered as a variety of T. candida. This type also resemble Type H of the yeasts isolated by Cordes (1920). However, his description is not sufficiently detailed to permit establishment of definite identity.

Description of Type 14

Two strains, B503 and B504, were encountered in this type.

Morphology

Form and size

Potato agar slant: oval to round; 5 to 10 X 6 to 14 microns

Wort agar slant: oval to round; 4 to 10 X 6 to 14 microns

Arrangement: singly, single bud attached

Staining reaction: gram positive

Spores: none

Slide cultures: no mycelium observed

Cultural characteristics

Potato agar slant: filiform, raised, smooth, slightly glistening, and white, turning light tan with age

Potato agar colony: circular, entire, convex, smooth, slightly glistening, and whitish; 1½ to 3 mm. in diameter

Gelatin stab

Growth in line of puncture: papillate

Liquefaction: none

Malt extract broth

24 hours: no surface growth; slightly viscid sediment

10 days: thin film; slightly viscid to slightly flocculent sediment

Biochemical features

Carbohydrate fermentation: no gas production from any sugars

Carbohydrate utilization: glucose utilized

Nitrogen utilization: only peptone utilized

Hydrolysis of fat: negative

Proteolysis: negative

Ethyl alcohol utilization: negative

Litmus milk: reduction only

Growth temperature

Oxygen relationship: aerobic

Growth temperature: optimum at 30° C.; growth at 21° and 37° C. but not at 10° C. or 45° C.

Heat resistance: no survival at 61.7° C. for 5 minutes

Action in cream. Strain B503 did not develop any defect in 7 days at 21° C. In the presence of S. lactis, a yeasty flavor was produced in 7 days.

Action in butter. Strain B503 developed astringency in 7 days at 21° C. It did not develop any defect in one month at 4° C.

Identity of Type 14. The characteristics of this type showed that it belongs to the genus Torulopsis as proposed by Lodder (1934). However, these organisms could not be identified with any of the yeast species described. The dimension of the cells, 5 to 10 microns in breadth by 6 to 14 microns in length, definitely set these organisms apart. It is suggested that this morphological difference entitles Type 14 to a new specific designation.

Description of Type 15

Seventeen strains, C20, C32, C66, B82, B98, B115, B170, B181, B185, B200, B206, C238, B254, B274, B329, B354, and B430, were encountered in this type.

Morphology

Form and size

Potato agar slant: oblong to cylindrical; 1.8 to 2.5 x 2.8 to 4.5 microns

Wort agar slant: oval; 1.4 to 1.8 x 3.0 to 5.0 microns

Arrangement: singly, single bud attached

Staining reaction: gram positive

Spores: none

Slide cultures: some short chains of cells observed (Figure 6)

Cultural characteristics

Potato agar slant: filiform, convex, smooth, glistening, and cream-colored

Potato agar colony: circular, entire, convex, smooth, glistening, and whitish; 1 to 1½ mm. in diameter

Gelatin stab

Growth in line of puncture: beaded to papillate

Liquefaction: none

Malt extract broth

24 hours: no surface growth; slight sediment

10 days: no surface growth; slightly viscid to slightly compact sediment

Biochemical features

Carbohydrate fermentation: no gas production from any sugars

Carbohydrate utilization: glucose utilized

Nitrogen utilization: only peptone utilized by 4 strains; peptone, asparagine, ammonium sulphate, and urea utilized by the rest of the strains

Hydrolysis of fat: negative

Proteolysis: negative

Ethyl alcohol utilization: negative

Litmus milk: slight reduction followed by alkaline reaction in about 4 weeks

Growth conditions

Oxygen relationship: aerobic

Growth temperature: optimum 30° to 37° C.; growth at 21° C. but not at 10° or 45° C.

Heat resistance: no survival at 61.7° C. for 5 minutes

Identity of Type 15. Organisms of this type conform in many details to Torulopsis uvae (Pollacci and Nannizzi) as described by Lodder (1934). The forms are similar but she observed larger cell dimensions. Short chains of cells with slide culture procedure were obtained in both studies. She mentioned weak growth in alcohol; the organisms in the present study did not utilize alcohol. This type may be regarded as T. uvae.

Description of Type 16

Eleven strains, C21, C29, B161, B162, C277, C286, C289. C295, C307, C308, and C311, were encountered in this type.

Morphology

Form and size: oval, pleomorphic, oidia and mycelium; oval-2.5 to 3.5 x 4.0 to 7.0 microns

Arrangement: singly, single bud attached

Staining reaction: gram positive; some cells did not hold the stain very well

Spores: none

Slide cultures: oidia-like mycelium; no blastospores observed on mycelium. Figure 7 shows both budding cells and formation of mycelium; Figure 8 shows oidia-like structure.

Cultural characteristics

Potato agar slant: filiform with undulating edge, rugose to slightly verrucose, slightly glistening and cream-colored to light tan; 3 strains were filiform with very slightly rough growth

Potato agar colony: circular with lobate margin, raised, rugose, slightly glistening, and cream-colored; 3 to 4 mm. in diameter

Gelatin stab

Growth in line of puncture: arborescent

Liquefaction: most strains showed slight liquefaction in the first week followed by drying of the surface without further liquefaction; 3 strains liquified the medium completely.

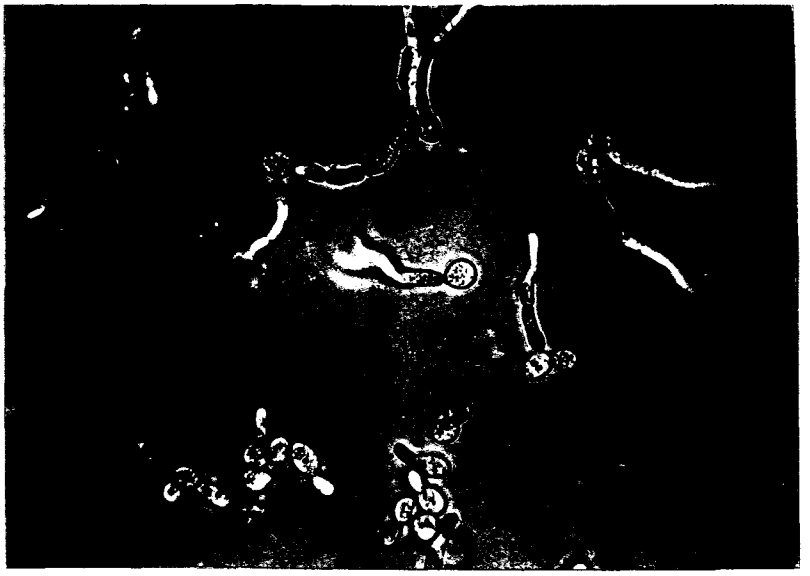

Fig. 7. Strain B162 of Type 16. Budding cells and formation of mycelia. 555x.

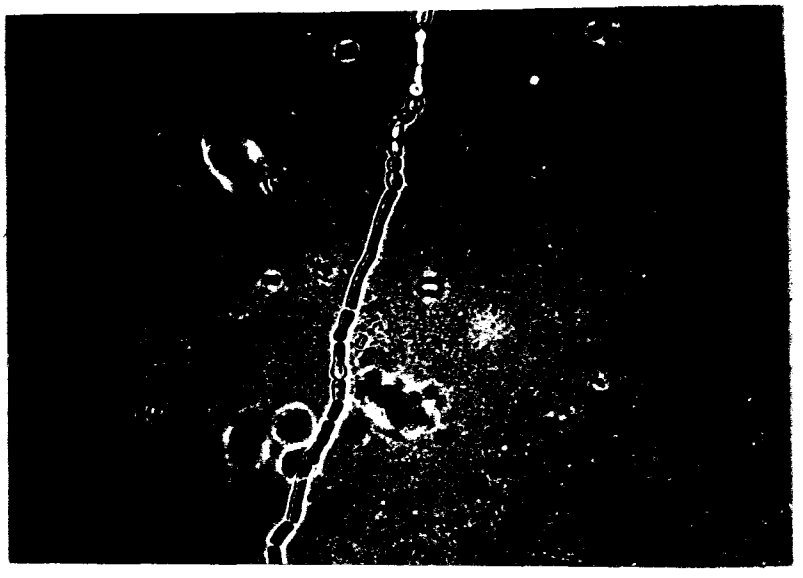

Fig. 8. Strain B162 of Type 16. Oidia-like mycelium. 555x.

Malt extract broth

24 hours: thin film; slightly granular sediment

10 days: ring; flaky to flocculent sediment

Biochemical features

Carbohydrate fermentation: no gas production from any sugars

Carbohydrate utilization: glucose, galactose, sucrose, maltose, and lactose utilized

Nitrogen utilization: peptone, asparagine, and ammonium sulphate utilized; urea utilized in presence of accessory growth factors

Hydrolysis of fat: positive

Proteolysis: negative

Ethyl alcohol utilization: negative

Litmus milk: total reduction in about 2 weeks followed by coagulation and partial or complete digestion

Growth conditions

Oxygen relationship: aerobic

Growth temperature: optimum 21° to 37° C.; growth at 10° C. but not at 45° C.

Heat resistance: one strain survived 61.7° C. for 5 and 10 but not 30 minutes; 2 strains survived 61.7° C. for 5 but not 10 minutes; 8 strains did not survive 61.7° C. for 5 minutes

Action in cream. Three strains, B161, C295, and C308, were studied. In 4 days at 21° C. only strain C308 developed a slightly unclean flavor whereas in 7 days all 3 strains developed bitter and slightly rancid

flavors. In the presence of S. lactis, the development of defect was similar in nature.

Action in butter. Strains B161, C295 and C308 required 7 days at 21° C. to develop very slight rancidity or slight oilness. In one month at 4° C., strain C308 developed unclean and acid flavors whereas the other 2 strains did not develop any defect.

Identity of Type 16. The characteristics of the organisms in this type place them in the genus Trichosporon as defined by Diddens and Lodder (1939). Since in their short paper these authors did not present any detailed descriptions, definite identity on species basis should await the appearance of the monograph of the subfamily Mycotoruloideae by them.

These organisms resemble in some respects the 6 groups of Trichosporon studied by Puntoni (1938) in physiological characteristics such as gelatin liquefaction, milk digestion and surface growth in liquid media. However, the crateriform growth exhibited by 5 groups and the smooth and shiny growth exhibited by the other group of Puntoni's cultures set them apart from organisms of Type 16 which developed raised, rugose and slightly glistening colony growth. Lastra (1939) isolated a new species Trichosporon proteolyticum which also had some characteristics in common with the cultures isolated in this study. The inability of T. proteolyticum to hydrolyze animal or vegetable fat and failure to grow on the surface of potato agar remove the possibility of close relationship between it and the cultures studied. Type 16 does not appear to belong to any recognized species and further characterization is needed before establishment of a new species.

Description of Type 17

Nineteen strains, C59, B153, B159, B160, C244, C269, B319, B350, B353, B357, B388, B401, B406, B408, B413, B416, B423, B486, and B487, were encountered in this type.

Morphology

Form and size: oval and sausage-shaped; oval - 1.4 to 2.4 x 3.0 to 5.0 microns, sausage-shaped - 2.0 to 8.0 x 10.0 or more microns

Arrangement: singly, single bud attached

Staining reaction: gram positive

Spores: none

Slide cultures: oblong to cylindrical blastospores, singly, in pairs, or in small clusters at the nodes of pseudomycelium

Cultural characteristics

Potato agar slant: spreading, raised, smooth, slightly glistening, and cream-colored; dull lustre on some strains; 2 strains exhibited slightly verrucose surface

Potato agar colony: circular, entire, pulvinate, smooth, glistening, and cream-colored; 1 to 2 mm. in diameter

Gelatin stab

Growth in line of puncture: arborescent

Liquefaction: none

Malt extract broth

24 hours: dry, matte, wrinkled or corrugated membrane; slightly viscid sediment

10 days: dry, matte, wrinkled or corrugated membrane

extending up the side of the tubes to a distance of 5 to 10 mm. above the surface of the liquid; flocculent sediment

Biochemical features

Carbbhydrate fermentation: gas production from glucose

Carbohydrate utilization: glucose utilized

Nitrogen utilization: peptone, asparagine, ammonium sulphate, and urea utilized

Hydrolysis of fat: negative

Proteolysis: negative

Ethyl alcohol utilization: positive; also forming a membrane on surface

Litmus milk: no change

Growth conditions

Oxygen relationship: aerobic

Growth temperature: optimum 21° to 45° C.; growth at 10° C.

Heat resistance: no survival at 61.7° C. for 5 minutes

Action in cream. Strain B401 did not develop any defect in 7 days at 21° C., either alone or in the presence of S. lactis.

Action in butter. Strain B401 did not develop any defect in 7 days at 21° C. The butter was criticized for a slightly acid flavor in one month at 4° C.

Identity of Type 17. Organisms of this type closely resemble Candida krusei (Castellani). Yeast-like fungi, identified as Candida krusei, have been isolated from human sources by Martin, et al (1937), grapes by Mrak and McClung (1940), wine by MacKinnon and Artagaveytia-

Allende (1945), and a number of other sources. Cordes' (1920) description of Type C Mycoderma from dairy sources corresponds remarkably well with all the strains studied. The production of a dull wrinkled film extending up the wall of the test tube and other characteristics were identical. Baker (1923) isolated 66 yeast forms from cream and stable air and considered them as Mycoderma monosa. His description indicates close relationships between his organisms and those of this study. However, he found no growth in whey agar slant at 45° C., whereas Type 17 produced excellent growth on wort agar slant at that temperature. Both Cordes and Baker described the "radial thread" type of sub-surface growth.

The auxanographic method shows only assimilation of glucose, fructose, and mannose. MacKinnon and Artagaveytia-Allende (1945) relied on Laurent liquid medium to demonstrate assimilation of glucose, maltose, and galactose. They mention slow fermentation of glucose, sometimes after 10 days; Type 17 fermented glucose rapidly. They stated that optimum growth temperature is between 30° to 37° C.; Type 17 grew equally well from 21° to 45° C. on wort agar. In spite of slight variations in their characteristics, Type 17 may be regarded tentatively as Candida krusei.

Description of Type 18

Sixteen strains, B76, B96, B119, B154, B178, B183, B186, B188, B197, B207, C221, B264, B324, B358, B407, and B499, were encountered in this type.

Morphology

Form and size: oval; 1.3 to 2.3 x 2.5 to 5.0 microns

Arrangement: singly, single bud attached

Staining reaction: gram positive

Spores: none

Slide cultures: oval and oblong blastospores in short chains or small clusters at the nodes and irregularly along pseudomycelium. Figure 9 illustrates oblong blastospores along the pseudomycelium; Figure 10 shows tree-like structure of oval and oblong blastospores on pseudomycelium.

Cultural characteristics

Potato agar slant: filiform, convex, smooth, somewhat glistening and cream-colored

Potato agar colony: circular, entire, convex, smooth, glistening and cream-colored; 1 to 2 mm. in diameter

Gelatin stab

Growth in line of puncture: beaded

Liquefaction: none

Malt extract broth

24 hours: no surface growth; slightly viscid sediment

10 days: no surface growth; slightly viscid sediment

Biochemical features

Carbohydrate fermentation: gas production from glucose

Carbohydrate utilization: glucose utilized

Nitrogen utilization: only peptone utilized

Hydrolysis of fat: negative

Proteolysis: negative

Ethyl alcohol utilization: negative

Litmus milk: growth without change

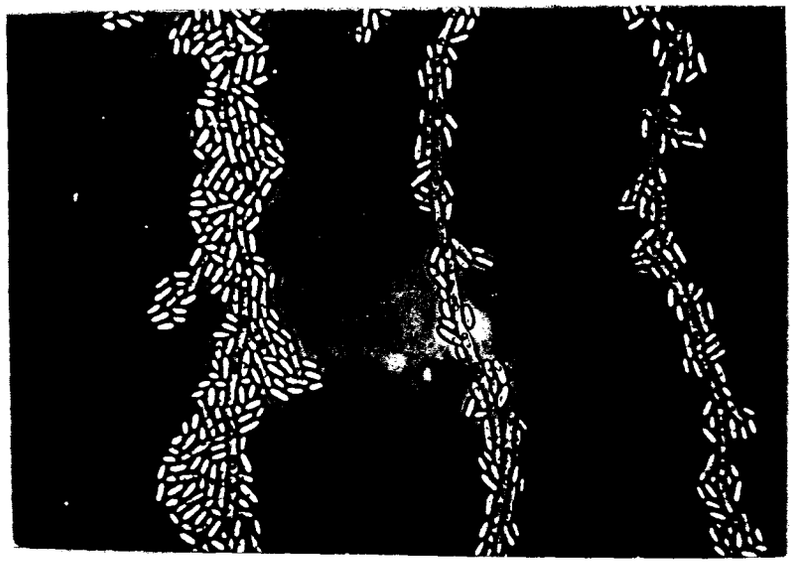

Fig. 9. Strain B76 of Type 18. Oblong blastospores on pseudomycelium. 555x.

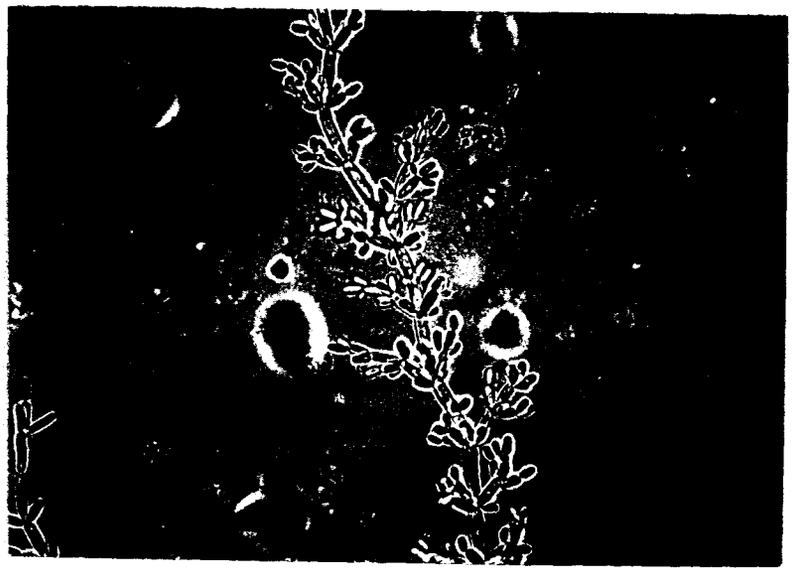

Fig. 10. Strain B119 of Type 18. Treelike structure on pseudomycelium. 555x.

Growth conditions

Oxygen relationship: aerobic

Growth temperature: optimum 21° to 45° C.; growth at 10° C.

Heat resistance: 2 strains survived 61.7° C. for 5 and 10 but not 30 minutes; 2 strains survived 61.7° C. for 5 but not 10 minutes; 12 strains did not survive 61.7° C. for 5 minutes

Action in cream. Strains B76 and B96 developed slightly unclean flavor in 4 days and yeasty and slightly rancid flavors in 7 days at 21° C. In the presence of S. lactis, similar defects were produced by strain B76, while a slightly cheesy flavor was produced by strain B96.

Action in butter. Strains B76 and B96 required 7 days at 21° C. to develop a slightly ester-like flavor. No defect developed in one month at 4° C.

Identity of Type 18. Organisms of this type evidently belong to the krusei group of Langeron and Guerra (1938) because of production of gas from glucose, fructose, and mannose. Nevertheless, their ability to assimilate only peptone sets them apart from any of the three species, Candida krusei, C. parakrusei, and C. aldoi, which are included in this group. Organisms of Type 18 differ further from C. krusei in their inability to utilize alcohol and from Type 19, which is considered as C. parakrusei, in respect to their development at 45° C. Conant (1940) and MacKinnon and Artagaveytia-Allende (1945) considered C. aldoi as synonymous with C. albicans, thus removing it from the krusei group. In view of the number of important differentiating characteristics, it is suggested that these organisms may properly be considered a new species.

However, a specific designation for this type should await further study and comparison.

Description of Type 19

Fifty-two strains, B3, B6, C35, C60, B75, B85, B87, B91, B92, B97, B105, B106, B113, B120, B122, B123, B126, B133, B149, B150, B157, B174, B182, B194, B208, C223, C226, C233, B243, B245, B256, B257, B263, B336, B342, B344, B348, B355, B356, B387, B389, B395, B397, B402, B421, C451, C482, B488, B489, B497, B501, and B502, were encountered in this type.

Morphology

Form and size: oval and a few sausage-shaped; oval - 2.0 to 2.5 x 3.0 to 4.5 microns, sausage-shaped - 1.8 to 2.0 x 5.0 to 7.0 microns

Arrangement: singly, single bud attached

Staining reaction: gram positive

Spores: none

Slide cultures: oval, oblong, or cylindrical blastospores, singly, in pairs, or small bunches at nodes of slightly branching pseudomycelium. Figure 11 shows oval blastospores on pseudomycelium; Figure 12 shows cylindrical blastospores on pseudomycelium; and Figure 13 illustrates both oval and cylindrical blastospores on pseudomycelium.

Cultural characteristics

Potato agar slant: filiform, convex, smooth, glistening , and white

Potato agar colony: circular, entire, convex to pulvinate, smooth, glistening, and cream-colored; 1 to 2 mm. in diameter

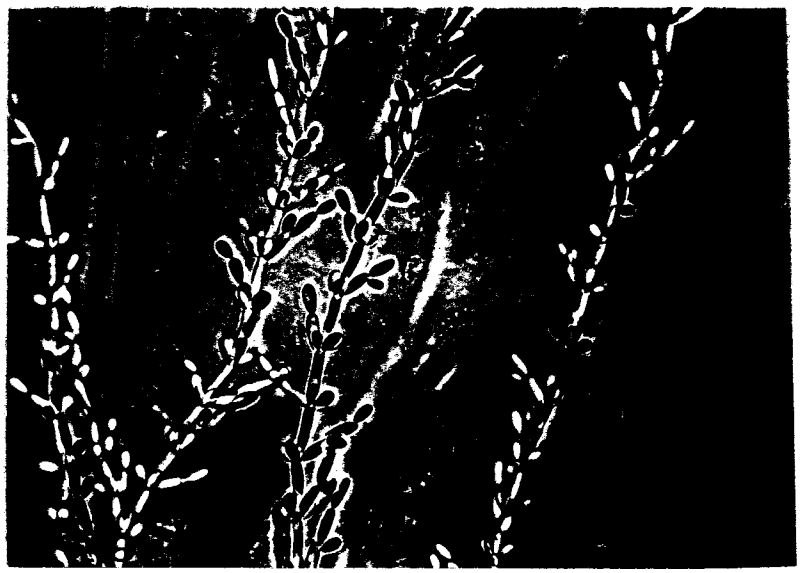

Fig. 11. Strain C451 of Type 19. Oval blastospores on pseudomycelium. 562x.

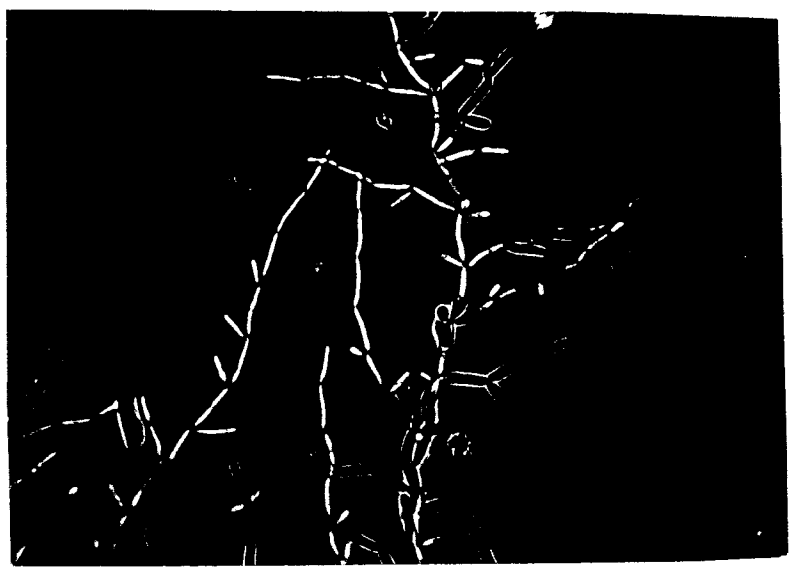

Fig. 12. Strain B208 of Type 19. Cylindrical blastospores on pseudomycelium. 555x.

Fig. 13. Strain B421 of Type 19. Oval blastospores on pseudomycelium. 562x.

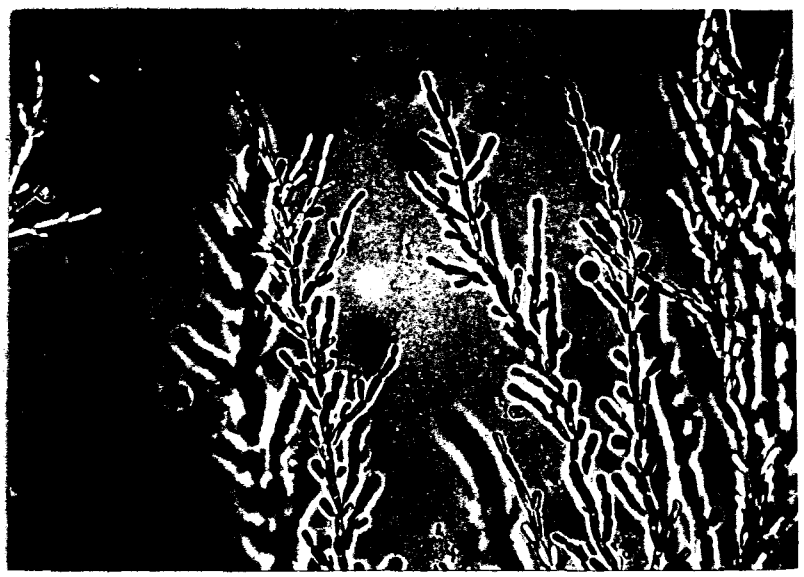

Fig. 14. Strain B141 of Type 20. Treelike structures. 555x.

Gelatin stab

Growth in line of puncture: arborescent

Liquefaction: none

Malt extract broth

24 hours: no surface growth; slightly viscid sediment

10 days: ring; slightly viscid to flocculent sediment

Biochemical features

Carbohydrate fermentation: gas production from glucose by all strains and from galactose by 25 strains

Carbohydrate utilization: glucose, galactose, sucrose, and maltose utilized

Nitrogen utilization: peptone, asparagine, and ammonium sulphate utilized; urea utilized in presence of accessory growth factor

Hydrolysis of fat: negative

Proteolysis: negative

Ethyl alcohol utilization: none

Litmus milk: slight to total reduction; most strains caused alkaline reaction in 2 months

Growth conditions

Oxygen relationship: aerobic

Growth temperature: optimum 21° to 37° C.; growth at 10° C. but not at 45° C.

Heat resistance: no survival at 61.7° C. for 5 minutes

Action in cream. Seven strains, B3, B92, B122, B126, B245, B355, and B502, were studied. At 21° C., all strains developed slightly yeasty,

slightly unclean, cheesy, and/or putrid flavors in 2 to 4 days, and moderate or definite rancid, cheesy, yeasty, unclean and/or bitter flavors in 7 days. In the presence of S. lactis, the defects produced were much the same.

Action in butter. In 7 days at 21° C. strains B3 and B245 deveoped a slightly acid flavor; B355 and B502, an acid flavor; B126, a very slightly unclean flavor; B92, a slightly unclean flavor; and B122, a very slightly rancid flavor. In one month at 4° C., an acid flavor was produced by strains, B245 and B355 but no defect was caused by any of the other strains.

Identity of Type 19. Organisms of this type have the characteristics of Candida parakrusei (Castellani) as described by Langeron and Guerra (1938). These investigators obtained negative results with urea by the auxanogram technic; the cultures isolated in the present study also did not utilize urea under usual experimental conditions but in the presence of accessory growth factor, the assimilation was positive. MacKinnon and Artagaveytia-Allende (1945) noted that by using reclaimed agar the auxanogram with urea was positive with Candida parakrusei whereas with new "Bacto" agar it was negative. Gas was produced from galactose by nearly half of the cultures in the present investigation; previous workers did not consider this characteristic of diagnostic value.

Description of Type 20

One strain, B141, was encountered in this type.

Morphology

Form and size: oval and sausage-shaped; oval - 1.5 to 3.0 x 3.0 to 5.0 microns, sausage-shaped - 2.0 x 4.0 to 6.0 microns

Arrangement: singly, single bud attached

Staining reaction: gram positive

Spores: none

Slide cultures: oblong blastospores in short chains on pseudomycelium presenting a treelike structure (Figure 14)

Cultural characteristics

Potato agar slant: filiform, convex, smooth, glistening, and whitish

Potato agar colony: circular with myceloid edge, convex, smooth, glistening, dirty white; 1½ to 2 mm. in diameter

Gelatin stab

Growth in line of puncture: arborescent

Liquefaction: none

Malt extract broth

24 hours: no surface growth; slightly flocculent sediment

10 days: no surface growth; viscid sediment

Biochemical features

Carbohydrate fermentation: gas production from glucose and sucrose

Carbohydrate utilization: glucose, sucrose, maltose, and raffinose utilized

Nitrogen utilization: all nitrogen sources utilized

Hydrolysis of fat: negative

Proteolysis: negative

Ethyl alcohol utilization: positive

Litmus milk: reduction in about one month

Growth conditions

Oxygen relationship: aerobic

Growth temperature: optimum 21° to 37° C.; growth at 10° C. but not at 45° C.

Heat resistance: no survival at 61.7° C. for 5 minutes

Identity of Type 20. Apparently this organism belongs to the guilliermondi group of the genus Candida as defined by Langeron and Guerra (1938) because of production of gas from glucose and sucrose. Of the 3 species in the group, this type resembles in most respects Candida guilliermondi but differs from it in that galactose was not utilized, the auxanogram was positive with all nitrogen sources, and there was growth in alcohol. These differences in characteristics set Type 20 apart from previously described species and are of such magnitude that the organisms probably should not be considered analogous to species recognized at the present time.

Description of Type 21

Thirteen strains, C63, B158, B201, B204, C258, C276, B320, B334, B351, B417, B428, C459, and C471, were encountered in this type.

Morphology

Form and size: oval and sausage-shaped; oval - 1.7 to 2.5 x 2.5 to 4.0 microns, sausage-shaped - 1.8 to 2.2 x 5.0 to 8.0 microns

Arrangement: singly, single bud attached

Staining reaction: gram positive

Spores: none

Slide cultures: oval, oblong, or cylindrical blastospores, singly or small clusters at nodes of pseudomycelium. Figure 15 shows cylindrical blastospores on pseudomycelium; Figure 16 shows oval blastospores on pseudomycelium.

Cultural characteristics

Potato agar slant: filiform, convex, smooth, glistening, and white; 5 strains produced slightly glistening, verrucose growth

Potato agar colony: circular, entire, pulvinate, smooth, glistening, and white; 1 to 1½ mm. in diameter; those strains developing verrucose growth on slants formed coiled and extruded colony growth and were dull in lustre

Gelatin stab

Growth in line of puncture: arborescent

Liquefaction: none

Malt extract broth

24 hours: no surface growth; slightly viscid sediment by "smooth" strains, slightly granular sediment by "rough" strains

10 days: slight film and slightly viscid sediment by "smooth" strains, ring and slightly granular to slightly flocculent sediment by "rough" strains

Biochemical features

Carbohydrate fermentation: gas production from glucose and sucrose by all strains and from galactose also by 7 strains

Carbohydrate utilization: glucose, galactose, sucrose, and maltose utilized

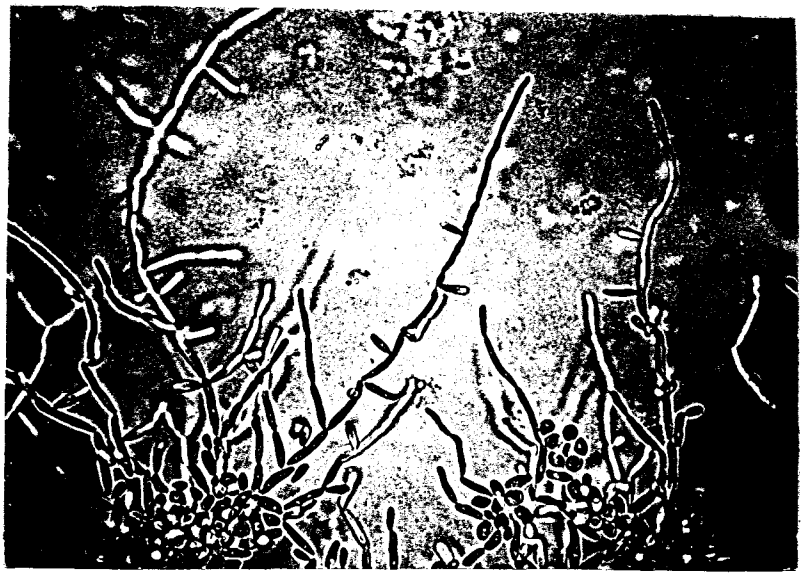

Fig. 15. Strain B204 of Type 21. Cylindrical blastospores on pseudomycelium. 555x.

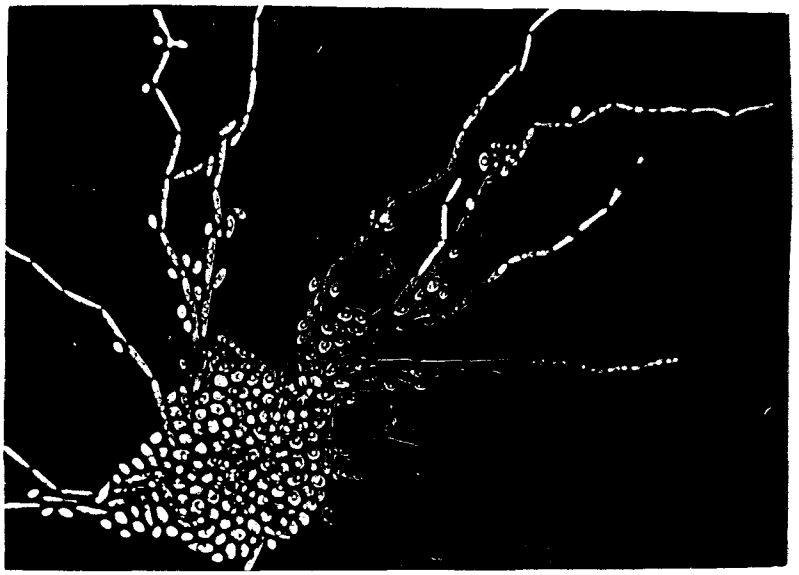

Fig. 16. Strain B351 of Type 21. Oval blastospores on pseudomycelium. 562x.

Nitrogen utilization: peptone, asparagine and ammonium sulphate utilized; urea utilized in presence of accessory growth factors

Hydrolysis of fat: negative

Proteolysis: negative

Ethyl alcohol utilization: negative

Litmus milk: slight reduction; some strains developed a slightly alkaline reaction

Growth conditions

Oxygen relationship: aerobic

Growth temperature: optimum 21° to 37° C.; growth at 10° C. but not at 45° C.

Heat resistance: no survival at 61.7° C. for 5 minutes

Action in cream. Strains B204, B320 and C459, were studied. Strains B204 and B320 developed slight bitterness in 4 days at 21° C. In 7 days, moderate rancidity was produced by B204 and moderate bitterness by B320. Strain C459 required 7 days to develop slightly yeasty and slightly rancid flavors. In the presence of S. lactis, all defects were slightly more pronounced.

Action in butter. Strain B204 required 7 days at 21° C. to develop a moderately unclean flavor. Strains B320 and C459 did not develop any defects in 7 days. All 3 strains did not develop any defects in one month at 4° C.

Identity of Type 21. Organisms of this type closely resemble Candida chalmersi (Castellani) Basgal as described by MacKinnon and Artagaveytia-Allende (1945). They found that the auxanogram with nitrogen sources was

positive for ammonium sulphate, peptone, and asparagine. In the present study urea was also utilized when in the presence of accessory growth factors. Mrak, Phaff, and Vaughn (1942) stated that strains identified as C. chalmersi utilized urea, whereas MacKinnon and Artagaveytia-Allende (1945) using these same strains were not able to confirm the results. This may be due to the materials used. Type 21 may be regarded as C. chalmersi.

Description of Type 22

One strain, B146, was encountered in this type.

Morphology

Form and size: oval and sausage-shaped; oval - 1.5 to 2.5 x 3.0 to 4.0 microns, sausage-shaped - 1.8 x 5.0 to 6.0 microns

Arrangement: singly, single bud attached

Staining reaction: gram positive

Spores: none

Slide cultures: oval blastospores in small clusters at nodes of pseudomycelium

Cultural characteristics

Potato agar slant: filiform, convex, smooth, glistening, and white

Potato agar colony: circular, entire, convex, smooth, glistening, and white; 2 to 3 mm. in diameter

Gelatin stab

Growth in line of puncture: beaded

Liquefaction: none

Malt extract broth:

24 hours: no surface growth; slightly flocculent sediment

10 days: film; viscid sediment

Biochemical features

Carbohydrate fermentation: gas production from glucose, galactose, sucrose and maltose

Carbohydrate utilization: glucose, galactose, sucrose, and maltose utilized

Nitrogen utilization: all nitrogen sources utilized

Hydrolysis of fat: negative

Proteolysis: negative

Ethyl alcohol utilization: positive

Litmus milk: reduction in about 4 weeks

Growth conditions

Oxygen relationship: aerobic

Growth temperature: optimum 21° to 30° C.; growth at 10° C. and 37° C. but not at 45° C.

Heat resistance: no survival at 61.7° C. for 5 minutes

Action in cream. Strain B146 required 7 days at 21° C. to develop very slightly rancid and very slightly ester-like flavors. In the presence of S. lactis, slightly fermented flavor was produced in 4 days and very rancid and slightly bitter flavors in 7 days.

Action in butter. Strain B146 developed slightly acid and slightly unclean flavors in 7 days at 21° C. but no defects in one month at 4° C.

Identity of Type 22. Organisms of this type belong to the tropicalis group of the genus Candida as defined by Langeron and Guerra (1938)

because of production of gas from glucose, fructose, sucrose, and maltose. Other differences in biochemical features set these organisms apart from the 3 species, Candida tropicalis, C. intermedia, and C. pelliculosa, which are recognized in the tropicalis group. Type 22 differs from the first and third species in optimum growth range and utilization of alcohol and from the second species in its inability to utilize lactose. It differs from the 3 species in its ability to utilize urea and from all described species of Candida in its ability to utilize potassium nitrate. These differences in characteristics are of such significance as probably to justify placing this organism in a new species. Such action should be withheld pending detail knowledge of the monograph of the subfamily Mycotoruloideae by Diddens and Lodders.

Description of Type 23

One strain, C367, was encountered in this type.

Morphology

Form and size: oval; 1.7 to 3.0 x 4.0 to 5.0 microns

Arrangement: singly, single bud attached

Staining reaction: gram positive

Spore: none

Slide culture: oval blastospores, singly or small clusters on septate true mycelium (cross walls very light)

Cultural characteristics

Potato agar slant: filiform, convex, smooth, glistening, and cream-colored

Potato agar colony: circular, entire, convex, smooth, whitish; 1½ to 2 mm. in diameter

Gelatin stab

Growth in line of puncture: arborescent

Liquefaction: none

Malt extract broth

24 hours: no surface growth; slightly viscid sediment

10 days: very thin film; flocculent sediment

Biochemical features

Carbohydrate fermentation: gas production from glucose, galactose, sucrose, and maltose

Carbohydrate utilization: glucose, galactose, sucrose, and maltose utilized

Nitrogen utilization: peptone, asparagine, ammonium sulphate, and urea utilized

Hydrolysis of fat: negative

Proteolysis: negative

Ethyl alcohol utilization: negative

Litmus milk: slight reduction in about one week with little subsequent change

Growth conditions

Oxygen relationship: aerobic

Growth temperature: optimum 21° to 45° C.; growth at 10° C.

Heat resistance: no survival at 61.7° C. for 5 minutes

Identity of Type 23. This organism resembles Candida tropicalis (Castellani) as described by Langeron and Guerra (1938) and MacKinnon and Artagaveytia-Allende (1945). The auxanogram with urea by Type 23 is

negative, but positive in the presence of accessory growth factors. This corresponded somewhat with the findings of MacKinnon and Artagaveytia-Allende who obtained a negative auxanogram with urea when new Bacto agar was used in the preparation of the medium but positive results when reclaimed agar was used. The cultures isolated developed only septate true mycelium with single and occasionally clusters of oval blastospores attached to the sides of the hyphae. MacKinnon and Artagaveytia-Allende also described pseudomycelium with verticils of blastospores. Type 23 may be regarded as Candida tropicalis.

Description of Type 24

One strain, C466, was encountered in this type.

Morphology

Form and size: oval, pleomorphic and filamentous; oval - 1.2 to 3.2 x 1.4 to 5.0 microns

Arrangement: singly, single bud attached

Staining reaction: gram positive

Spore: none

Slide culture: oval blastospores, singly on septate true mycelium (Figure 17)

Cultural characteristics

Potato agar slant: filiform to spreading, raised, slightly villous with verrucose ridge, dull and whitish.

Potato agar colony: circular, entire, capitate, slightly villous, dull and whitish; 1½ to 2½ mm. in diameter

Fig. 17. Strain C466 of Type 24. Oval blastospores on septate true mycelium. 555x.

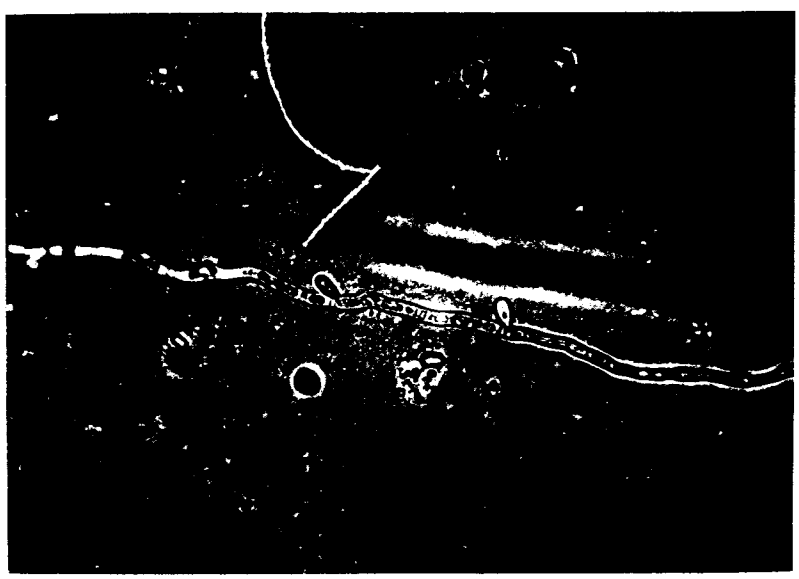

Fig. 18. Strain B323 of Type 25. Oval blastospores on septate true mycelium. 555x.

Gelatin stab

Growth in line of puncture: plumose

Liquefaction: none

Malt extract broth

24 hours: no surface growth; flocculent sediment

10 days: ring; lumpy, flocculent sediment

Biochemical features

Carbohydrate fermentation: gas production from glucose, galactose, sucrose, and maltose

Carbohydrate utilization: glucose, galactose, sucrose, and maltose utilized

Nitrogen utilization: peptone, asparagine, ammonium sulphate, and urea utilized

Hydrolysis of fat: negative

Proteolysis: negative

Ethyl alcohol utilization: negative

Litmus milk: reduction and pellicle formation in 4 weeks followed by slow digestion

Growth conditions

Oxygen relationship: aerobic

Growth temperature: optimum 21° to 37° C.; growth at 10° C. but not at 45° C.

Heat resistance: no survival at 61.7° C. for 5 minutes

Identity of Type 24. This organism resembles Candida tropicalis (Castellani) to some extent. Martin, et al (1937), Langeron and Guerra (1938) and MacKinnon and Artagaveytia-Allende (1945), described smooth

growth on solid medium. This is typical of Type 23 which is considered as C. tropicalis. Organisms of Type 24 developed spreading, dull, and villous growth. This is not the "rough" phase of dissociation because of the spreading and villous characteristics rather than verrucose growth which seemingly accompanies roughness. This organism utilized urea even in the absence of accessory growth factors. The cultural characters set this type apart from the typical C. tropicalis although apparently closely related to this species.

Description of Type 25

Thirteen strains, C17, C46, C57, B100, B145, C220, C229, B316, C47, B323, B328, C375, and C438, were encountered in this type.

Morphology

Form and size: oval and spherical; oval - 2.0 to 3.0 x 5.0 to 7.0 microns, spherical - 3.0 to 3.5 microns

Arrangement: singly, single bud attached

Staining reaction: gram positive

Spore: none

Slide culture: oval blastospores, singly on septate true mycelium (Figure 18)

Cultural characteristics

Potato agar slant: spreading, raised, smooth, glistening, and white

Potato agar colony: circular, entire, convex to pulvinate, smooth, glistening, and whitish; 1½ to 4 mm. in diameter

Gelatin stab

Liquefaction: stratiform liquefaction conspicuous in 2 days, completely liquefied in one month

Malt extract broth

24 hours: dry, matte membrane; slightly viscid sediment

10 days: thin film; slightly viscid to slightly flocculent sediment

Biochemical features

Carbohydrate fermentation: no gas production from any sugars

Carbohydrate utilization: glucose utilized

Nitrogen utilization: peptone, asparagine, ammonium sulphate, and urea utilized

Hydrolysis of fat: positive

Proteolysis: positive

Ethyl alcohol utilization: positive without membrane formation

Litmus milk: slight reduction; digestion accompanied by soft coagulation in 2 days; digestion completed in about 2 weeks

Growth conditions

Oxygen relationship: aerobic

Growth temperature: optimum 21° to 30° C.; growth at 10° C. but not at 37° C.

Heat resistance: no survival at 61.7° C. for 5 minutes

Action in cream. Strains C46 and C220 were studied. At 21° C. both strains developed slightly unclean flavors in 2 days, unclean, rancid and cheesy flavors in 4 days and very bitter and rancid flavors in 7 days. In the presence of S. lactis, similar defects resulted.

Action in butter. At 21° C., strains, C46 and C220 developed slightly cheesy and slightly rancid flavors in 4 days and cheesy and rancid flavors in 7 days. In one month at 4° C., strain C46 developed slightly rancid and slightly cheesy flavors, while strain C220 developed a slightly unclean flavor.

Identity of Type 25. Organisms of this type resemble Mycotorula lipolytica (Harrison) which was studied by Harrison (1928) and Long (1936). The earlier investigators mentioned very slight growth at 37° C.; Type 25 did not grow at that temperature. Type 25 developed essentially the same defects in cream and butter as were found by Long. The organisms isolated in this study may then be regarded as identical with Mycotorula lipolytica. Diddens and Lodder (1940) in a very short paper included Candida lipolytica as one of the 23 species in the genus Candida. They did not give any description or the sources of the culture. In view of the mycelial and blastospore structures observed, Mycotorula lipolytica undoubtedly should be placed in the genus Candida. Type 25 thus can be regarded as Candida lipolytica.

Description of Type 26

Five strains, C55, C56, C67, C218, and C477, were encountered in this type.

Morphology

Form and size: oval to ellpsoidal; 1.2 to 2.0 x 2.2 to 3.0 microns

Arrangement: singly, single bud attached

Staining reaction: gram positive

Spore: none

Slide culture: cylindrical blastospores, singly, in pairs or short chains at nodes of pseudomycelium (Figure 19)

Cultural characteristics

Potato agar slant: spreading, raised, smooth at first but soon radiating, causing superficial furrows, very glistening, and cream-colored

Potato agar colony: circular, entire, pulvinate, smooth, glistening, and cream-colored

Gelatin stab

Growth in line of puncture: arborescent

Liquefaction: none

Malt extract broth

24 hours: no surface growth; finely granular sediment

10 days: ring with or without shiny pellicle; flaky and flocculent sediment

Biochemical features

Carbohydrate fermentation: no gas production from any sugars

Carbohydrate utilization: all sugars utilized

Nitrogen utilization: peptone, asparagine, and ammonium sulphate utilized; urea utilized in the presence of accessory growth factors

Hydrolysis of fat: positive

Proteolysis: negative

Ethyl alcohol utilization: negative

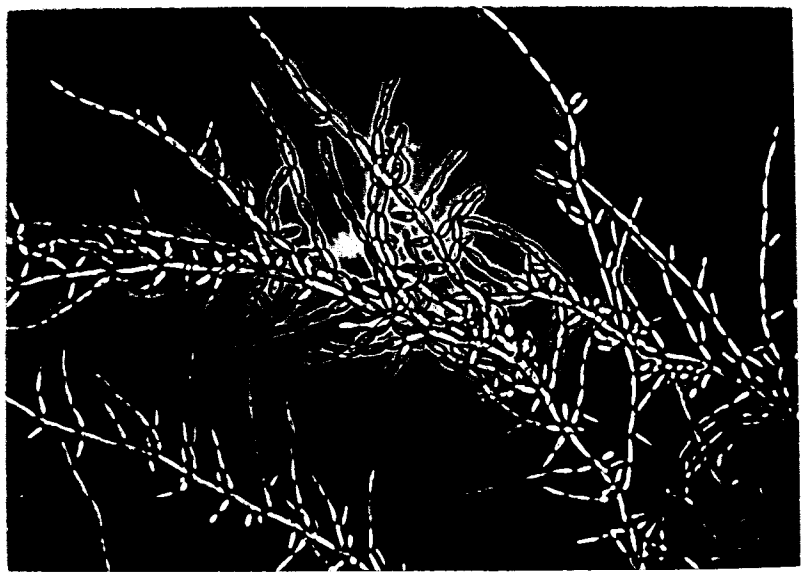

Fig. 19. Strain C67 of Type 26. Cylindrical blastospores on pseudomycelium. 555x.

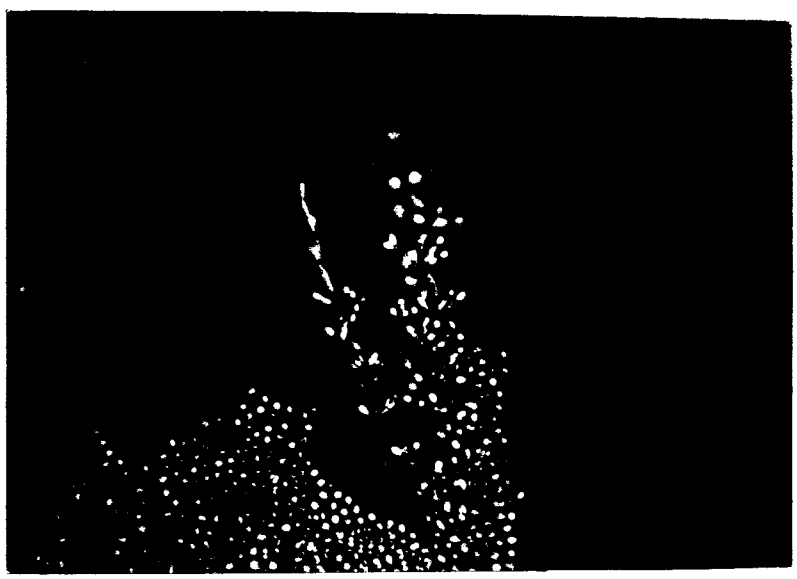

Fig. 20. Strain C235 of Type 28. Oval blastospores on pseudomycelium. 555x.

Litmus milk: complete reduction in about 3 weeks; usually pellicle formation; coagulation followed by partial or complete digestion in about 6 weeks

Growth conditions

Oxygen relationship: aerobic

Growth temperature: optimum 21° to 30° C.; growth at 10° C. but not at 45° C.

Heat resistance: no survival at 61.7° C. for 5 minutes

Action in cream. Strains C56 and C218 were studied. At 21° C. strain C56 required 7 days to develop a slightly bitter flavor while C218 caused a slightly yeasty flavor in 2 days and rancid and slightly bitter flavor in 7 days. Presence of S. lactis did not influence defect production by C56, but resulted in a fermented flavor in addition to other defects with C218.

Action in butter. Strains C56 and C218 developed slightly unclean and slightly fermented flavors in 4 days and unclean flavor in 7 days at 21° C. Slightly cheesy and slightly rancid flavors were detected in one month at 4° C.

Identity of Type 26. Organisms of Type 26 resemble Candida flarei (Redaelli and Ciferri) as described by MacKinnon and Artagaveytia-Allende (1945) in nearly all details. In both studies growth was smooth at first but radiating and superficial furrows soon appeared. The auxanogram with nitrogen sources is identical with that of the above authors, even with respect to the need of accessory growth factors for urea utilization. In the auxanogram with sugars, a difference occurred in that organisms

of Type 26 utilize lactose, while those reported earlier do not. Other similarities such as occurrence of chains of blastospores closely related Type 26 to Candida flarei.

Description of Type 27

Twenty-eight strains, B79, B127, B131, B134, B187, B192, B195, B198, B199, B203, B209, B211, C230, B251, B252, B260, B271, B272, C280, C296, B360, C379, B391, B394, B415, B427, C456, and B498, were encountered in this type.

Morphology

Form and size: oval, pointed at one pole and ellipsoidal; 1.7 to 3.0 x 3.0 to 7.0 microns

Arrangement: singly, single bud attached

Staining reaction: gram positive

Spore: none

Slide culture: oval and oblong blastospores, singly, pairs, or in short chains at nodes of pseudomycelium

Cultural characteristics

Potato agar slant: 18 strains were filiform, convex, smooth, glistening, and whitish; 10 strains were beaded, convex, verrucose, slightly glistening, and cream-colored

Potato agar colony: circular, entire, convex, glistening, and whitish growth: 1 to 1½ mm. in diameter; those strains exhibiting roughness on slants were coiled and extruded with slightly glistening luster

Gelatin stab

Growth in line of puncture: arborescent

Liquefaction: none

Malt extract broth

24 hours: no surface growth; slight sediment

10 days: slight ring and slightly viscid sediment by "smooth" strains; glistening pellicle or granular islets and slightly flocculent to slightly granular sediment by "rough" strains

Biochemical features

Carbohydrate fermentation: no gas production from any sugars

Carbohydrate utilization: glucose and galactose (weakly) utilized

Nitrogen utilization: peptone utilized weakly

Hydrolysis of fat: 11 strains positive; 17 strains negative

Proteolysis: negative

Ethyl alcohol utilization: negative

Litmus milk: slight reduction only

Growth temperature

Oxygen relationship: aerobic

Growth temperature: optimum 21° to 37° C.; growth at 10° C. but not at 45° C.

Heat resistance: no survival at 61.7° C. for 5 minutes

Action in cream. Three lipolytic strains, B199, B203, and B272, and one non-lipolytic strain, B260, were studied. At 21° C. the lipolytic strains produced slightly bitter and slightly rancid flavors in 4 days

and cheesy, moderately bitter, and rancid flavors in 7 days. Except for the absence of rancidity, the non-lipolytic strains developed similar defects. In the presence of S. lactis, no difference in development of defects was detected.

Action in butter. At 21° C., strains B199 and B203 developed slightly unclean flavor in 4 days and rancid and moderately cheesy flavors in 7 days; strains B260 and B272 caused a slightly unclean flavor in 4 days but no additional deterioration was detected in 7 days. In one month at 4° C., strains B199 and B203 did not develop any defect while B260 and B272 caused a slightly unclean and slightly acid flavor.

Identity of Type 27. Strains from this type conform in all characteristics to Candida zeylanoides of Langeron and Guerra (1938) who mentioned creamy white, soft, shiny, and smooth colonies corresponding to the smooth strains and those that show sectors with furrows corresponding to the rough strains encountered in this study.

Description of Type 28

Forty-seven strains, B1, B2, B4, B7, B8, B9, B11, C30, C34, C50, C51, C54, B69, B88, B94, B102, B103, B109, B110, B111, B116, B118, B137, B163, B165, B176, B187, C215, C217, C222, C231, C235, C236, C241, B248, C293, B349, C369, B390, B414, B422, B429, C457, C461, B490, B491, and B494, were encountered in this type.

Morphology

Form and size: oval; 1.6 to 2.3 x 2.5 to 4.0 microns

Arrangement: singly, single bud attached

Staining reaction: gram positive

Spores: none

Slide cultures: oval blastospores in short chains or clusters at nodes of mycelium; in some cases mycelium formation was hard to obtain. Figure 20 illustrates light formation of pseudomycelium.

Cultural characteristics

Potato agar slant: filiform, convex, smooth, glistening, and cream-colored to whitish

Potato agar colony: circular, entire, convex, smooth, glistening, and cream-colored to white; 1 to 1½ mm. in diameter

Gelatin stab

Growth in line of puncture: arborescent

Liquefaction: none

Malt extract broth

24 hours: no surface growth; slightly viscid sediment

10 days: ring; slightly viscid to flocculent sediment

Biochemical features

Carbohydrate fermentation: no gas production from any sugars

Carbohydrate utilization: glucose utilized

Nitrogen utilization: peptone, asparagine, ammonium sulphate, and urea were utilized; some strains required accessory growth factors to utilize urea

Hydrolysis of fat: negative with the exception of 5 strains

Proteolysis: negative

Ethyl alcohol utilization: negative

Litmus milk: slight reduction; most strains caused alkaline reaction in about 6 weeks

Growth conditions

Oxygen relationship: aerobic

Growth temperature: optimum 21° to 30° C.; growth at 10° C. but not at 37° C.

Heat resistance: no survival at 61.7° C. for 5 minutes

Action in cream. Four strains, B111, C217, C241, and C369 were studied. They required 7 days at 21° C. to develop yeasty, slightly bitter or slightly rancid flavors. In the presence of S. lactis there was no difference in development of defect.

Action in butter. Strains B111, C217, and C369 did not develop any defect in 7 days at 21° C. and C241 required 7 days at 21° C. to develop a slightly unclean flavor. In one month at 4° C., strain B111 caused astringency and slightly unclean flavor while strains C217, C241, and C369 did not develop any defect.

Identity of Type 28. Organisms of this type resemble in many respects Candida zeylanoides of Langeron and Guerra (1938). The auxanogram on nitrogen is positive for urea, peptone, asparagine, and ammonium sulphate; strains studied by Langeron and Guerra utilized only peptone. These organisms also differ from Type 27, which is considered identical to C. zeylanoides, in that organisms of Type 28

cannot develop at 37° C. In view of these differentiating characteristics, it is suggested that these organisms may properly be considered a new species. However, a specific designation for this type should await further study.

Summary of the 28 types

Table 4 summarized the data on identity and occurrence of each of the types recognized. Of the 28 types, 14 were identified with known species with a considerable degree of certainty, 6 were closely related to known species, differing in one or more characteristics of less than major importance from the known organism and thus probably not more distantly related than as a variety of the central type and 8 were sufficiently different from organisms previously described in detail to suggest that they might represent new species. However, no attempt was made to assign specific names to the apparently new types, for more careful comparison with previously described cultures and a more complete search of the literature would be necessary before new names would be justified. Three of these new types were represented by considerable numbers of strains, 11 in the case of Type 16, 16 in the case of Type 18 and 47 in the case of Type 28; the other 5 types, 1, 12, 14, 20, and 22, were represented by only one or 2 organisms each. Of the 28 types recognized, 2 (3 cultures) were placed in the genus Saccharomyces, 2 (41 cultures) in the genus Rhodotorula, 9 (81 cultures) in the genus Torulopsis, 2 (9 cultures) tentatively

TABLE 4

Summary of the Occurrence of Strains in Each of the 28 Types and Their Identity

Type No.	No. of Strains	Genus Designation	Species Designation
1	1	Saccharomyces	***
2	2	"	S. cerevisiae (Hansen) Presshefe a Delft
3	31	Rhodotorula	R. mucilaginosa (Jörgensen)
4	10	"	R. mucilaginosa var. Carbonei
5	4	Pullularia*	P. pullulans var. fusca**
6	5	"	Dematium nigrum (P. nigrum)**
7	18	Torulopsis	T. Molischiana
8	2	"	T. Holmii**
9	1	"	T. colliculosa**
10	10	"	T. Laurentii
11	9	"	T. rotundata
12	1	"	***
13	21	"	T. candida**
14	2	"	***
15	17	"	T. uvae
16	11	Trichosporon	***
17	19	Candida	C. krusei
18	16	"	***
19	52	"	C. parakrusei
20	1	"	***
21	13	"	C. chalmersi
22	1	"	***
23	1	"	C. tropicalis
24	1	"	C. tropicalis**
25	13	"	C. lipolytica
26	5	"	C. flarei
27	28	"	C. zeylanoides
28	47	"	***

* Designation as Pullularia tentative
** Related or a variety of the species
*** Species not described previously

in the genus Pullularia, one (11 cultures) in the genus Trichosporon, and 12 (197 cultures) in the genus Candida.

DISCUSSION

Identity of Organisms

In this study the primary division of non-lactose fermenting yeasts and yeast-like fungi was on the basis of ascospore formation. Only 3 of the 342 cultures were found to be ascosporogenous, and these 3 cultures were placed in 2 types. Repeated unsuccessful attempts were made, using 2 media, to induce ascospore formation, indicating the comparative rarity of sporogenous strains in the material studied. The literature indicates that some yeasts usually classed as being asporogenous seemingly can be induced to form spores. Todd and Herrmann (1936) claimed that Debaryomyces neoformans formed spores after the agar had dried, and Windisch (1938) reported the same result with Torulopsis pulcherrima. In a study of nearly 300 strains classified as Mycotoruloideae, Diddens and Lodder (1939) found that 9 of the strains were able to form spores and thus had been classified improperly previously. Mrak, et al (1942) considered that Kloeckera lindneri may be a non-sporulating form of Hanseniaspora melligeri. At times the environment unquestionably may be unfavorable for sporulation of certain strains. Sporulation also can be delayed. With our present knowledge of yeast physiology there seems to be no basis superior to spore formation in making the primary division. Consequently, Types 1 and 2 are placed in the family Endomycetaceae as defined by Stelling-Dekker (1931).

The 2 types encountered in the family Endomycetaceae could be identified readily as belonging to the genus Saccharomyces according to Stelling-Dekker (1931) because none of the cultures studied developed any mycelium, exhibited any sexual phenomena, or showed any appreciable difference in the morphology of the cells. Types 1 and 2 were differentiated mainly on their fermentation characteristics. This division was substantiated by the auxanograms with nitrogen sources.

Initial division of the asporogenous yeasts was based on chromogenesis. This character was easily determined because the colors were relatively constant, although one strain of black yeast lost the ability to grow as a completely black culture after a number of transfers. In this study, the cultures were divided into the carotinoid-pigmented group as represented by the family Rhodotorulaceae, the black-pigmented group as represented tentatively by the genus Pullularia, and those essentially lacking in pigmentation as represented by the family Torulopsidaceae.

All of the cultures in the family Rhodotorulaceae gave identical auxanograms with the sugars and the nitrogen sources tested. Only small differences in the size and shape of cells were observed; therefore, these characteristics were omitted in the separation of the various strains. Shade of the pigment and the consistency of the growth on solid medium were the criteria employed for division. Type 3 with pink, slimy and fluid growth and Type 4 with deep pink and butyrous to slightly slimy growth were obtained on these bases. According to Henrici (1941) these characteristics are highly variable, but in this study they were constant through a number of transfers. Other characteristics which are of

significance to the dairy bacteriologists are the ability to coagulate and/or digest milk. However, these characteristics were not the same for all strains within a type. For instance, in Type 3, only 4 of 31 strains coagulated milk without further digestion, while in Type 4, only 4 of 10 strains digested casein. Because of this non-homogeniety of reaction, these characteristics cannot be used for separation at levels previously established.

The black yeasts can be separated into 2 definite types on the bases of staining reaction, gelatin liquefaction, action on milk, utilization of raffinose and appearance of growth on slants. At present, these organisms hold no well-recognized systematic position. The black yeasts isolated in this study possess a mycelium structure with blastospore arrangement somewhat similar to those of certain of the genus Candida. Placement in the genus Pullularia therefore is subject to review when more information is available. These organisms are encountered sufficiently frequently to justify some curiousity as to their proper taxonomic position, but that must await a study in greater detail than was possible in this investigation.

The ability or inability to develop mycelium by the various strains in the family Torulopsidaceae serves to divide the family into 2 subfamilies. The development of mycelium seemingly depends upon many factors. The oxygen tension apparently is a major factor, since hyphae were produced chiefly in the covered or partially anaerobic portions of the slide cultures. Wickerham and Rettger (1939) observed that in the presence of air and an abundance of easily available foods, certain yeasts may exist

entirely as budding yeast-like cells while under less favorable environments, mycelium was formed. Langeron and Guerra (1938) mentioned that filaments are not easily formed on slide culture with Candida zeylanoides, while MacKinnon and Artagaveytia-Allende (1945) claimed the same difficulty with C. guilliermondi and C. chalmersi. Despite the admitted limitations of the use of formation of mycelium as a taxonomic criterion, all the strains of the Torulopsidaceae which reproduce only by budding usually are placed in the subfamily Torulopsidoideae, while those that also form mycelium are placed in the subfamily Mycotoruloideae.

All the strains of the Torulopsidoideae encountered in this study possessed cells which were either round, oval, or cylindrical and were without tubular processes. Furthermore, no dry, matte membrane was formed. Therefore, all these strains were considered as belonging to the genus Torulopsis, as defined by Lodder (1934).

Further differentiations within the genus Torulopsis were on the basis of sugar fermentation, sugar utilization, nitrogen utilization, morphological characteristics, appearance on agar slants and temperature relationships. Temperature relationships do not seem to have been used by most of those studying the taxonomy of yeasts and yeast-like fungi. In this study differences in growth at various temperatures commonly confirmed separation on other bases. A total of 9 types were placed in the genus Torulopsis of the subfamily Torulopsidoideae.

The 9 types of the genus Torulopsis were divided into 2 groups, one fermenting one or more carbohydrates while the other fermented none of the test carbohydrates. In the first group are 3 types which can be differentiated on the basis of sugars each type ferments, either alone or in

combination with the auxanograms with nitrogen sources or on differences in growth ranges. Type 9 can be segregated from Types 7 and 8 because its auxanogram is positive only with peptone whereas the auxanograms of the latter 2 types are positive with urea, peptone, asparagine, and ammonium sulphate. Furthermore, the optimum growth range of Type 7 extended to 45° C., while that of Type 9 extended only to 37° C., and that of Type 8 only to 30° C. The differences in characteristics are numerous and definite; therefore, there is no question of their separation.

In the second group of the Torulopsis are 3 types which are differentiated from any of the other types in the genus on the appearance of growth on agar slants. Their cultural characteristics were quite definite and set them apart. Type 10 developed a ropy and dirty cream-colored growth on agar slant at the beginning. After about 2 weeks' storage at 4° C., the consistency was very slimy and the color was brownish. Both Types 11 and 12 developed cream-colored pigments at the start but in about 10 days the pigment changed to a very light pink. Furthermore, Type 10 utilized all sugars while Type 11 was not able to utilize galactose and lactose and the sugar auxanogram with Type 12 was not satisfactory. Other differences between Type 11 and 12 have been discussed on pages 67 and 69. The remaining 3 types, 13, 14, and 15, are differentiated on their ability to utilize various sugars and on the size of cells. Types 15 and 14 utilized only glucose while Type 13 utilized a number of other sugars. There is no doubt but that the size of cells set Type 15 apart from Type 14. The appearance of growth on slants substantiated this division.

In the Mycotoruloideae, the group which formed arthrospores belongs to the genus Trichosporon and the group which does not form arthrospores

belongs to the genus Candida, according to Diddens and Lodder (1940). Strains within the genus Trichosporon were not further separated because correlated characteristics of possible taxonomic value were not found.

In this study, the fermentation of sugars seems to serve well for primary division within the genus Candida, although the development of true mycelium also may be used for this purpose. Certain mycologists would favor the morphological over the physiological characteristics for such a division. Langeron and Talice (1932) divided the subfamily Mycotoruloideae into six genera solely on the basis of morphology, but later Langeron and Guerra (1938) divided the genus Candida of the same subfamily into 7 groups on the basis of sugar fermentation.

The plurality of genera obtained in the earlier study of Langeron and Talice is confusing. Relatively slight differences in size, shape, or arrangement of blastospores or mycelium set one genus apart from another, whereas, some of these differences may be a result of environmental conditions. At present it seems that the fermentation of carbohydrate serves as the most reliable procedure by which this group of yeast-like fungi may be differentiated. Accordingly, the procedure in the later study of Langeron and Guerra was followed. Further division of the 7 groups within the genus Candida was by utilization of alcohol, sugars, and nitrogen sources, appearance of growth on slants, proteolysis on milk plates or liquefaction of gelatin, and differences in growth temperatures.

The growth temperatures, although not used generally by yeast taxonomists, were found in this study to be of considerable value, especially when substantiated by other differentiating characteristics. For instances, Type 18, which grew at 45° C., differs from Type 19, which would not grow

at 45° C., not only in growth temperature but also in the auxanograms with sugars and nitrogen sources. In the auxanogram with sugars, Type 18 was positive only with glucose while Type 19 was positive with glucose, galactose, sucrose, and maltose. In the auxanogram with nitrogen sources, Type 18 was positive only with peptone while Type 19 was positive with urea, peptone, asparagine, and ammonium sulphate.

Utilization of alcohol served as a satisfactory basis for division, especially when correlated with other differences. For instance, Type 17 utilized alcohol and the auxanogram was positive with urea, peptone, asparagine, and ammonium sulphate whereas Type 18 could not utilize alcohol and its auxanogram with nitrogen sources was positive only with peptone.

The utilization of sugars and nitrogen sources was a favorable basis for classification, especially when substantiated by other characteristics as just discussed.

In the genus Candida, Type 24 which developed a villous growth on agar slant was separated from Type 23 which possessed a smooth surface. This difference in appearance on agar slants seems to be more definite for differentiation than such differences as degree of sliminess or shade of pigmentation as discussed in a previous section. When separation of types on the basis of appearance on agar slants is supported by differences in growth temperatures, as with Type 24 which grew at 37° C. but not at 45° C. and Type 23 which grew at 45° C., the separation is more conclusive.

The study of growth temperature has also brought out the fact that there is much closer relationship between Type 7, a Torulopsis and Type 18, a Candida, than with any other of the types isolated, whether they be Candida or Torulopsis species. These 2 types were the same in all characteristics

except that those strains placed in the genus Torulopsis did not develop mycelium. Even their heat resistance indicates a potential relationship. Some organisms of these 2 types were the only ones except for a number of Trichosporon cultures which were able to survive 61.7° C. for 5 minutes. Possibly with continued study under different environments, Type 7 would form mycelium.

Taxonomists give little, if any, attention to the changes produced by various yeast forms on milk agar plates, litmus milk, and lipolysis plates. The interest of the dairy bacteriologists in these changes is obvious. In this study, all strains in Types 5 and 25 showed a clear zone of proteolysis on the milk plates, whereas none of the strains in the rest of the 28 types showed this change. The action was definite and there was no variation within types. Therefore, in some instances more attention might be given to the changes produced on the milk plate as a differentiating characteristic.

All strains in Types 5 and 25 were able to coagulate and completely digest litmus milk. All strains in Types 16 and 26 were able to coagulate milk but digestion ranged from partial to complete. Some of the strains in Types 4, 10, and 11 coagulated and digested milk, and 4 of the strains in Type 3 coagulated the milk without digestion. Alkaline growth in litmus milk was not a sufficiently consistent characteristic to be of importance in differentiation. Growth of this group of organisms in litmus milk permits some conclusions with respect to identity with a number of types but has very marked limitations with others because of the variations within a type.

The ability of strains in any one type to hydrolyze fat was variable in many instances. All strains in Types 16, 25, and 26, and only certain

strains in Types 11, 27, and 28, exhibited lipolysis. The latter finding is in agreement with results of Hussong, Long and Hammer (1937) who found differences in the lipolytic ability of the variants of Pseudomonas fragi. Lipolytic ability thus may be considered a secondary characteristic of occasional value in identification of organisms in this group.

In this study, variation was noted not only in biochemical features but also in cultural characteristics. In Types 17, 21, and 27, both "smooth" and "rough" strains were encountered. The "smooth" strains developed smooth surface growth on agar slants and agar plates and slightly viscid sediment in malt extract broth, while the "rough" strains gave verrucose surface growth on agar slant and agar plates, and usually a granular sediment in malt extract broth. The existence of variation whether by true dissociation or hereditary changes in some species of the yeasts and yeast-like fungi is a fact which cannot be denied. Punkari and Henrici (1933) reported spontaneous variation with Torulopsis pulcherrima developing variants not only in texture (smooth or rough) but also in color (red and white). MacKinnon and Artagaveytia-Allende (1945) described smooth and rough variants in a number of strains of Candida. Numerous other authors have reported similar observations. No change or reversion was observed throughout this study. Probably the inciting agent for such a transition was not encountered.

Further study of the cultures isolated in this study and also a careful comparison with those previously identified should be made before attempting to assign species designations to some of the apparently new types encountered in this study. Type 1 of the genus Saccharomyces, Types 12 and 14 of the genus Torulopsis, Type 16 of the genus Trichosporon, and

Types 18, 20, 22, and 28 of the genus Candida may have been studied and identified previously but descriptions were not found in the literature.

The following key is proposed for use in separating the 28 types which were studied and as a summary of certain differential characteristics which were used:

KEY FOR THE IDENTIFICATION OF NON-LACTOSE FERMENTING YEASTS AND YEAST-LIKE FUNGI FROM CREAM AND BUTTER

1. a. Ascosporogenous yeasts. (2)
   b. Asporogenous yeasts. (3)
2. a. Ferment only glucose. Type 1
   b. Ferment glucose, galactose, sucrose, maltose, and "1/3 raffinose". Type 2
3. a. Produce carotinoid pigments on wort agar slant. (4)
   b. Produce dark pigments on wort agar slant. (5)
   c. Do not produce pigments on wort agar slant; cream, white, or turning slight tan or very slight pink with age. (6)
4. a. Very slimy, fluid growth on agar slant. Type 3
   b. Slightly slimy, butyrous growth on agar slant. Type 4
5. a. Raffinose utilized; very proteolytic. Type 5
   b. Raffinose not utilized; not proteolytic. Type 6
6. a. Neither mycelium nor pseudomycelium formed. (7)
   b. True mycelium and/or pseudomycelium formed; blastospores usually present. (13)

7.	a. Ferment sugars.	(8)	
	b. Ferment no sugars.	(9)	
8.	a. Ferment only glucose.		Type 7
	b. Ferment glucose, galactose, and sucrose.		Type 8
	c. Ferment glucose, galactose, sucrose, and maltose.		Type 9
9.	a. Growth on wort agar very slimy; turning brownish with age.		Type 10
	b. Growth on wort agar moderately slimy; turning light pinkish with age.	(10)	
	c. Growth on wort agar not slimy; very little change in pigmentation.	(11)	
10.	a. Grow at 30° C.		Type 11
	b. Do not grow at 30° C.		Type 12
11.	a. Utilize only glucose.	(12)	
	b. Also utilize other sugars.		Type 13
12.	a. Cells relatively large.		Type 14
	b. Cells relatively small.		Type 15
13.	a. Arthrospores present.		Type 16
	b. Arthrospores absent.	(14)	
14.	a. Ferment sugars.	(15)	
	b. Ferment no sugars.	(21)	
15.	a. Ferment glucose, galactose$^{\pm}$	(16)	
	b. Ferment glucose, sucrose, and galactose$^{\pm}$	(18)	
	c. Ferment glucose, sucrose, galactose, and maltose.	(19)	

16. a. Utilize alcohol. Type 17

    b. Do not utilize alcohol. (17)

17. a. Grow at 45° C. Type 18

    b. Do not grow at 45° C. Type 19

18. a. Utilize all nitrogen sources. Type 20

    b. Utilize only some nitrogen sources. Type 21

19. a. Utilize alcohol. Type 22

    b. Do not utilize alcohol. (20)

20. a. Grow at 45° C.; smooth growth on agar slant. Type 23

    b. Do not grow at 45° C.; villous growth on agar slant. Type 24

21. a. Liquefy gelatin; positive on milk plate. Type 25

    b. Do not liquefy gelatin; negative on milk plate. (22)

22. a. Utilize all sugars. Type 26

    b. Utilize glucose and galactose± (23)

23. a. Utilize only peptone (weakly). Type 27

    b. Utilize other nitrogen sources. Type 28

The key permits separation of all the types of yeasts and yeast-like fungi isolated in this study, but it must be admitted such a key cannot be used to identify all the yeasts which might be isolated from dairy products.

Significance of the Organisms in Cream and Butter

The relatively large proportion of non-lactose fermenting yeasts and yeast-like fungi encountered in this study is in agreement with the observation of investigators who had repeatedly found them in considerable numbers in their studies on distribution of yeast forms in dairy products. Only 27 of the 369 yeasts isolated from cream and butter were of the lactose fermenting type. The occurrence of the lactose fermenting types in cream was more frequent in the summer than in the other seasons. It seems that comparatively high temperature and acid conditions favor their development. On the other hand, these environmental conditions apparently are favorable to at least some of the yeasts which are unable to ferment lactose. Certain types of non-lactose fermenting organisms isolated during the course of study are able to grow at 45° C., others only at lower temperatures. Their acid tolerance was obvious since they were isolated from potato agar plates which had a pH of 3.5; also, they tend to cause more pronounced changes when growing in association with S. lactis than when growing alone. Consequently, during the summer months when cream was held at relatively high temperatures, both lactose and non-lactose fermenting yeasts should be able to develop. Type of initial contamination undoubtedly would be of a major factor in determining the predominant organism or organisms of the yeast group. During the winter months the small number of isolations of lactose fermenting type organisms may be due to their comparative absence or to higher contamination with the more prevalent non-lactose fermenting types, some of which can develop quite well at lower temperatures.

The number of lactose fermenting yeasts in butter was very low, and

they constituted only a small portion of the total population. Their occurrence in butter probably results from chance contamination from a number of sources, such as churns and other equipment with growth a minor factor.

Most non-lactose fermenting yeasts and yeast-like fungi have been considered a more or less inert group of organisms usually producing little or no change in cream and butter. The results obtained show that a small but significant number of the cultures possesses lipolytic and/or proteolytic properties. Extensive flavor defects were produced in cream by certain of the organisms in relatively short periods, and some lots of unsalted butter made from cream inoculated with selected organisms showed deterioration rather rapidly.

Good correlation was obtained in some cases but not throughout the study between the lipolytic and proteolytic tests and the actual defect development in cream and butter. For instance, organisms of Type 25, a lipolytic and proteolytic type, developed corresponding defects as cheesiness, bitterness, uncleanliness, and rancidity in cream and butter. On the other hand, organisms of Type 5, a proteolytic but not lipolytic type, developed not only the expected proteolytic deterioration, but also rancidity. Furthermore, organisms of Type 19, which were both non-lipolytic and non-proteolytic, produced cheesy, yeasty, bitter, rancid, and unclean flavor defects in cream and somewhat milder defects in butter.

When lipolytic strains of Type 11 were inoculated into cream and butter, they were not able to develop any rancid flavor. This suggests that the organisms may utilize the lower fatty acids, or it possibly may be that selective fat hydrolysis occurs in which the higher molecular weight fatty acids are liberated rather than the lower weight ones. The development of

rancidity in cream and butter by cultures showing no fat hydrolysis in the test medium may have been due to differences in the composition of the various substrata.

Proteolytic flavor defects, such as cheesiness and bitterness, are not necessarily correlated with final changes observed in proteolytic tests. These defects may be evidence of preliminary or intermediate proteolysis. The composition of the various substrata may be important. Another possible explanation is that the procedures employed in testing for lipolysis and proteolysis usually do not detect these changes unless a considerable portion of the substratum is attacked. These organisms may be sufficiently lipolytic and proteolytic to cause defects without giving positive results on test media. However, the rapid rate of flavor deteriorations, some within 2 to 4 days at 21° C. does not agree entirely with such an explanation. Probably the proper environment has not been attained to produce identical results in the two types of tests. Since mycelium formation with blastospore arrangement is obtained to a greater degree under somewhat reduced oxygen tension, it could be that defects would be produced in a mass of cream or butter, while the organism may not hydrolyze fat or protein to the same degree on the surface of an agar plate.

In numerous instances, development of defects in cream was enhanced by the presence of S. lactis. This illustrates the potential significance of these organisms in sour milk and cream. The acid produced by the S. lactis serves not as an inhibitor, as in the case of some bacteria, but as a source of food supply for the yeasts and yeast-like organisms. The ability of these organisms to assimilate organic acids such as lactic acid would make possible their wide distribution in nature, for there is an abundance of such

acids in decaying or fermenting materials. An appreciable portion of bitterness, yeastiness, rancidity, and similar defects found in much old sour cream may be the result of growth of yeasts and yeast-like fungi.

A number of strains of yeasts and yeast- like fungi evidently secrete a rennin-like enzyme since they were able to cause coagulation in milk tube without formation of acid. In all such cases, digestion occurred only after soft or firm coagula were formed.

The growth of yeasts at low temperatures is demonstrated by the development of nearly all the strains at 10° C. and the fairly common production of defects in experimental butter held at 4° C. Under commercial conditions, salt and the physical condition of the substratum undoubtedly would minimize growth in butter. However, these organisms might be the cause of defect production under special conditions, such as prolonged storage of unsalted butter at temperatures slightly above freezing, especially if the butter were not well-worked.

In the present study, none of the strains survived exposure to 61.7° C. for 30 minutes, and only a very small number survived 61.7° C. for 5 or 10 minutes. This agrees closely with the findings of Edwards (1913) who reported that none of the yeast forms isolated by him were able to resist heat exposure between 60° to 70° C. for 10 minutes. However, Keiper (1938) observed 13 unnamed strains of Monilia (Candida), probably of medical importance, were able to resist temperature exposure at 60° C. for 60 minutes and some of the 13 strains, 70° C. for 10 minutes. In view of the results obtained in the present study, the occurrence of yeast-like organisms of this type in butter may be considered due to faulty pasteurization or to subsequent contamination.

In general, yeasts and yeast-like fungi have been thought of as being facultative aerobes. In this study the organisms were able to develop only on or very close to the surface of the deep agar tubes. They were also seen to develop beneath the surface of potato agar plates. Thus it seems these organisms apparently preferred aerobic conditions but were able to grow in an environment of slightly reduced oxygen tension. In a mass of cream or butter the development of these organisms then would be greater at or close to the surface, and this could be a limiting factor in the development of a large population.

SUMMARY AND CONCLUSIONS

Three hundred and sixty-nine cultures of yeasts and yeast-like fungi were isolated from cream and butter. One hundred and thirty-nine of the cultures were obtained from 124 samples of cream and 230 cultures were obtained from 203 samples of butter. Of the total number of cultures isolated, 342 (92.7%) were unable to ferment lactose and 27 (7.3%) were able to ferment this sugar.

An attempt was made to identify the 342 non-lactose fermenting yeasts and yeast-like fungi on the bases of cell morphology and dimension, sporulation, chromogenesis, ability or inability to form mycelium, types of mycelial formation, carbohydrate fermentation and utilization, nitrogen utilization, alcohol utilization, appearance of growth on agar slant, growth temperature, proteolysis, and lipolysis. Twenty-eight types were recognized, the majority of which undoubtedly could be considered species.

Six genera, Saccharomyces, Rhodotorula, Pullularia (tentative), Torulopsis, Trichosporon, and Candida, were recognized.

Organisms identified as Saccharomyces cerevisiae (Hansen) Presshefe a Delft, Rhodotorula mucilaginosa (Jörgensen), R. mucilaginosa var. Carbonei, Torulopsis Molischiana, T. Laurentii, T. rotundata, T. uvae, Candida krusei, C. parakrusei, C. chalmersi, C. tropicalis, C. lipolytica, C. flarei, and C. zeylanoides were found.

Six types could not be identified definitely with previously described species because of minor differences in characteristics or incomplete

description in the literature, but they may be regarded tentatively as being related to previously described species or as a variety of these species.

Eight type could not be identified with previously described species. On the basis of correlated differences, new specific designation seem warranted. However, further studies, especially comparisons with known cultures, such as those listed by Diddens and Lodder (1940) but not yet available, should be made before suggesting definite names.

A key to the identification of the 28 types of non-lactose fermenting yeasts and yeast-like fungi is given.

Lipolysis and action on litmus milk frequently vary within a type and are of importance to the dairy industry but apparently of limited taxonomic significance.

No great significance can be attached to the colony characteristics since irregularities occurred so frequently within some types.

Heat resistance in most cases supports differences established on other bases. All strains in 25 types were destroyed in 5 minutes at 61.7° C. but a number of strains in 3 types resisted 5 or 10 minutes but not 30 minutes at this temperature. This indicated that these yeasts and yeast-like fungi were unable to resist minimum pasteurization exposures.

All of the organisms were aerobic but could develop under a somewhat reduced oxygen tension.

The action of 47 strains of representative non-lactose fermenting yeasts and yeast-like fungi on cream at 21° C. was studied. Four types developed very pronounced defects such as rancidity, putridness, cheesiness, and uncleanliness. Seven types developed fairly pronounced off-flavors such as

moderate bitterness, moderate rancidity, and yeastiness. Six types caused only mild defects such as slightly yeasty, slightly rancid, slightly unclean, and slightly bitter flavors. Four types caused no defects. Slight variations of defect development occurred within some types. In a number of instances, where defects were developed, the presence of S. lactis enhanced the off-flavors.

The action of 47 strains of representative non-lactose fermenting yeasts and yeast-like fungi on unsalted butter was studied at 21° C. and at 4° C. At 21° C. 2 types produced very pronounced defects such as rancid and cheesy flavors, 3 types developed fairly pronounced defects such as unclean and moderately cheesy flavors, 8 types caused mild off-flavors such as slight bitterness, astringency, slight acidity, slight uncleanliness, and slight rancidity, and 8 types developed no defect. Slight variations of off-flavors occurred within some types. When the butter was incubated at 4° C., one type developed a very pronounced rancid defect, 10 types caused mild defects such as slightly unclean, slightly acid, slightly cheesy, slightly rancid and astringent flavors, and 10 types were not criticized for any defect. Slight variations of defect development occurred within some types.

Non-lactose fermenting yeasts and yeast-like fungi are distributed widely in butter and cream and are capable of causing pronounced defects under some circumstances.

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