Chapter 2

Taxonomy, Role, and Significance of Microorganisms in Foods

Because human food sources are of plant and animal origin, it is important to understand the biological principles of the microbial biota associated with plants and animals in their natural habitats and respective roles. Although it sometimes appears that microorganisms are trying to ruin our food sources by infecting and destroying plants and animals, including humans, this is by no means their primary role in nature. In our present view of life on this planet, the primary function of microorganisms in nature is self-perpetuation. During this process, the heterotrophs and autotrophs carry out the following general reaction:

All organic matter
(carbohydrates, proteins, lipids, etc.)
↓
Energy + Inorganic compounds
(nitrates, sulfates, etc.)

This, of course, is essentially nothing more than the operation of the nitrogen cycle and the cycle of other elements. The microbial spoilage of foods may be viewed simply as an attempt by the food biota to carry out what appears to be their primary role in nature. This should not be taken in the teleological sense. In spite of their simplicity when compared to higher forms, microorganisms are capable of carrying out many complex chemical reactions essential to their perpetuation. To do this, they must obtain nutrients from organic matter, some of which constitutes our food supply.

If one considers the types of microorganisms associated with plant and animal foods in their natural states, one can then predict the general types of microorganisms to be expected on this particular food product at some later stage in its history. Results from many laboratories show that untreated foods may be expected to contain varying numbers of bacteria, molds, or yeasts, and the question often arises as to the safety of a given food product based on total microbial numbers. The question should be twofold: What is the total number of microorganisms present per gram or milliliter and what *types* of organisms are represented in this number? It is necessary to know which organisms are associated with a particular food in its natural state and which of the organisms present are not normal for that particular food. It is, therefore, of value to know the general distribution of bacteria in nature and the general types of organisms normally present under given conditions where foods are grown and handled.

BACTERIAL TAXONOMY

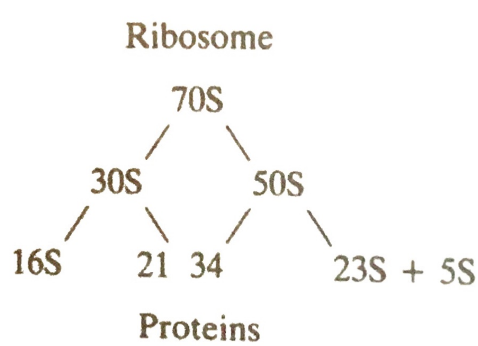
Many changes have taken place in the classification or taxonomy of bacteria in the past two decades. Many of the new taxa have been created as a result of the employment of molecular genetic methods, alone or in combination with some of the more traditional methods:

1. DNA homology and mol% G + C content of DNA
2. 23S, 16S, and 5S rRNA sequence similarities
3. Oligonucleotide cataloging
4. Numerical taxonomic analysis of total soluble proteins or of a battery of morphological and biochemical characteristics
5. Cell wall analysis
6. Serological profiles
7. Cellular fatty acid profiles

Although some of these have been employed for many years (e.g., cell wall analysis and serological profiles) others (e.g., ribosomal RNA [rRNA] sequence similarity) came into wide use only during the 1980s. The methods that are the most powerful as bacterial taxonomic tools are outlined and briefly discussed below.

rRNA Analyses

Taxonomic information can be obtained from RNA in the production of nucleotide catalogs and the determination of RNA sequence similarities. First, the prokaryotic ribosome is a 70S (Svedberg) unit, which is composed of two separate functional subunits: 50S and 30S. The 50S subunit is composed of 23S and 5S RNA in addition to about 34 proteins, whereas the 30S subunit is composed of 16S RNA plus about 21 proteins.



The 16S subunit is highly conserved and is considered to be an excellent chronometer of bacteria over time.[53] Using reverse transcriptase, 16S rRNA can be sequenced to produce long stretches (about 95% of the total sequence) to allow for the determination of precise phylogenetic relationships.[31] Alternatively, the 16S rDNA may be sequenced after amplification of specific regions by polymerase chain reaction (PCR)-based methods.

To sequence 16S rRNA, a single-stranded DNA copy is made by use of reverse transcriptase with the RNA as template. When the single-stranded DNA is made in the presence of dideoxynucleotides, DNA fragments of various sizes result that can be sequenced by the Sanger method. From the DNA sequences, the template 16S rRNA sequence can be deduced. It was through studies of 16S rRNA sequences that led Woese and his associates to propose the establishment of three kingdoms of life forms: Eukaryotes, Archaebacteria, and Prokaryotes. The last include the cyanobacteria and the eubacteria, with the bacteria of importance in foods being eubacteria. Sequence similarities of 16S rRNA are widely employed, and some of the new foodborne taxa were created primarily by its use along with other information. It appears that the sequencing of 23S rDNA will become more widely used in bacterial taxonomy.

Nucleotide catalogs of 16S rRNA have been prepared for a number of organisms, and extensive libraries exist. By this method, 16S rRNA is subjected to digestion by RNase T1, which cleaves the molecule at G(uanine) residues. Sequences (-mers) of 6–20 bases are produced and separated, and similarities S_{AB} (Dice-type coefficient) between organisms can be compared. Although the relationship between S_{AB} and percentage similarity is not good below S_{AB} value of 0.40, the information derived is useful at the phylum level. The sequencing of 16S rRNA by reverse transcriptase is preferred to oligonucleotide cataloging, as longer stretches of rRNA can be sequenced.

Analysis of DNA

The mol% G + C of bacterial DNA has been employed in bacterial taxonomy for several decades, and its use in combination with 16S and 5S rRNA sequence data makes it even more meaningful. By 16S rRNA analysis, the Gram-positive eubacteria fall into two groups at the phylum level: one group with mol% G + C >55, and the other <50.[53] The former includes the genera *Streptomyces*, *Propionibacterium*, *Micrococcus*, *Bifidobacterium*, *Corynebacterium*, *Brevibacterium*, and others. The group with the lower G + C values include the genera *Clostridium*, *Bacillus*, *Staphylococcus*, *Lactobacillus*, *Pediococcus*, *Leuconostoc*, *Listeria*, *Erysipelothrix*, and others. The latter group is referred to as the *Clostridium* branch of the eubacterial tree. When two organisms differ in G + C content by more than 10%, they have few base sequences in common.

DNA–DNA or DNA–RNA hybridization has been employed for some time, and this technique continues to be of great value in bacterial systematics. It has been noted that the ideal reference system for bacterial taxonomy would be the complete DNA sequence of an organism.[49] It is generally accepted that bacterial species can be defined in phylogenetic terms by use of DNA–DNA hybridization results, where 70% or greater relatedness and 5°C or less T_m (melting point) defines a species.[50] When DNA–DNA hybridization is employed, phenotypic characteristics are not allowed to override except in exceptional cases.[50] Although a genus is more difficult to define phylogenetically, 20% sequence similarity is considered to be the minimum level of DNA–DNA homology.[50]

Even if there is not yet a satisfactory phylogenetic definition of a bacterial genus, the continued application of nucleic acid techniques, along with some of the other methods listed above, should lead ultimately to a phylogenetically based system of bacterial systematics. In the meantime, changes in the extant taxa may be expected to continue to occur.

The Proteobacteria

The Gram-negative bacteria of known importance in foods belong to the class *Proteobacteria*, which was established following extensive studies on the rRNA sequences of numerous genera of

Table 2–1 Subclasses of the *Proteobacteria* to Which Many Foodborne Genera Belong. *Campylobacter* and *Helicobacter* Belong to the δ-Subclass

Alpha	*Beta*	*Gamma*
Acetobacter	*Acidovorax*	*Acinetobacter*
Asaia	*Alcaligenes*	*Aeromonas*
Brevundimonas	*Burkholderia*	*Alteromonas*
Devosia	*Chromobacterium*	*Azomonas*
Gluconobacter	*Comamonas*	*Bacteriodes*
Paracoccus	*Delftia*	*Carnimonas*
Pseudoaminobacter	*Hydrogenophaga*	*Enterobacteriaceae*[a]
Sphingomonas	*Janthinobacterium*	*Flavobacterium*
Xanthobacter	*Pandoraea*	*Halomonas*
Zymomonas	*Pseudomonas* (plant pathogens)	*Moraxella*
	Ralstonia	*Plesiomonas*
	Telluria	*Pseudoalteromonas*
	Variovorax	*Pseudomonas*
	Vogesella	*Psychrobacter*
	Wautersia	*Photobacterium*
	Xylophilus	*Shewanella*
		Stenotrophomonas
		Vibrio
		Xanthomonas
		Xylella

[a]Include *Escherichia, Citrobacter, Salmonella, Shigella, Proteus, Raoultella, Proteus, Klebsiella, Edwardsiella*, etc.

Gram-negative bacteria.[43] The class is divided into five subclasses designated α, β, γ, etc. The subclasses are defined on the basis of their 16S rRNA sequences.[54–56] By extensive use of signature sequences (conserved inserts and deletions) of different proteins, an evolutionary relationship of the *Proteobacteria* has been proposed.[20] It has been suggested that the first eubacteria were low G + C Gram positives (e.g., *Clostridium, Bacillus, Lactobacillus*), followed by high G + C Gram positives (e.g., *Micrococcus, Propionibacterium, Rubrobacter*), and then by *Deinococcus-Thermus*. Next arose three groups that are not foodborne (not listed here), and then the *Proteobacteria* with ϵ and σ followed by α, β, and γ.[20] It has been stressed that these groups are related to each other in a linear rather than a tree-like manner.[20] It can be seen from Table 2–1 that most foodborne bacteria (especially foodborne pathogens) belong to the γ-subclass. The earliest prokaryotes are estimated to have arisen 3.5–3.8 billion years ago.[20]

Some of the important genera known to occur in foods are listed below in alphabetical order. Some are desirable in certain foods; others bring about spoilage or cause gastroenteritis. It should be noted that the bacterial genera in this list along with those in Table 2–2 are now somewhat problematic since most were defined largely on phenotypic data. They are placed here mainly on historical reports but the list may be expected to change as more phylogenetic data are employed.

Bacteria

Acinetobacter
Aeromonas
Alcaligenes
Arcobacter
Bacillus
Brevibacillus
Brochothrix
Burkholderia
Campylobacter
Carnobacterium
Citrobacter
Clostridium
Corynebacterium
Enterobacter
Enterococcus
Erwinia
Escherichia
Flavobacterium
Hafnia
Kocuria
Lactococcus
Lactobacillus
Leuconostoc
Listeria
Micrococcus
Moraxella
Paenibacillus
Pandoraea
Pantoea
Pediococcus
Proteus
Pseudomonas
Psychrobacter
Salmonella
Serratia
Shewanella
Shigella
Sphingomonas
Stenotrophomonas
Staphylococcus
Vagococcus
Vibrio
Weissella
Yersinia

Molds

Alternaria
Aspergillus
Aureobasidium
Botrytis
Byssochlamys
Cladosporium
Colletotrichum
Fusarium
Geotrichum
Monilia
Mucor
Penicillium
Rhizopus
Trichothecium
Wallemia
Xeromyces

Yeasts

Brettanomyces/Dekkera
Candida
Cryptococcus
Debaryomyces
Hanseniaspora
Issatchenkia
Kluyveromyces
Pichia
Rhodotorula
Saccharomyces
Schizosaccharomyces
Torulaspora
Trichosporon
Yarrowia
Zygosaccharomyces

Protozoa

Cryptosporidium parvum
Cyclospora cayetanensis
Entamoeba histolytica
Giardia lamblia
Toxoplasma gondii

PRIMARY SOURCES OF MICROORGANISMS FOUND IN FOODS

The genera and species previously listed are among the most important normally found in food products. Each genus has its own particular nutritional requirements, and each is affected in predictable ways by the parameters of its environment. Eight environmental sources of organisms to foods are listed below, and these, along with the genera of bacteria and protozoa noted, are presented in Table 2–2 to reflect their primary food-source environments.

Soil and Water. These two environments are placed together because many of the bacteria and fungi that inhabit both have a lot in common. Soil organisms may enter the atmosphere by the action of wind and later enter water bodies when it rains. They also enter water when rainwater flows over soils into bodies of water. Aquatic organisms can be deposited onto soils through the actions of cloud formation and subsequent rainfall. This common cycling results in soil and aquatic organisms being one and the same to a large degree. Some aquatic organisms, however, are unable to persist in soils, especially those that are indigenous to marine waters. *Alteromonas* spp. are aquatic forms that require seawater salinity

Table 2–2 Relative Importance of Eight Sources of Bacteria and Protozoa to Foods

Organisms	Soil and Water	Plants/Products	Food Utensils	Gastrointestinal Tract	Food Handlers	Animal Feeds	Animal Hides	Air and Dust
Bacteria								
Acinetobacter	XX	X	X				X	X
Aeromonas	XX[a]	X						
Alcaligenes	X	X	X	X			X	
Alteromonas	XX[a]							
Arcobacter	X							
Bacillus	XX[b]	X	X		X	X	X	XX
Brochothrix		XX	X					
Brevibacillus	X	X						X
Burkholderia		XX						
Campylobacter				XX	X			
Carnobacterium	X	X	X					
Citrobacter	X	XX	X	XX				
Clostridium	XX[b]	X	X	X	X	X	X	XX
Corynebacterium	XX [b]	X	X		X		X	X
Enterobacter	X	XX	X	X			X	
Enterococcus	X	X	X	XX	X	X	X	X
Erwinia	X	XX	X					
Escherichia	X	X		XX	X			
Flavobacterium	X	XX					X	
Hafnia	X	X		XX				
Kocuria	X	X	X		X		X	X
Lactococcus		XX	X	X			X	
Lactobacillus		XX	X	X			X	
Leuconostoc		XX	X	X			X	
Listeria	X	XX			X	X	X	
Micrococcus	X	X	X		X	X	X	XX
Mycobacterium[c]		X						
Moraxella	X	X					X	
Mycobacterium								

Paenibacillus	XX	X	X					XX
Pandoraea		X						
Pectobacterium	X	XX						
Pantoea	X	X		X				
Pediococcus		XX	X	X			X	
Proteus	X	X	X	X	X		X	
Pseudomonas	XX	X	X			X	X	
Psychrobacter	XX	X	X				X	
Salmonella				XX		XX		
Serratia	X	X	X	X		X	X	
Shewanella	X	X						
Sphingomonas	X	X						
Shigella				XX				
Stenotrophomonas	X	XX						
Staphylococcus				X	XX		X	
Vagococcus	XX			XX				
Vibrio	XX			X				
Weissella		XX	X					
Yersinia	X	X		X				
Protozoa								
C. cayetanensis	X	X		X				
C. parvum	XX			X	X			
E. histolytica	XX			X	X			
G. lamblia	XX			X	X			
T. gondii		X		XX				

Note: XX indicates a very important source.

[a]Primarily water

[b]Primarily soil.

[c]Nontuberculous.

for growth and would not be expected to persist in soils. The bacterial biota of seawater is essentially Gram-negative, and Gram-positive bacteria exist there essentially only as transients. Contaminated water has been implicated in *Cyclospora* contamination of fresh raspberries.

Plants and Plant Products. It may be assumed that many or most soil and water organisms contaminate plants. However, only a relatively small number find the plant environment suitable to their overall well-being. Those that persist on plant products do so by virtue of a capacity to adhere to plant surfaces so that they are not easily washed away and because they are able to obtain their nutritional requirements. Notable among these are the lactic acid bacteria and some yeasts. Among others that are commonly associated with plants are bacterial plant pathogens in the genera *Corynebacterium*, *Curtobacterium*, *Pectobacterium*, *Pseudomonas*, and *Xanthomonas*; and fungal pathogens among several genera of molds.

Food Utensils. When vegetables are harvested in containers and utensils, one would expect to find some or all of the surface organisms on the products to contaminate contact surfaces. As more and more vegetables are placed in the same containers, a normalization of the microbiota would be expected to occur. In a similar way, the cutting block in a meat market along with cutting knives and grinders are contaminated from initial samples, and this process leads to a buildup of organisms, thus ensuring a fairly constant level of contamination of meat-borne organisms.

Gastrointestinal Tract. This biota becomes a water source when polluted water is used to wash raw food products. The intestinal biota consists of many organisms that do not persist as long in waters as do others, and notable among these are pathogens such as salmonellae. Any or all of the Enterobacteriaceae may be expected in fecal wastes, along with intestinal pathogens, including the five protozoal species already listed.

Food Handlers. The microbiota on the hands and outer garments of handlers generally reflect the environment and habits of individuals, and the organisms in question may be those from soil, water, dust, and other environmental sources. Additional important sources are those that are common in nasal cavities, the mouth, and on the skin, and those from the gastrointestinal tract that may enter foods through poor personal hygiene practices.

Animal Feeds. This is a source of salmonellae to poultry and other farm animals. In the case of some silage, it is a known source of *Listeria monocytogenes* to dairy and meat animals. The organisms in dry animal feed are spread throughout the animal environment and may be expected to occur on animal hides.

Animal Hides. In the case of milk cows, the types of organisms found in raw milk can be a reflection of the biota of the udder when proper procedures are not followed in milking and of the general environment of such animals. From both the udder and the hide, organisms can contaminate the general environment, milk containers, and the hands of handlers.

Air and Dust. Although most of the organisms listed in Table 2–2 may at times be found in air and dust in a food-processing operation, the ones that can persist include most of the Gram-positive organisms listed. Among fungi, a number of molds may be expected to occur in air and dust, along with some yeasts. In general, the types of organisms in air and dust would be those that are constantly reseeded to the environment. Air ducts are not unimportant sources.

SYNOPSIS OF COMMON FOODBORNE BACTERIA

These synopses are provided to give the reader glimpses of bacterial groups that are discussed throughout the textbook. They are not meant to be used for culture identifications. For the latter, one or more of the cited references should be consulted. Some of the phylogenetic features of these bacteria are presented in the Appendix.

Acinetobacter (A • ci • ne'to • bac • ter; Gr. *akinetos*, unable to move). These Gram-negative rods show some affinity to the family Neisseriaceae, and some that were formerly achromobacters and moraxellae are placed here. Also, some former acinetobacters are now in the genus *Psychrobacter*. They differ from the latter and the moraxellae in being oxidase negative. They are strict aerobes that do not reduce nitrates. Although rod-shaped cells are formed in young cultures, old cultures contain many coccoid-shaped cells. They are widely distributed in soil and water and may be found on many foods, especially refrigerated fresh products. The mol% G + C content of DNA for the genus is 39–47. (See Chapter 4 for a further discussion relative to meats.) It has been proposed, based on DNA–rRNA hybridization data, that the genera *Acinetobacter*, *Moraxella*, and *Psychrobacter* be placed in a new family (Moraxellaceae), but this proposal has not been approved.

Aeromonas (ae • ro • mo'nas; *gas producing*). These are typically aquatic Gram-negative rods formerly in the family Vibrionaceae but now in the family Aeromonadaceae.[32] As the generic name suggests, they produce copious quantities of gas from the fermented sugars. They are normal inhabitants of the intestines of fish, and some are fish pathogens. The mol% G + C content of DNA is 57–65. (The species that possesses pathogenic properties is discussed in Chapter 31.)

Alcaligenes (al • ca • li'ge • nes; *alkali producers*). Although Gram negative, these organisms sometimes stain Gram positive. They are rods that do not, as the generic name suggests, ferment sugars but instead produce alkaline reactions, especially in litmus milk. Nonpigmented, they are widely distributed in nature in decomposing matter of all types. Raw milk, poultry products, and fecal matter are common sources. The mol% G + C content of DNA is 58–70, suggesting that the genus is heterogeneous.

Alteromonas (al • te • ro • mo'nas; *another monad*). These are marine and coastal water inhabitants that are found in and on seafoods; all species require seawater salinity for growth. They are Gram-negative motile rods that are strict aerobe.[17]

Arcobacter (Ar'co • bac • ter; L. *arcus*, bow). This genus was created during revision of the genera *Campylobacter*, *Helicobacter*, and *Wolinella*,[45] and the three species were once classified as *Campylobacter*. They are Gram-negative curved or S-shaped rods that are quite similar to the campylobacters except they can grow at 15°C and are aerotolerant. They are found in poultry, raw milk, shellfish, and water; and in cattle and swine products.[51,52] These oxidase- and catalase-positive organisms cause abortion and enteritis in some animals, and the latter in humans is associated with *A. butzleri*.

Bacillus (ba • cil'lus). These are Gram-positive spore-forming rods that are aerobes in contrast to the clostridia, which are anaerobes. Although most are mesophiles, psychrotrophs and thermophiles exist. The genus contains only two pathogens: *B. anthracis* (cause of anthrax) and *B. cereus*. Although most strains of the latter are nonpathogens, some cause foodborne gastroenteritis (further discussed in Chapter 24). This genus has been delimited by the transfer of a number of its former species to eight new genera: *Alicyclobacillus*, *Aneurinibacillus*, *Brevibacillus*, *Gracilibacillus*, *Paenibacillus*, *Virgibacillus*, and *Salibacillus*.[5] Also, the former group 5 *Bacillus* species are now in the genus *Geobacillus*, and the former *B. stearothermophilus* is now *G. stearothermophilus*.[36]

Brevibacillus (Bre • vi • ba • cil'lus). Previously classified as *Bacillus* spp. as noted above, these organisms occur in soil and water, and are common on plants, and in air, and dust. At least nine species are recognized.

Brochothrix (bro • cho • thr'ix; Gr. *brochos*, loop; *thrix*, thread). These Gram-positive nonsporeforming rods are closely related to the genera *Lactobacillus* and *Listeria*,[40] and some of the common features are discussed in Chapter 25. Although they are not true coryneforms, they bear resemblance to this group. Typically, exponential-phase cells are rods, and older cells are coccoids, a feature typical of coryneforms. Their separate taxonomic status has been reaffirmed by rRNA data, although only two species are recognized: *B. thermosphacta* and *B. campestris*. They share some features with the genus *Microbacterium*. They are common on processed meats and on fresh and processed meats that are stored in gas-impermeable packages at refrigerator temperatures. In contrast to *B. thermosphacta*, *B. campestris* is rhamnose and hippurate positive.[44] The mol% G + C content of DNA is 36. They do not grow at 37°C.

Burkholderia (Burkholder • ia). Gram-negative rods that occur on plants (especially certain flowers), in raw milk, and cause vegetable spoilage. In a study of raw cow's milk in Northern Ireland, 14 out of 26 (54%) samples contained *B. cepacia*.[34] They are significant pathogens in cystic fibrosis patients. They were formerly classified in the genus *Pseudomonas*.

Campylobacter (cam • py' • lo • bac • ter; Gr. *campylo*, curved). Although most often pronounced "camp'lo • bac • ter," the technically correct pronunciation should be noted. These Gram-negative, spirally curved rods were formerly classified as vibrios. They are microaerophilic to anaerobic. The genus has been restructured since 1984. The once *C. nitrofigilis* and *C. cryaerophila* have been transferred to the new genus *Arcobacter*; the once *C. cinnaedi* and *C. fenneliae* are now in the genus *Helicobacter*; and the once *Wolinella carva* and *W. recta* are now *C. curvus* and *C. rectus*.[45] The mol% G + C content of DNA is 30–35. For more information, see reference 32 and Chapter 28.

Carnobacterium (car • no • bac • terium; L. *carnis*, of flesh-meat bacteria). This genus of Gram-positive, catalase-negative rods was formed to accommodate some organisms previously classified as lactobacilli. They are phylogenetically closer to the enterococci and vagococci than the lactobacilli.[6,12] They are heterofermentative, and most grow at 0°C and none at 45°C. Gas is produced from glucose by some species, and the mol% G + C for the genus is 33.0–37.2. They differ from the lactobacilli in being unable to grow on acetate medium and in their synthesis of oleic acid. They are found on vacuum-packaged meats and related products, as well as on fish and poultry meats.[11,23,48]

Citrobacter (cit • ro • bac'ter). These enteric bacteria are slow lactose-fermenting, Gram-negative rods that typically produce yellow colonies on plate count agar. All members can use citrate as the sole carbon source. *C. freundii* is the most prevalent species in foods, and it and the other species are not uncommon on vegetables and fresh meats. The mol% G + C content of DNA is 50–52.

Clostridium (clos • tri'di • um; Gr. *closter*, a spindle). These anaerobic spore-forming rods are widely distributed in nature, as are their aerobic counterparts, the bacilli. The genus contains many species, some of which cause disease in humans (see Chapter 24 for *C. perfringens* food poisoning and botulism). Mesotrophic, psychrotrophic, and thermophilic species/strains exist; their importance in the thermal canning of foods is discussed in Chapter 17. A reorganization of the genus created the following five new genera: *Caloramater*, *Filifactor*, *Moorella*, *Oxobacter*, and *Oxalophagus*.[8] The clostridial species of known importance in foods remain in the genus at this time. The five new genera appear to be unimportant in foods.

Corynebacterium (co • ry • ne • bac • ter' • i • um; Gr. *coryne*, club). This is one of the true coryneform genera of Gram-positive, rod-shaped bacteria that are sometimes involved in the spoilage of vegetable and meat products. Most are mesotrophs, although psychrotrophs are known, and one, *C. diphtheriae*, causes diphtheria in humans. The genus has been reduced in species with the transfer of some of the plant pathogens to the genus *Clavibacter* and others to the genus *Curtobacterium*. The mol% G + C content of DNA is 51–63.

Enterobacter (en • te • ro • bac'ter). These enteric Gram-negative bacteria are typical of other Enterobacteriaceae relative to growth requirements, although they are not generally adapted to the gastrointestinal tract. They are further characterized and discussed in Chapter 20. *E. agglomerans* has been transferred to the genus *Pantoea*. *E. sakazakii* is discussed in Chapter 31.

Enterococcus (en • te • ro • coc'cus). This genus was erected to accommodate some of the Lancefield serologic group D cocci. It has since been expanded to more than 16 species of Gram-positive ovoid cells that occur singly, in pairs, or in short chains. They were once in the genus *Streptococcus*. Some species do not react with group D antisera. The genus is characterized more thoroughly in Chapter 20, and its phylogenetic relationship to other lactic acid bacteria can be seen in Figure 25–1.

Erwinia (er • wi'ni • a). These Gram-negative enteric rods are especially associated with plants. At least three species have been transferred to the genus *Pantoea*,[33] and the former *E. carotovora* and *E. chrysanthemi* are now in the genus *Pectobacterium* as *P. carovovorum* and *P. chrysanthemi* (see Chapter 6).

Escherichia (esch • er • i'chi • a). This is clearly the most widely studied genus of all bacteria. Those strains that cause foodborne gastroenteritis are discussed in Chapter 27, and *E. coli* as an indicator of food safety is discussed in Chapter 20.

Flavobacterium (fla • vo • bac • te'ri • um). These Gram-negative rods are characterized by their production of yellow to red pigments on agar and by their association with plants. Some are mesotrophs, and others are psychrotrophs, where they participate in the spoilage of refrigerated meats and vegetables. Some of the former flavobacterial species have been placed in the following five new genera: *Empedobacter*, *Chryseobacterium*, *Myroides*, *Sphingomonas*, and *Sphingobacterium*.

Hafnia (haf'ni • a). These Gram-negative enteric rods are important in the spoilage of refrigerated meat and vegetable products; *H. alvei* is the only species at this time. It is motile and lysine and ornithine positive, and it has a mol% G + C content of DNA of 48–49.

Kocuria (Ko • cu'ri • a, after M. Kocur). A new genus split off from the genus *Micrococcus*.[42] The three species (*K. rosea*, *K. varians*, and *K. kristinae*) are oxidase negative and catalase positive, and the mol% G + C content of DNA is 66–75.

Lactobacillus (lac • to • ba • cil'lus). Taxonomic techniques that came into wide use during the 1980s have been applied to this genus, resulting in some of those in the ninth edition of *Bergey's Manual* being transferred to other genera. Based on 16S rRNA sequence data, three phylogenetically distinct clusters are revealed,[10] with one cluster encompassing *Weissella*. In all probability, this genus will undergo further reclassification. They are Gram-positive, catalase-negative rods that often occur in long chains. Although those in foods are typically microaerophilic, many true anaerobic strains exist, especially in the colon and the rumen. They typically occur on most, if not all, vegetables, along with some of the other lactic acid bacteria. Their occurrence in dairy products is common. One species, *L. suebicus*, was recovered from apple and pear mashes; it grows at pH 2.8 and in 12–16% ethanol.[28] Many fermented products are produced, and these are discussed in Chapter 7. Those that are common on refrigerator-stored, vacuum-packaged meats are discussed in Chapters 5 and 14.

Lactococcus (lac • to • coc'cus). The nonmotile Lancefield serologic group N cocci once classified in the genus *Streptococcus* have been elevated to generic status. They are Gram-positive, nonmotile, and catalase-negative, spherical, or ovoid cells, that occur singly, in pairs, or as chains. They grow at 10°C but not at 45°C, and most strains react with group N antisera. L-Lactic acid is the predominant end-product of fermentation.

Leuconostoc (leu • co • nos'toc; *colorless nostoc*). Along with the lactobacilli, this is another of the genera of lactic acid bacteria. They are Gram-positive, catalase-negative cocci that are heterofermentative. The genus has been reduced in number of species (see *Weissella* below). The former *L. oenos* has been transferred to a new genus, *Oenococcus* as *O. oeni*,[16] and the former *L. paramesenteroides* has been transferred to the new genus *Weissella*. These cocci are typically found in association with the lactobacilli.

Listeria (lis • te'ri • a). This genus of six species of Gram-positive, nonsporing rods is closely related to *Brochothrix*. The six species show 80% similarity by numerical taxonomic studies; they have identical cell walls, fatty acid, and cytochrome composition. They are more fully described and discussed in Chapter 25.

Micrococcus (mi • cro • coc'cus). These Gram-positive and catalase-positive cocci are inhabitants of mammalian skin and can grow in the presence of high levels of NaCl. This genus has been reduced by the creation of the following five new genera: *Dermacoccus*, *Kocuria*, *Kytococcus*, *Nesterenkonia*, and *Stomatococcus*. At the present time, *M. luteus* and *M. lylae* are the only two micrococcal species.

Moraxella (mo • rax • el'la). These short Gram-negative rods are sometimes classified as *Acinetobacter*. They differ from the latter in being sensitive to penicillin and oxidase positive and having a mol% G + C content of DNA of 40–46. The genus *Psychrobacter* includes some that were once placed in this genus. Their metabolism is oxidative, and they do not form acid from glucose.

Paenibacillus (pae • ba • cil'lus; *almost a bacillus*). This newly established genus comprises organisms formerly in the genera *Bacillus* and *Clostridium*, and it includes the following species: *P. alvei*, *P. amylolyticus*, *P. azotofixans*, *P. circulans*, *P. durum*, *P. larvae*, *P. macerans*, *P. macquariensis*, *P. pubuli*, *P. pulvifaciens*, and *P. validus*.[2,8] Recently, two new species were added (*P. lautus* and *P. peoriae*[22]). The paenibacilli are notable for their degradation of a number of macromolecules, their production of antibacterial and antifungal agents, and the capacity of some to fix N_2 in association with plants. A newly-named species was isolated from raw and UHT-treated milk.[38]

Pandoraea (Pan • do • rae'a). Although first isolated from sputa of cystic fibrosis patients,[7] these organisms are related to some of the pseudomonads. Although not demonstrated to be common in foods, one species, *P. norimbergenesis*, has been isolated from powdered milk.[35]

Pantoea (pan • toe'a). This genus consists of Gram-negative, noncapsulated, nonsporing straight rods, most of which are motile by peritrichous flagella. They are widely distributed and are found on plants and in seeds, in soil, water, and human specimens. Some are plant pathogens. The four recognized species were once classified as enterobacters or erwinias. *P. agglomerans* includes the former *Enterobacter agglomerans*, *Erwinia herbicola*, and *E. milletiae*; *P. ananas* includes the former *Erwinia ananas* and *E. uredovora*; *P. stewartii* was once *E. stewartii*; and *P. dispersa* is an original species.[18] The G + C content of DNA ranges from 49.7 to 60.6 mol%.[33]

Pediococcus (pe • di • o • coc'cus; *coccus growing in one plane*). These homofermentative cocci are lactic acid bacteria that exist in pairs and tetrads resulting from cell division in two planes. *P. acidilactici*, a common starter species, caused septicemia in a 53-year-old male.[19] Their mol% G + C content of DNA is 34–44; they are further discussed in Chapter 7. The once *P. halophilus* is now in the genus *Tetragenococcus* as *T. halophilus*. It can grow in 18% NaCl.

Proteus (pro'te • us). These enteric Gram-negative rods are aerobes that often display pleomorphism, hence the generic name. All are motile and typically produce swarming growth on the surface of moist agar plates. They are typical of enteric bacteria in being present in the intestinal tract of humans and animals. They may be isolated from a variety of vegetable and meat products, especially those that undergo spoilage at temperatures in the mesophilic range.

Pseudomonas (pseu • do'mo • nas; *false monad*). These are typical soil and water bacteria and they are widely distributed among fresh foods, especially vegetables, meats, poultry, and seafood products. Although once the largest genus of foodborne bacteria, the genus has been delimited by the transfer of many former species to at least 13 new genera: *Acidovorax*, *Aminobacter*, *Brevundimonas*, *Burkholderia*, *Comamonas*, *Delftia*, *Devosia*, *Herbaspirillium*, *Hydrogenophaga*, *Marinobacter*, *Ralstonia*, *Sphingomonas*, *Telluria*, and *Wautersia*. *P. fluorescens* and *P. aeruginosa* remain in the original genus (see reference 24).

Psychrobacter (psy • chro' • bac • ter). This genus was created primarily to accommodate some of the nonmotile Gram-negative rods that were once classified in the genera *Acinetobacter* and *Moraxella*. They are plump coccobacilli that often occur in pairs. Also, they are aerobic, nonmotile, and catalase and oxidase positive, and generally do not ferment glucose. Growth occurs in 6.5% NaCl and at 1°C, but generally not at 35°C or 37°C. They hydrolyze Tween 80, and most are egg-yolk positive (lecithinase). They are sensitive to penicillin and utilize γ-aminovalerate, whereas the acinetobacters do not. They are distinguished from the acinetobacters by being oxidase positive and aminovalerate users and from nonmotile pseudomonads by their inability to utilize glycerol or fructose. Because they closely resemble the moraxellae, they have been placed in the family Neisseriaceae. The genus contains some of the former achromobacters and moraxellae, as noted. They are common on meats, poultry, and fish, and in water.[26,39]

Salmonella (sal • mon • el'la). All members of this genus of Gram-negative enteric bacteria are considered to be human pathogens. It should be noted that the salmonellae have been placed in two species with those that affect humans placed in the species *Salmonella enterica*. The serotypes (serovars) of more than 2,400 are listed as follows: *Salmonella enterica* serotype Newport, or *Salmonella* Newport (note that the serotype is not italicized). (See Chapter 26 for a more detailed explanation.) The mol% G + C content of DNA is 50–53.

Serratia (ser • ra'ti • a). These Gram-negative rods that belong to the family Enterobacteriaceae are aerobic and proteolytic, and they generally produce red pigments on culture media and in certain foods, although nonpigmented strains are not uncommon. *S. liquefaciens* is the most prevalent of the foodborne species; it causes spoilage of refrigerated vegetables and meat products. The mol% G + C content of DNA is 53–59. Interestingly, a spore-forming *S. marcescens* isolate has been reported and named *S. marcescens* subsp. *sakuensis*.[1]

Shewanella (she • wa • nel'la). The bacterium once classified as *Pseudomonas putrefaciens* and later as *Alteromonas putrefaciens* has been placed in this genus as *S. putrefaciens*. They are Gram-negative, straight or curved rods, nonpigmented, and motile by polar flagella. They are oxidase positive and have a mol% G + C of 44–47. The other three species in this genus are *S. hanedai*, *S. benthica*, and *S. colwelliana*. All are associated with aquatic or marine habitats, and the growth of *S. benthica* is enhanced by hydrostatic pressure.[14,32]

Shigella (shi • gel'la). All members of this genus are presumed to be human enteropathogens; they are discussed further in Chapter 26.

Sphingomonas (Sphin • go • monas). There are at least 33 species of this genus of Gram-negative bacteria that typically produce yellow pigment, and which formerly were in the genus *Flavobacterium*. They are found in water, on certain vegetables, and cause human disease.[58]

Staphylococcus (staph • y • lo • coc'cus; *grape-like coccus*). These Gram-positive, catalase-positive cocci include *S. aureus*, which causes several disease syndromes in humans, including foodborne gastroenteritis. It and other members of the genus are discussed further in Chapter 23. The former *S. caseolyticus* has been transferred to the new genus *Macrococcus* as *M. caseolyticus*.[29]

Stenotrophomonas (Ste • no • tro • pho • mo'nas, a unit feeding on few substrates). These Gram-negative rods are common inhabitants of plants and they have been recovered from soil, water, and milk. They are growth-promoting or symbionts in the rhizosphere of several crop plants.[21] *S. maltophila* is regarded as the second most common nosocomial bacterium after *Pseudomonas aeruginosa* (see reference 21). One species, *S. rhizophila*, has been used to control fungal diseases in plants (see reference 57).

Vagococcus (va • go • coc'cus; *wandering coccus*). This genus was created to accommodate the group N lactococci based on 16S sequence data.[11] They are motile by peritrichous flagella, are Gram positive and catalase negative, and grow at 10°C but not at 45°C. They grow in 4% NaCl but not 6.5%, and no growth occurs at pH 9.6. The cell wall peptidoglycan is Lys-D-Asp, and the mol% G + C is 33.6. At least one species produces H_2S. They are found on fish, in feces, and in water and may be expected to occur on other foods.[11,47] Information on the phylogenetic relationship of the vagococci to other related genera is presented in Figure 25–1.

Vibrio (vib'ri • o). These Gram-negative straight or curved rods are members of the family Vibrionaceae. Several former species have been transferred to the genus *Listonella*.[32] Several species cause gastroenteritis and other human illness; they are discussed in Chapter 28. The mol% G + C content of DNA is 38–51. (See reference 13 for environmental distribution.)

Weissella (Weiss'ella, after N. Weiss). This genus of lactic acid bacteria was established in 1993 in part to accommodate the "leuconostoc branch" of the lactobacilli.[9] The seven species are closely related to the leuconostocs, and with the exception of *W. paramesenteroides* and *W. hellenica*, they produce DL-lactate from glucose. All produce gas from carbohydrates. *W. hellenica* is a new species associated with fermented Greek sausages.[9] The former *Leuconostoc paramesenteroides* is now *W. paramesenteroides*, and the following five species were formerly classified as *Lactobacillus* spp.: *W. confusa*, *W. halotolerans*, *W. kandleri*, *W. minor*, and *W. viridescens*. The G + C content of DNA is 37–47 mol%.

Yersinia (yer • si'ni • a). This genus includes the agent of human plague, *Y. pestis*, and at least one species that causes foodborne gastroenteritis, *Y. enterocolitica*. All foodborne species are discussed in Chapter 28. The mol% G + C content of DNA is 45.8–46.8. The sorbose-positive biogroup 3A strains have been elevated to species status as *Y. mollaretti* and the sorbose-negative strains as *Y. bercovieri*.[49]

SYNOPSIS OF COMMON GENERA OF FOODBORNE MOLDS

Molds are filamentous fungi that grow in the form of a tangled mass that spreads rapidly and may cover several inches of area in 2 to 3 days. The total of the mass or any large portion of it is referred to as *mycelium*. Mycelium is composed of branches or filaments referred to as *hyphae*. Those of greatest importance in foods multiply by ascospores, zygospores, or conidia. The *ascospores* of some genera are notable for their extreme degrees of heat resistance. One group forms pycnidia or acervuli (small, flask-shaped, fruiting bodies lined with conidiophores). *Arthrospores* result from the fragmentation of hyphae in some groups.

There were no radical changes in the systematics of foodborne fungi during the 1980s. The most notable changes involve the discovery of the sexual or perfect states of some well-known genera and species. In this regard, the *ascomycete* state is believed by mycologists to be the more important reproductive state of a fungus, and this state is referred to as the *teleomorph*. The species name given to a teleomorph takes precedence over that for the *anamorph*, the imperfect or conidial state. *Holomorph* indicates that both states are known, but the teleomorph name is used.

The taxonomic positions of the genera described are summarized below. (Consult references 3, 4, and 37 for identifications; see reference 25 for the types that exist in meats.)

Division: Zygomycota
  Class: Zygomycetes (nonseptate mycelium, reproduction by sporangiospores, rapid growth)
    Order: Mucorales
      Family: Mucoraceae
        Genus: *Mucor*
          Rhizopus
          Thamnidium

Division: Ascomycota
  Class: Plectomycetes (septate mycelium, ascospores produced in asci usually number 8)
    Order: Eurotiales
      Family: Trichocomaceae
        Genus: *Byssochlamys*
          Emericella
          Eupenicillium
          Eurotium

Division: Deuteromycota (the "imperfects," anamorphs; perfect stages are unknown)
- Class: Coelomycetes
  - Genus: *Colletotrichum*
- Class: Hypomycetes (hyphae give rise to conidia)
  - Order: Hyphomycetales
    - Family: Moniliaceae
      - Genus: *Alternaria*
        - *Aspergillus*
        - *Aureobasidium* (*Pullularia*)
        - *Botrytis*
        - *Cladosporium*
        - *Fusarium*
        - *Geotrichum*
        - *Helminthosporium*
        - *Monilia/Sclerotinium*
        - *Penicillium*
        - *Stachybotrys*
        - *Trichothecium*

Some of the genera are listed below in alphabetical order.

Alternaria. Septate mycelia with conidiophores and large brown conidia are produced. The conidia have both cross and longitudinal septa and are variously shaped. They cause brown to black rots of stone fruits, apples, and figs. Stem-end rot and black rot of citrus fruits are also caused by species/strains of this genus. This is a field fungus that grows on wheat. Additionally, it is found on red meats. Some species produce mycotoxins (see Chapter 30).

Aspergillus. Chains of conidia are produced. Where cleistothecia with ascospores are developed, the perfect stage of those found in foods is *Emericella*, *Eurotium*, or *Neosartorya*. *Eurotium* (the former *A. glaucus* group) produces bright yellow cleistothecia, and all species are xerophilic. *E. herbariorum* has been found to cause spoilage of grape jams and jellies.[41] *Emericella* produces white cleistothecia, and *E. nidulans* is the teleomorph of *Aspergillus nidulans*. *Neosartorya* produces white cleistothecia and colorless ascospores. *N. fischeri* is heat resistant, and resistance of its spores is similar to those of *Byssochlamys*.[37] The aspergilli appear yellow to green to black on a large number of foods. Black rot of peaches, citrus fruits, and figs is one of the fruit spoilage conditions produced. They are found on country-cured hams and on bacon. Some species cause spoilage of oils, such as palm, peanut, and corn. *A. oryzae* and *A. soyae* are involved in the shogu fermentation and the former in koji. *A. glaucus* produces katsuobushi, a fermented fish product. The *A. glaucus–A. restrictus* group contains storage fungi that invade seeds, soybeans, and common beans. *A. niger* produces β-galactosidase, glucoamylase, invertase, lipase, and pectinase: and *A. oryzae* produces α-amylase. Several species produce aflatoxins, and others produce ochratoxin A and sterigmatocystin (see Chapter 30).

Aureobasidium (Pullularia). Yeast-like colonies are produced initially. They later spread and produce black patches. *A. pullulans* (*Pullularia pullulans*) is the most prevalent in foods. They are found in shrimp, are involved in the "black spot" condition of long-term stored beef, and are common on fruits and vegetables.

Botrytis. Long, slender, and often pigmented conidiophores are produced. Mycelium is septate; conidia are borne on apical cells and are gray in color, although black, irregular sclerotia are sometimes produced. *B. cinerea* is the most common in foods. They are notable as the cause of gray mold rot of apples, pears, raspberries, strawberries, grapes, blueberries, citrus, and some stone fruits (see Chapter 6).

Byssochlamys. This genus is the teleomorph of certain species of *Paecilomyces*, but the latter does not occur in foods.[37] The ascomycete *Byssochlamys* produces open clusters of asci, each of which contains eight ascospores. The latter are notable for their heat resistance, resulting in spoilage of some high-acid canned foods. In their growth, they can tolerate low oxidation–reduction potential (Eh) values. Some are pectinase producers, and *B. fulva* and *B. nivea* spoil canned and bottled fruits. These organisms are almost uniquely associated with food spoilage, and *B. fulva* possesses a thermal D value at 90°C between 1 and 12 minutes with a z value of 6–7°C.[37]

Cladosporium. Septate hyphae with dark, tree-like, budding conidia variously branched, characterize this genus. In culture, growth is velvety and olive colored to black. Some conidia are lemon shaped. *C. herbarum* produces "black spot" on beef and frozen mutton. Some spoil butter and margarine, and some cause restricted rot of stone fruits and black rot of grapes. They are field fungi that grow on barley and wheat grains. *C. herbarum* and *C. cladosporiodes* are the two most prevalent on fruits and vegetables.

Colletotrichum. They belong to the class Coelomycetes and form conidia inside acervuli. Simple but elongated conidiophores and hyaline conidia that are one celled, ovoid, or oblong are produced. The acervuli are disc or cushion shaped, waxy, and generally dark in color. *C. gloeosporioides* is the species of concern in foods; it produces anthracnose (brown/black spots) on some fruits, especially tropical fruits such as mangos and papayas.

Fusarium. Extensive mycelium is produced that is cottony with tinges of pink, red, purple, or brown. Septate fusiform to sickle-shaped conidia (macroconidia) are produced. They cause brown rot of citrus fruits and pineapples and soft rot of figs. As field fungi, some grow on barley and wheat grains. Some species produce zearalenone, fumonisins, and trichothecenes (see Chapter 30).

Geotrichum (once known as *Oidium lactis* and *Oospora lactis*). These yeast-like fungi are usually white. The hyphae are septate, and reproduction occurs by formation of arthroconidia from vegetative hyphae. The arthroconidia have flattened ends. *G. candidum*, the anamorph of *Dipodascus geotrichum*, is the most important species in foods. It is variously referred to as "dairy mold" because it imparts flavor and aroma to many types of cheese, and as "machinery mold" because it builds up on food-contact equipment in food-processing plants, especially tomato canning plants. They cause sour rot of citrus fruits and peaches and the spoilage of dairy cream. They are widespread and have been found on meats and many vegetables. Some participate in the fermentation of gari.

Monilia/Sclerotinium. Pink, gray, or tan conidia are produced. *M. sitophila* is the conidial stage of *Neurospora intermedia*. *Monilia* is the conidial state of *Monilinia fructicola*. They produce brown rot of stone fruits such as peaches. *Monilina* sp. causes mummification of blueberries.

Mucor. Nonseptate hyphae are produced that give rise to sporangiophores that bear columella with a sporangium at the apex. No rhizoids or stolons are produced by members of this large genus. Cottony colonies are often produced. The conditions described as "whiskers" of beef and "black spot" of frozen mutton are caused by some species. At least one species, *M. miehei*, is a lipase producer. It is found in fermented foods, bacon, and many vegetables. One species ferments soybean whey curd.

Penicillium. When conidiophores and conidia are the only reproductive structures present, this genus is placed in the Deuteromycota. They are placed with the ascomycetes when cleistochecia with ascospores are formed as either *Talaromyces* or *Eupenicillium*. Of the two teleomorphic genera, *Talaromyces* is the most important in foods.[37] *T. flavus* is the teleomorph of *P. dangeardii*, and it has been involved in the spoilage of fruit juice concentrates.[27] It produces heat-resistant spores. When conidia are formed in the penicillus, they pinch off from *phialides*. Typical colors on foods are blue to blue-green. Blue and green mold rots of citrus fruits and blue mold rot of apples, grapes, pears, and stone fruits, are caused by some species. One species, *P. roqueforti*, produces blue cheese. Some species produce citrinin, yellow rice toxin, ochratoxin A, rubratoxin B, and other mycotoxins (see Chapter 30).

Rhizopus. Nonseptate hyphae are produced that give rise to stolons and rhizoids. Sporangiophores typically develop in clusters from ends of stolons at the point of origin of rhizoids. *R. stolonifer* is by far the most common species in foods. Sometimes referred to as "bread molds," they produce watery soft rot of apples, pears, stone fruits, grapes, figs, and others. Some cause "black spot" of beef and frozen mutton. They may be found on bacon and other processed meats. Some produce pectinases, and *R. oligosporus* is important in the production of oncom, bongkrek, and tempeh.

Thamnidium. These molds produce small sporangia borne on highly branched structures. *T. elegans* is the only species, and it is best known for its growth on refrigerated beef hindquarters where its characteristic growth is described as "whiskers." It is less often found in decaying eggs.

Trichothecium. Septate hyphae that bear long, slender, and simple conidiophores are produced. *T. roseum* is the only species, and it is pink in color and causes pink rot of fruits. It also causes soft rot of cucurbits and is common on barley, wheat, corn, and pecans. Some produce mycotoxins (see Chapter 30).

Other Molds. Two categories of organisms are presented here, the first being some miscellaneous genera that are found in some foods but are generally not regarded as significant. These are *Cephalosporium*, *Diplodia*, and *Neurospora*. *Cephalosporium* is a deuteromycete often found on frozen foods. The microspores of some *Fusarium* species are similar to those of this genus. *Diplodia* is another deuteromycete that causes stem-end rot of citrus fruits and water tan-rot of peaches. *Neurospora* is an ascomycete, and *N. intermedia* is referred to as the "red bread" mold. *Monilia sitophila* is the anamorph of *N. intermedia*. The latter is important in the oncom fermentation and has been found on meats. The "white spot" of beef is produced by *Sporotrichum* spp.; and rots of various fruits are caused by *Gloeosporium* spp. Some *Helminthosporium* spp. are plant pathogens and some are saprophytes.

Neosartorya fischeri (anamorph *Aspergillus fischerianus*) was first recognized in the early 1960s as the cause of spoilage of fruit products. Its ascospores are very heat resistant, being able to withstand boiling in distilled water for up to an hour. It has a D_{87} C of around 11 minutes in phosphate buffer. Interestingly, it produces several mycotoxins—fumitremorgin A, B, and C; terrein; verruculogen; and fischerin.

The second category consists of xerophilic molds, which are very important as spoilage organisms. In addition to *Aspergillus* and *Eurotium*, Pitt and Hocking[37] include six other genera among the xerophiles:

Basipetospora, *Chrysosporium*, *Eremascus*, *Polypaecilum*, *Wallemia*, and *Xeromyces*. These molds are characterized by the ability to grow below a_w (water activity) = 0.85. They are of significance in foods that owe their preservation to a low a_w. Only *Wallemia* and *Xeromyces* are discussed further below.

Wallemia produces deep-brown colonies on culture media and on foods. *W. sebi* (formerly *Sporendonema*), the most notable species, can grow at an a_w of 0.69. It produces the "dun" mold condition on dried and salted fish.

Xeromyces has only one species, *X. bisporus*. It produces colorless cleistothecia with evanescent asci that contain two ascospores. This organism has the lowest a_w growth of any other known organisms.[37] Its a_w high is ~0.97, its optimum is 0.88, and its minimum is 0.61. Its thermal *D* at 82.2°C is 2.3 minutes. It causes problems in licorice, prunes, chocolate, syrup, and other similar products.

SYNOPSIS OF COMMON GENERA OF FOODBORNE YEASTS

Yeasts may be viewed as being unicellular fungi in contrast to the molds, which are multicellular; however, this is not a precise definition, as many of what are commonly regarded as yeasts actually produce mycelia to varying degrees.

Yeasts can be differentiated from bacteria by their larger cell size and their oval, elongate, elliptical, or spherical cell shapes. Typical yeast cells range from 5 to 8 μm in diameter, with some being even larger. Older yeast cultures tend to have smaller cells. Most of those of importance in foods divide by budding or fission.

Yeasts can grow over wide ranges of acid pH and in up to 18% ethanol. Many grow in the presence of 55–60% sucrose. Many colors are produced by yeasts, ranging from creamy, to pink, to red. The *asco-* and *arthrospores* of some are quite heat resistant. (Arthrospores are produced by some yeast-like fungi.)

Regarding the taxonomy of yeasts, newer methods have been employed in the past decade or so consisting of 5S rRNA, DNA base composition, and coenzyme Q profiles. Because of the larger genome size of yeasts, 5S rRNA sequence analyses are employed more than for larger RNA fractions. Many changes have occurred in yeast systematics, due in part of the use of newer methods but also to what appears to be a philosophy toward grouping rather than splitting taxa. One of the most authoritative works on yeast systematics is that edited by Kreger-van Rij and published in 1984.[30] In this volume, the former *Torulopsis* genus has been transferred to the genus *Candida*, and some of the former *Saccharomyces* have been transferred to *Torulaspora* and *Zygosaccharomyces*. The teleomorphic or perfect states of more yeasts are now known, and this makes references to the older literature more difficult.

The taxonomy of 15 or so foodborne genera is summarized below. For excellent discussions on foodborne yeasts, the publications by Deak and Beuchat,[15] Beneke and Stevenson,[3] and Pitt and Hocking[37] should be consulted. For identification, Deak and Beuchat[15] have presented an excellent simplified key to foodborne yeasts.

Division: Ascomycotina
- Family: Saccharomycetaceae (ascospores and arthrospores formed; vegetative reproduction by fission or budding)
  - Subfamily: Nadsonioideae
    - Genus: *Hanseniaspora*

Subfamily: Saccharomycotoideae
Genus: *Debaryomyces*
Issatchenkia
Kluyveromyces
Pichia
Saccharomyces
Torulaspora
Zygosaccharomyces
Subfamily: Schizosaccharomycetoideae
Genus: *Schizosaccharomyces*

Division: Deuteromycotina
Family: Cryptococcaceae (the "imperfects"; reproduce by budding)
Genus: *Brettanomyces*
Candida
Cryptococcus
Rhodotorula
Trichosporon

The above genera are listed below in alphabetical order.

Brettanomyces (The perfect stage is *Dekkera*). These asporogenous yeasts form ogival cells and terminal budding, and produce acetic acid from glucose only under aerobic conditions. *B. intermedius* is the most prevalent, and it can grow at a pH as low as 1.8. They cause spoilage of beer, wine, soft drinks, and pickles, and some are involved in afterfermentation of some beers and ales. *D. bruxellensis* is involved in some sourdough fermentations, and it contributes to biogenic amines in red wines.

Candida. This genus was erected in 1923 by Berkhout and has since undergone many changes in definition and composition.[46] It is regarded as being a heterogenous taxon that can be divided into 40 segments comprising 3 main groups, based mainly on fatty acid composition and electrophoretic karotyping.[47] The generic name means "shining white," and cells contain no carotenoid pigments. The ascomycetous imperfect species are placed here, including the former genus *Torulopsis*, as follows:

Candida famata (Torulopsis candida; T. famata)
Candida kefyr (Candida pseudotropicalis, T. kefyr; Torula cremoris)
Candida stellata (Torulopsis stellata)
Candida holmii (Torulopsis holmii)

Many of the *anamorphic* forms of *Candida* are now in the genera *Kluyveromyces* and *Pichia*.[15] *Candida lipolytica* is the anamorph of *Saccharomycopsis lipolytica*.

Members of this genus are the most common yeasts in fresh ground beef and poultry, and *C. tropicalis* is the most prevalent in foods in general. Some members are involved in the fermentation of cacao beans, as a component of kefir grains, and in many other products, including beers, ales, and fruit juices.

Cryptococcus. This genus represents the anamorph of *Filobasidiella* and other *Basidiomycetes*. They are asporogenous, reproduce by multilateral budding, and are nonfermenters of sugars. They are hyaline and red or orange, and they may form arthrospores. They have been found on plants, in soils, on strawberries and other fruits, marine fish, shrimp, and fresh ground beef.

Debaryomyces. These ascosporogenous yeasts sometimes produce a pseudomycelium and reproduce by multilateral budding. They are one of the two most prevalent yeast genera in dairy products. *D. hansenii* represents what was once *D. subglobosus* and *Torulaspora hansenii*, and it is the most prevalent foodborne species. It can grow in 24% NaCl and at an a_w as low as 0.65. It forms slime on wieners, grows in brines and on cheeses, and causes spoilage of orange juice concentrate and yogurt.

Hanseniaspora. These are apiculate yeasts whose anamorphs are *Kloeckera* spp. They exhibit bipolar budding, and, consequently, lemonshaped cells are produced. The asci contain two to four hat-shaped spores. Sugars are fermented, and they can be found on a variety of foods, especially figs, tomatoes, strawberries, citrus fruits, and the cacao bean fermentation.

Issatchenkia. Members of this genus produce pseudomycelia and multiply by multilateral budding. Some species once in the genus *Pichia* have been placed here. The teleomorph of *Candida krusei* is *I. orientalis*. They typically form pellicles in liquid media. They contain coenzyme Q-7 and are prevalent on a wide variety of foods.

Kluyveromyces (*Fabospora*). These ascospore-forming yeasts reproduce by multilateral budding, and the spores are spherical. *K. marxianus* now includes the former *K. fragilis*, *K. lactis*, *K. bulgaricus*, *Saccharomyces lactis*, and *S. fragilis*. *K. marxianus* is one of the two most prevalent yeasts in dairy products. *Kluyveromyces* spp. produce β-galactosidase and are vigorous fermenters of sugars, including lactose. *K. marxianus* contains coenzyme Q-6 and is involved in the fermentation of kumiss. It is also used for lactase production from whey and as the organism of choice for producing yeast cells from whey. They are found on a wide variety of fruits, and *K. marxianus* causes cheese spoilage.

Pichia. This is the largest genus of true yeasts. They reproduce by multilateral budding, and the asci usually contain four spheroidal, hat- or saturn-shaped spores. Pseudomycelia and arthrospores may be formed. Some of the hat-shaped spore formers may be *Williopsis* spp., and some of the former species are now classified in the genus *Debaryomyces*. *P. guilliermondii* is the perfect state of *Candida guillermondii*. The anamorph of *P. membranaefaciens* is *Candida valida*. *Pichia* spp. typically form films on liquid media and are known to be important in producing indigenous foods in various parts of the world. Some have been found on fresh fish and shrimp, and they are known to grow in olive brines and to cause spoilage of pickles and sauerkraut.

Rhodotorula. These yeasts are anamorphs of Basidiomycetes. The teliospore producers are in the genus *Rhodosporidium*. They reproduce by multilateral budding and are nonfermenters. *R. glutinis* and *R. mucilaginosa* are the two most prevalent species in foods. They produce pink to red pigments, and most are orange or salmon pink in color. The genus contains many psychrotrophic species/strains that are found on fresh poultry, shrimp, fish, and beef. Some grow on the surface of butter.

Saccharomyces. These ascosporogenous yeasts multiply by multilateral budding and produce spherical spores in asci. They are diploid and do not ferment lactose. Those once classified as *S. bisporus* and *S. rouxii* are now in the genus *Zygosaccharomyces*, and the former *S. rosei* is now in the genus *Torulaspora*. All bakers', brewers', wine, and champagne yeasts are *S. cerevisiae*. They are found in kefir grains and can be isolated from a wide range of foods, such as dry-cured salami and numerous fruits. *S. cerevisiae* rarely causes spoilage.

Schizosaccharomyces. These ascosporogenous yeasts divide by lateral fission of cross-wall formation and may produce true hyphae and arthrospores. Asci contain from four to eight bean-shaped spores, and no buds are produced. They are regarded as being only distantly related to the true yeasts. *S. pombe* is the most prevalent species; it is osmophilic and resistant to some chemical preservatives.

Torulaspora. Multilateral budding is the method of reproduction with spherical spores in asci. Three haploid species formerly in the genus *Saccharomyces* are now in this genus. They are strong fermenters of sugars, and contain coenzyme Q-6. *T. delbrueckii* is the most prevalent species.

Trichosporon. These nonascospore-forming oxidative yeasts multiply by budding and by arthroconidia formation. They produce a true mycelium, and sugar fermentation is absent or weak. They are involved in cacao bean and idli fermentations and have been recovered from fresh shrimp, ground beef, poultry, frozen lamb, and other foods. *T. pullulans* is the most prevalent species, and it produces lipase.

Yarrowia. Formerly *Saccharomycopsis*, these yeasts belong to the order *Endomycetales* and they are common on fruits, vegetables, meats, and poultry. *Candida lipolytica* is the anamorph, and *Y. lipolytica* is the teleomorphic (perfect) stage.

Zygosaccharomyces. Multilateral budding is the method of reproduction, and the bean-shaped ascospores formed are generally free in asci. Most are haploid and they are strong fermenters of sugars. *Z. rouxii* is the most prevalent species, and it can grow at an a_w of 0.62, second only to *Xeromyces bisporus* in its ability to grow at a low a_w.[37] Some are involved in shoyu and miso fermentations, and some are common spoilers of mayonnaise and salad dressing, especially *Z. bailii*, which can grow at a pH of 1.8.[37]

REFERENCES