Bacterial and Actinomycete Flora of Kansas-Nebraska and Pacific Northwest Wheat and Wheat Flour[1]

R. R. GRAVES, RUTH F. ROGERS, A. J. LYONS, Jr., and C. W. HESSELTINE,
Northern Regional Research Laboratory, Peoria, Illinois

ABSTRACT

Wheat and flour samples from 11 representative flour mills in the Kansas-Nebraska and Pacific Northwest wheat-producing areas were examined for their bacteriological content. Examinations were made for total aerobic bacteria, psychrophiles, fecal streptococci, salmonellae, coagulase-positive staphylococci, catalase-negative bacteria, thermophilic spores, and actinomycetes. The standard method for estimating total aerobic bacteria was improved through the use of cycloheximide (Actidione) as a fungal inhibitor. In general, total and differential counts were low. Counts for total bacteria ranged from 220 to 20,000/g. in flour and from 15,000 to 660,000 in wheat. These counts were consistently lower in the finished flours than in the parent grains. Actinomycete counts ranged from 0 to 5,300/g. of flour and from 0 to 300/g. of wheat. Tests for salmonellae and coagulase-positive staphylococci were negative in the samples examined.

Some 532 cultures of bacteria were isolated for taxonomic studies. The most frequently encountered and widely distributed bacteria in wheat belonged to the genus *Flavobacterium*; in flour the dominant organism was *Paracolobactrum aerogenoides*. Of the 228 actinomycete cultures isolated, *Streptomyces albus* predominated.

Literature reviews by Semeniuk (1) and by Hesseltine and Graves (2) reveal that most of the bacteriological studies of domestic and Canadian wheats and flours were made during the first four decades of the century, a period when spoilage of breads by rope-producing bacteria was more common than it is today. Ropiness of bread, a type of spoilage caused by a mucoid variant of *Bacillus subtilis,* is now rare in commercially baked breads, primarily because of the use of calcium or sodium propionates in the flour. The treatment of wheat with chlorine at the tempering stage in milling has also been instrumental in reducing the incidence of "rope" in bread.

Renewed interest in the bacteriology of wheat and flour in recent years has come mainly from an expanding market for flour in refrigerated and frozen foods. Because flour is a major raw ingredient in many of these nonsterile foods, an evaluation of the numbers and kinds of microorganisms contributed by it is important to the milling industry. Processors are beginning to request that the miller guarantee the suitability of his flour for these special foods, and some of the larger ones are specifying flours with a maximum bacterial count of 5,000 per g. According to Doty (3), maximum bacterial counts being specified are 15,000 per g. for use in canned biscuits, 10,000 per g. for frozen fruit pies, and 5,000 per g. for "TV dinners" and frozen meat pies.

Bacterial counts reported in the literature for flour vary considerably, ranging from less than 1,000 per g. to more than 5 million (4-10). Com-

[1]Contribution from the Northern Regional Research Laboratory, Peoria, Illinois 61604. This is a laboratory of the Northern Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture. Mention of firm names or trade products does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

paratively little information is available regarding numbers and kinds of bacteria in wheat itself. In 19 samples of soft red winter wheat examined by Gustafson and Parfitt (6), counts ranged from 46,000 to 3,260,000 per g. James, Wilson, and Stark (11) found that bacterial populations on samples of Manitoba Northern wheat ranged from 43,000 to 108,000 per g. They noted that two types of bacteria consistently predominated and suggested that these species be considered epiphytic on wheat. One organism was identified as *Bacterium herbicola aureum* Duggeli *(Flavobacterium herbicola* Mack) (12), and the other as a species of *Pseudomonas.* Later, James (13) studied in detail the yellow chromogen originally classified as *B. herbicola aureum* Duggeli and concluded that it belonged in the genus *Xanthomonas.* He proposed the name *Xanthomonas trifolii* (Huss) comb. nov.

To establish the present microbiological status of domestic wheats and flours, a survey of wheats and commercial flours from various mills throughout the country is being made in co-operation with the Millers' National Federation. Six geographical areas representing a cross-section of the nation's major wheat-producing areas were selected for study. The bacteriology of wheats and flours from two of these study areas is reported here. A study of the fungi associated with flours from these same areas has been reported elsewhere (14).

MATERIALS AND METHODS

Sampling

Samples of unwashed wheats and of flours milled from them were received from representative mills in the Kansas-Nebraska and Pacific Northwest wheat-growing areas and prepared for examination as described in a previous paper (14). Samples from nine mills in Kansas-Nebraska and from two in the Pacific Northwest included: unwashed wheat, 11; patent flour, 13; first-clear flour, 3; and straight-grade flour, 3. Only two Pacific Northwest mills were involved in the survey, because they are the only ones processing locally grown wheat; the rest mill imported wheat.

Bacteriological Examination

The total bacterial population was estimated by preparing duplicate pour plates with Plate Count Agar (PCA, Difco) containing 100 p.p.m. cycloheximide (Actidione) (15) to inhibit fungal growth. The Seitz-filtered antibiotic solution was added to the melted, tempered agar before it was poured. Preliminary experiments showed that cycloheximide at this concentration had little or no effect on bacterial growth but suppressed fungal growth (Fig. 1). Incubation was at 32°C. for 3 days.

To enumerate psychrophilic bacteria, plates were poured with PCA plus cycloheximide and incubated at 5°C. for 10 days. As an indirect method for estimating the level of lactic acid bacteria present, catalase-negative bacteria were counted by inoculating 1-ml. quantities of diluted sample onto the dry surface of 7-day-old plates of Difco Orange Serum Agar (OSA) containing 100 p.p.m. cycloheximide. Plates poured a week in advance were dry enough to absorb the excess water of the 1-ml. inoculum. The plates were incubated at 32°C. for 3 days, and each colony was tested for catalase by adding a drop of hydrogen peroxide solution (10% v./v.). Actinomycete populations were

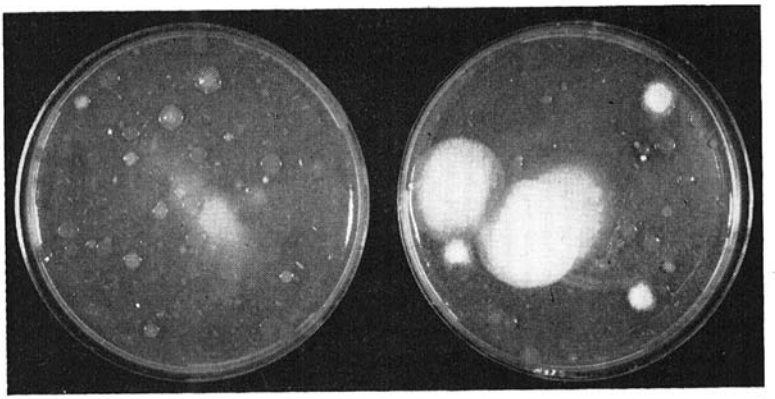

Fig. 1. Duplicate agar plates prepared from a 1:100 dilution of flour showing selective inhibition of fungi. Right, Plate Count Agar. Note presence of both mold and bacterial colonies. Left, Plate Count Agar plus 100 p.p.m. cycloheximide.

determined by surface-inoculating prepoured, 7-day-old plates of PCA plus cycloheximide. Colonies were counted after 14 days at 28°C. Although the fungal antibiotic virtually eliminates interference from yeasts and molds, bacteria grow uninhibited. After 2 weeks, however, the sporulating actinomycete colonies are easily distinguished from the dried, degenerate bacterial colonies. Counts of thermophilic spores, coagulase-positive staphylococci, salmonellae, and fecal streptococci were made according to the methods outlined in *Cereal Laboratory Methods* (16).

Isolation, Characterization, and Identification

Totals of 532 bacteria and 228 actinomycetes were isolated and purified for taxonomic study. Actinomycetes were isolated from the plates used for their enumeration and bacteria, from the plates used for determining the total bacterial population. An accurate appraisal of the relative incidence of each genus or species present in a given sample required that each colony developing on or in the agar medium be subcultured and identified. This appraisal was possible with actinomycetes, where colony numbers rarely exceeded 25 to 30 per plate, but was not practicable with bacteria, where the number of colonies was usually much greater. It was decided, therefore, that a reasonably accurate picture of species distribution could be obtained by subculturing and identifying a representative fraction of the total number of colonies present. With each sample, one of the plates used for the colony count was selected at random, and from it, 25 colonies were picked into tubes of Trypticase Soy Broth (BBL). A method described by Kirsch *et al.* (17) was used to select the colonies for subculturing. Purification was accomplished by streaking growth from the broth cultures onto plates of Trypticase Soy Agar (TSA, BBL) for single-colony isolations. Once purified, the cultures were maintained on TSA slants at 4°–5°C. Actinomycete colonies were subcultured and maintained on slants of either Asparagine Dextrose Agar (18) or Tomato Paste-Oatmeal Agar (19).

Methods based on those described in the *Manual of Microbiological*

Methods (20) were used to study the bacterial isolates. On the basis of morphological, cultural, and physiological characteristics, the cultures were grouped and enough replicates were eliminated to reduce to 265 the number of cultures to be studied. These were further characterized and classified into genera as far as feasible and into species where possible according to descriptions given in *Bergey's Manual of Determinative Bacteriology* (21).

Unless otherwise indicated, incubation of test media was at 28°C. Action on sugars and alcohols was observed in Purple Broth Base (Difco) containing 1% of the carbon source (lactose broth was incubated at 28°C. and 37°C.); the medium of Hugh and Leifson (22) was used to distinguish between oxidative and fermentative metabolism of carbohydrates. Protopectinase activity was detected by the method of Vaughn *et al.* (23). The cellulose-salts medium of Alarie and Gray (24) was used to demonstrate cellulolytic activity; observations were made weekly over a period of 35 days. Urea hydrolysis was tested in Urease Test Medium (BBL). Decomposition of alginate was determined according to the method of Waksman as modified by Skerman (25), and production of acetic acid from ethanol was detected on the agar medium of Shimwell, Carr, and Rhodes (26). Heterofermentative metabolism of glucose was tested in the tomato juice-gelatin medium of Gibson and Abd-El-Malek (27), and utilization of $NH_4H_2PO_4$, on the agar medium recommended by Hucker (28). Difco's Coagulase Plasma was used to determine coagulase activity. Leifson's method was used to determine flagellation; pigmentation was noted on TSA, potato slants, and on the "B" medium of King, Ward, and Raney (29). Growth at 37°C. (air temperature) and at 42°C. (water-bath temperature) was determined by observing slant cultures of Tryptone-Glucose-Yeast Extract Agar (TGY) (18) for growth at intervals up to 1 week. Each culture was carried through three transfers at each temperature; only those cultures showing growth on the third transfer were considered positive. Methods described by Smith, Gordon, and Clark (30) were used to study the aerobic spore-forming bacteria. Actinomycete isolates were studied by the methods described by Lyons and Pridham (31).

RESULTS

Total and Differential Counts

Results from preliminary studies of flour milled from the 1963 wheat crop (hard red winter) in the Kansas-Nebraska area are given in Table I. In general, total bacterial counts were low, the low-grade flours (high ash content) having a higher count, in most instances, than the high-grade flours (low ash content). Correlation between ash content, flour grade, and bacterial numbers has been reported elsewhere (6,8). Of the seven samples examined for psychrophiles, only one (14%) contained a detectable level of this group of organisms. Fecal streptococci were detected in less than half of the samples and were present in relatively low numbers. Thermophilic spore counts (total aerobic and flat-sour) were also low, the counts being somewhat higher in clear flours than in patent. All except one of the samples (88%) harbored actinomycetes, the average count being about 990 per g. of flour.

Data comparing total and differential counts of 1964 wheats and of

TABLE I

MICROBIAL COUNTS OF WHEAT FLOURS MILLED FROM THE 1963 CROP IN KANSAS-NEBRASKA

Mill No.	Flour	Ash	Total Aerobic Bacteria	Psychrophilic Bacteria	Actinomycetes	Fecal Streptococci	Thermophilic Spores: Total Aerobic	Thermophilic Spores: Flat-Sour
		%	*per g.*	*per g.*	*per g.*	*MPN[a] per g.*	*per 10 g.*	*per 10 g.*
1	Patent	0.40	3,100	0	0	0	125	75
	1st Clears	1.30	9,300	0	100	0	215	105
2	Patent	0.38	9,900	0	2,300	240	55	50
	1st Clears	0.62	20,000	145	5,300	3.6	160	120
3	Patent	0.40	4,800	0	85	0	5	5
	1st Clears	0.69	3,200	0	25	3.6	30	20
4	Patent	0.40	1,300	0	30	0	5	5
5	Straight-grade	.....	3,000	.....	65	.....	0	0

[a] Most probable number.

TABLE II

MICROBIAL COUNTS OF 1964 WHEATS AND OF FLOURS MILLED FROM THEM IN KANSAS-NEBRASKA AND THE PACIFIC NORTHWEST

Mill	Total Aerobic Bacteria[a]		Psychrophilic Bacteria[a]		Catalase-Negative Bacteria		Actinomycetes		Thermophilic Spores: Total Aerobic		Thermophilic Spores: Flat-Sour	
	Wheat	Flour	Wheat	Flour	Wheat	Flour	Wheat	Flour	Wheat	Flour	Wheat	Flour
	per g.	*per g.*	*per g.*	*per g.*	*per g.*	*per g.*	*per g.*	*per g.*	*per 10 g.*	*per 10 g.*	*per 10 g.*	*per 10 g.*
1	110	4.8	29	1.2	0	0	300	1,700	70	70	25	70
2	160	0.8	140	0.34	0	0	40	60	50	10	45	0
3	140	4.9	39	0.66	0	0	20	180	10	0	5	0
4	97	4.6	45	3.2	1,000	0	100	90	5	0	0	0
5	660	6.6	140	0.77	0	15	100	60	15	5	15	0
6[b]	15	0.22	0	0.0	0	0	0	10	0	0	0	0
7	600	1.4	140	0.83	0	20	85	85	5	20	0	0
8[b]	36	8.6	34	4.6	130	160	20	10	15	0	0	0
9	66	3.2	45	0.06	0	10	140	25	25	160	5	10
10	93	3.3	26	0.7	0	10	130	160	10	15	10	5
11	38	3.6	21	0.32	0	0	55	10	0	1,200	0	730

[a] Values $\times 10^3$.

[b] Mills located in the Pacific Northwest.

flours milled from them are presented in Table II. Total bacterial counts were considerably lower in finished flours than in wheats from which they were milled, the respective counts averaging 3,800 and 180,000 per g. Psychrophilic bacteria were present in 91% of the wheat and flour samples, with populations averaging 60,000 per g. of wheat and 1,200 per g. of flour. They accounted for as much as 94% of the bacterial population in wheat and 70% in flour. In both wheat and flour, catalase-negative bacteria comprised only a small proportion of the bacterial flora. They were found in only two wheat samples and in about half the flour samples. Thermophilic spores (total aerobic) were present in 82% of the wheat samples and averaged 19 per 10 g.; in the flours they were detected in 64% of the samples and averaged about 135 per 10 g. Six of the 11 wheat samples (55%) harbored flat-sour spores, the average count being 10 spores per 10 g.; four of the flour samples

(36%) contained these spores, the counts averaging 74 per 10 g. On the average, actinomycete counts were higher in the flours (220 per g.) than in the parent wheats (90 per g.). These organisms were encountered in all flour samples and in all wheat samples except one.

Fecal streptococci (not shown in table) were detected in only 18% of the wheats and flours examined. Counts (MPN/g.[2]) ranged from 0 to 43 in the wheats and from 0 to 3.6 in the flours. Tests for salmonellae and coagulase-positive staphylococci were not made.

Taxonomic Distribution of Bacteria

On the basis of morphological, cultural, and physiological tests, the bacterial isolates were grouped as in Fig. 2. Gram-positive cocci (group A) com-

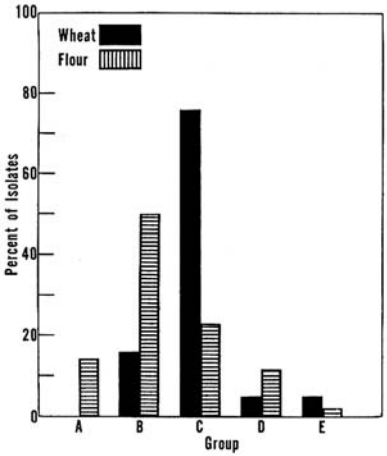

Fig. 2. Distribution of different groups of bacteria isolated from wheat and flour. A, cocci (micrococci, staphylococci, and leuconostocs); B, gram-negative gas-forming rods; C, gram-negative nongas-forming rods; D, aerobic spore-formers; E, other gram-positive rods.

prised about 14% of the bacterial population in flour, whereas this group was not detected in wheat. Organisms placed in group B, gram-negative rods that produced acid and gas from glucose, predominated in flour and represented 50% of the population. In wheat, they accounted for about 15% of the isolates. Organisms in this group included *Escherichia coli, Aerobacter cloacae, Paracolobactrum aerogenoides, P. coliforme,* and members of the genus *Erwinia.* Gram-negative, nongas-forming rods (group C) accounted for slightly more than 75% of the wheat flora, and about 23% of the bacteria from flour. These were primarily organisms belonging to the genera *Flavobacterium* and *Pseudomonas.* Although aerobic spore-formers (group D) were not present to any great extent in either wheat or flour, they accounted for slightly more than 10% of the cultures from flour and less than 5% of those from wheat. Gram-positive rods other than the spore-formers (group E) represented about 5% of the cultures from wheat and less than 2% of those

[2] Most probable number per g. (see reference 16).

TABLE III

DISTRIBUTION OF BACTERIA ISOLATED FROM WHEAT AND FLOUR

ORGANISM	WHEAT			FLOUR		
	No. of Isolates	Percent of Total	Percent of Samples Yielding Cultures	No. of Isolates	Percent of Total	Percent of Samples Yielding Cultures
		%	%		%	%
Achromobacter	0	0.0	0.0	2	0.7	18.2
Aerobacter cloacae	2	0.8	18.2	42	15.6	54.5
Alcaligenes faecalis	0	0.0	0.0	1	0.4	9.1
Bacillus brevis	1	0.4	9.1	1	0.4	9.1
B. cereus	3	1.1	27.3	3	1.1	18.2
B. circulans	6	2.3	9.1	0	0.0	0.0
B. coagulans	0	0.0	0.0	1	0.4	9.1
B. licheniformis	1	0.4	9.1	1	0.4	9.1
B. megaterium	0	0.0	0.0	1	0.4	9.1
B. pumilis	0	0.0	0.0	11	4.1	45.5
B. subtilis	0	0.0	0.0	9	3.3	36.4
B. subtilis var. *niger*	0	0.0	0.0	1	0.4	9.1
Bacillus (unidentified species)	0	0.0	0.0	3	1.1	27.3
Brevibacterium	2	0.8	18.2	1	0.4	9.1
Corynebacterium sp.	0	0.0	0.0	2	0.7	9.1
Erwinia	0	0.0	0.0	11	4.1	54.5
Escherichia coli	0	0.0	0.0	3	1.1	9.1
Flavobacterium spp.	140	53.0	90.9	39	14.6	90.9
Leuconostoc mesenteroides	0	0.0	0.0	2	0.7	9.1
Micrococcus candidus	0	0.0	0.0	8	2.9	45.5
M. caseolyticus	0	0.0	0.0	3	1.1	18.2
M. flavus	0	0.0	0.0	2	0.7	18.2
M. freudenreichii	0	0.0	0.0	10	4.0	9.1
M. ureae	0	0.0	0.0	3	1.1	18.2
M. varians	0	0.0	0.0	1	0.4	9.1
Micrococcus	0	0.0	0.0	4	1.5	27.3
Paracolobactrum aerogenoides	37	14.0	54.5	77	28.7	90.9
P. coliforme	0	0.0	0.0	1	0.4	9.1
Pseudomonas spp.	60	22.7	81.8	15	5.6	72.7
Staphylococcus aureus	0	0.0	0.0	4	1.5	18.2
Unidentified	12	4.5	36.4	6	2.2	27.3
Total	264	100.0		268	100.0	

recovered from flour. Most of the cultures in group E could not be identified; a few, however, were placed in the genera *Brevibacterium* and *Corynebacterium*.

Table III illustrates the relative abundance and distribution of the bacteria isolated from wheat and flour. The most frequently encountered and widely distributed bacteria in wheat were those belonging to the genus *Flavobacterium*. Members of this genus accounted for more than half (53%) of the isolates from wheat, and cultures were recovered from about 91% of the samples. *Paracolobactrum aerogenoides* and strains of *Pseudomonas* were the next most commonly isolated bacteria, representing 14 and 23% of the respective isolates.

In flour the dominant organism was *Paracolobactrum aerogenoides*. It comprised about 29% of the isolates and was found in 91% of the samples. Flavobacteria and pseudomonads were present in more than 70% of the

flours, the former constituting about 15% of the population and the latter, 6%. Coliform bacteria were recovered more often from flour than from wheat. More than half (54.5%) of the flour samples contained *Aerobacter cloacae,* whereas less than half (18.2%) of the wheats yielded cultures of this organism. Thomas and Hobson (32) found that aerogenes-cloacae strains were part of the normal bacterial flora of ears and panicles of growing cereals.

Only three cultures of *E. coli* were isolated and all came from one sample of flour. The majority of *Bacillus* species came from flour, *B. pumilis* being the principal species. Lactic acid bacteria were virtually absent. Only two cultures of *Leuconostoc mesenteroides* were recovered and these came from one flour sample. *Staphylococcus aureus* was found only in flour and represented less than 2% of the isolates. Species of *Micrococcus* accounted for about 12% of the cultures from flour, and of the six species identified, *M. candidus* was the most widely distributed. Accounting for less than 1% of the isolates from wheat and flour were strains of *Achromobacter, Alcaligenes, Brevibacterium,* and *Corynebacterium.*

Classification of Streptomycetes

All the actinomycete cultures isolated (228) from wheat and flour were members of the genus *Streptomyces.* Of these, 146 (64%) were *S. albus,* the type species of the genus. Although the remaining 82 *Streptomyces* cultures have not been identified, the majority (70%) of these are similar to *S. griseus* in mycelium coloration and morphology.

Examination of representative strains of *S. albus* isolates showed that, on the basis of various morphological and physiological characteristics, they could be divided into two distinct groups. In Tables IV and V, the principal characteristics of representative strains of each group are compared with those of the proposed (31) neotype strain of *S. albus,* strain ATCC 3004 (IMRU 3004, maintained as NRRL B-1811 in the permanent ARS Culture Collection at the Northern Laboratory). As shown in Table IV, the group represented by *S. albus* S-2006 was identical with the neotype strain with respect to aerial and substratal mycelia coloration. The other group, represented by *S. albus* S-1835, differed from the neotype strain in that the aerial mycelium was white with faint tinges of pink and the substratal mycelium was more yellowish than buff.

Except for a slight variation in the utilization of *i*-inositol, the carbon utilization pattern exhibited by *S. albus* S-2006 was the same as that of the

TABLE IV

COMPARISON OF MYCELIUM COLORATION[a] OF *Streptomyces albus* S-1835, *Streptomyces albus* S-2006, AND *Streptomyces albus* NRRL B-1811

AGAR MEDIUM	FEATURE (MYCELIUM)	*S. albus* S-2006	*S. albus* S-1835	*S. albus* NRRL B-1811
Inorganic salts-starch	Aerial	Cretaceous (chalk-white)	Pale pinkish buff XXIX	Cretaceous (chalk-white)
Glycerol-asparagine	Substratal	Light buff XV	Maize yellow IV	Light buff XV

[a] See reference 31.

TABLE V

COMPARISON OF PHYSIOLOGICAL CHARACTERISTICS AND ANTIBIOTIC ACTIVITY OF *Streptomyces albus* S-2006, *Streptomyces albus* S-1835, and *Streptomyces albus* NRRL B-1811[a]

CHARACTERISTIC	*S. albus* S-2006	*S. albus* S-1835	*S. albus* NRRL B-1811	CHARACTERISTIC	*S. albus* S-2006	*S. albus* S-1835	*S. albus* NRRL B-1811
Carbon utilization				Hydrolysis of starch	–	+	–
L-Arabinose	⊕	+	⊕	Production of H_2S	–	–	–
Raffinose	(–)	⊕	(–)	Antibiotic activity against:			
Rhamnose	(–)	(–)	(–)	*Bacillus subtilis*	+	+	–
D-Xylose	⊕	+	⊕	*Candida albicans*	–	+	–
Glucose	+	+	+	*Escherichia coli*	–	+	–
D-Galactose	+	+	+	*Mucor ramannianus*	–	+	–
Fructose	⊕	+	⊕	*Saccharomyces pasteurianus*	–	+	–
i-Inositol	⊕	(–)	(–)	*Sarcina lutea*	+	+	+
D-Mannitol	+	+	+				
Salicin	⊕	⊕	⊕				

[a]Symbols: –, Negative reaction or no growth; (–), faint growth, probably no utilization; ⊕, poor to fair growth; +, positive reaction or good growth and definite utilization.

neotype strain (Table V). The utilization pattern of the other strain (S-1835) varied somewhat more. With respect to diastatic and antibiotic activities, *S. albus* S-2006 resembled strain NRRL B-1811 more closely than did *S. albus* S-1835. Like the neotype strain, neither *S. albus* S-2006 nor *S. albus* S-1835 produced hydrogen sulfide.

Microscopic examination of the two representative strains showed that they formed small, compact to extended, coiled chains of spores. Both strains were placed in the morphological section *Spira* (33). Electron photomicrographs comparing the spore morphology of these strains with that of the neotype strain are shown in Fig. 3. Strains S-1835 and S-2006 both formed spores that were elongated and ovoid with smooth surfaces. Strain S-1835 differed from strains S-2006 and NRRL B-1811 in that it produced chains of spores in the form of compact spirals rather than extended spirals, and the spores were enclosed in a loose-fitting, conspicuous sheath.

DISCUSSION

Although general conclusions regarding the bacteriological condition of domestic wheats and flours cannot, and should not, be drawn until the survey has been completed, a comparison of counts with those reported earlier (1,2) seems to indicate a downward trend in the bacterial content of commercial flours. Improved handling of wheat, better milling practices, and the use of modern milling equipment (e.g., complete pneumatic conveying systems) have undoubtedly done much to upgrade mill sanitation and to reduce microbial populations in flour.

Fecal contamination, as indicated by the low counts for enterococci and the infrequent recovery of *E. coli,* was minimal in the samples examined. Although no salmonellae or coagulase-positive staphylococci were detected, the number of samples tested was limited; therefore, it should not be concluded

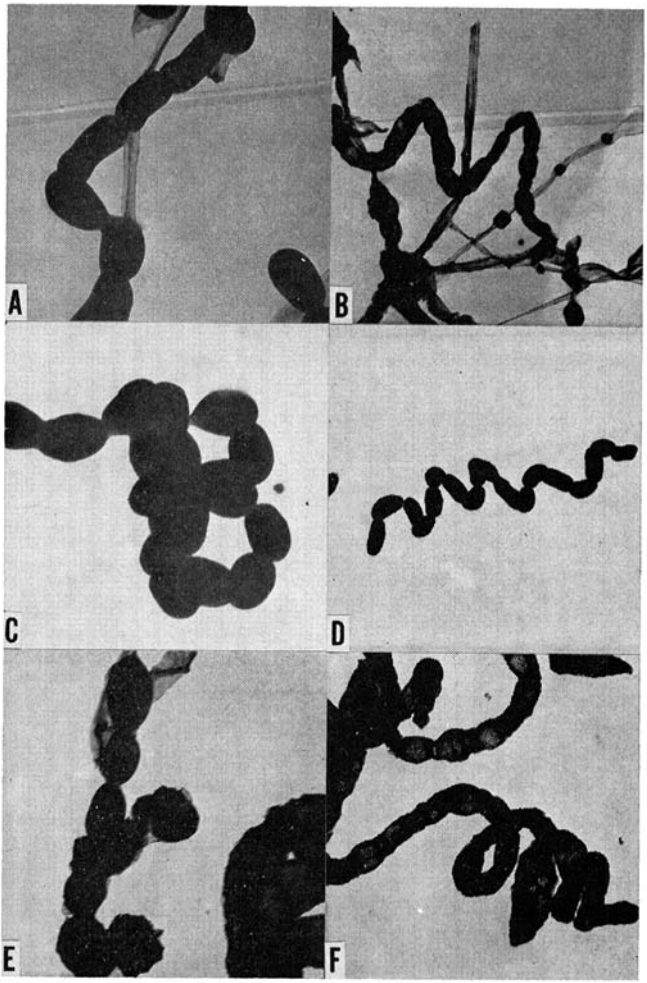

Fig. 3. Electron photomicrographs comparing spore morphology of *Streptomyces albus* strains S-2006 and S-1835 with *S. albus* NRRL B-1811. A, strain NRRL B-1811 (about 6,000 ×); B, strain NRRL B-1811 (about 2,000 ×); C, strain S-2006 (about 6,000 ×); D, strain S-2006 (about 2,000 ×); E, strain S-1835 (about 6,000 ×); F, strain S-1835 (about 3,600 ×).

that flour is always free from such contaminants. The incidence of these organisms in domestic flours is worthy of further investigation.

The finding that certain bacteria were consistently present on wheats and flours was not surprising. According to Semeniuk (1), numerous other investigators have reported similar results. The majority of these workers noted that the dominant bacterium on wheat was a yellow-pigmented, gram-negative bacillus which they designated *Bacterium herbicola aureum*. Gram-negative, yellow-pigmented rods were also the most abundant bacterial forms on wheat in our studies, but in accordance with descriptions given in *Bergey's Manual*

(21), they were identified as members of the genus *Flavobacterium*. Also in abundance on wheat was a yellow-pigmented, fluorescent strain of *Pseudomonas*, similar to that described by James *et al.* (11) as being one of the two chief species of bacteria present on wheats they examined in Canada.

Somewhat surprising was the finding that, in flour, the incidence of *Paracolobactrum* was higher than that of either *Flavobacterium* or *Pseudomonas*. Greater susceptibility of these two genera to the rigors of milling procedures, especially to the action of bleaching and maturing agents, may explain the apparent shift in the predominant species in flour. Another possible explanation is that species of *Paracolobactrum* are able to survive for longer periods of time in stored flour, so that even if *Flavobacterium* and *Pseudomonas* species predominated initially in the flour, *Paracolobactrum* would gradually gain ascendancy. Growth of certain microorganisms during the tempering and milling stages could, in some instances, account for such a shift within the microbial population. In winter, for example, the condensate from sweating pipes, boots, and other mill machinery may provide enough moisture for the growth of some bacteria within the milling system itself. The organism(s) that can grow readily under these conditions may then contaminate the flour stream and change the percentage of types found in the finished flour. With this condition, the flour may also be seeded with an organism not found in the parent wheat.

The relation of wheat and flour microfloras to geography is of interest, and it is anticipated that as the survey progresses, the effect of prevailing climatic conditions in the different regions on microfloral patterns will become evident.

Acknowledgment

We express our appreciation to the Millers' National Federation for assistance rendered in securing wheat and flour samples.

Literature Cited

1. SEMENIUK, G. Microflora. *In* Storage of cereal grains and their products, ed. by J. A. Anderson and A. W. Alcock; vol. II, chap. III, pp. 77–151. American Association of Cereal Chemists: St. Paul, Minnesota (1954).
2. HESSELTINE, C. W., and GRAVES, R. R. Microbiological research on wheat and flour. Econ. Bot. 20: 156–168 (1966).
3. DOTY, J. Bacteria control in the flour milling operation. Am. Miller Processor 89: 7, 20–21 (1961).
4. BELL, H. G. Fungus and bacterial growth on stored flour. Am. Miller Processor 37: 280–281 (1909).
5. TURLEY, H. The microscopy of flour. Baking Technol. 1: 327–329 (1922).
6. GUSTAFSON, C. B., and PARFITT, E. H. Effect of numbers of bacteria on the development of rancidity in soft wheat flour. Cereal Chem. 10: 233–238 (1933).
7. HOLTMAN, D. F. The microbiological content of soft wheat flour. J. Bacteriol. 30: 359–361 (1935).
8. JAMES, N., and SMITH, K. N. Studies on the microflora of flour. Can J. Res. 26C: 479–485 (1948).
9. THATCHER, F. S., COUTU, C., and STEVENS, F. The sanitation of Canadian flour mills and its relationship to the microbial content of flour. Cereal Chem. 30: 71–102 (1953).
10. DACK, G. M. Flour bacteriology. Cereal Sci. Today 6: 9–10 (1961).

11. JAMES, N., WILSON, JOYCE, and STARK, E. The microflora of stored wheat. Can. J. Res. 24C: 224–233 (1946).
12. MACK, E. Untersuchungen über *Bacterium herbicola*. Zentr. Bakt. Parasitenk. Abt. II 95: 218–261 (1936).
13. JAMES, N. Yellow chromogenic bacteria on wheat. II. Determinative studies. Can. J. Microbiol. 1: 479–485 (1955).
14. GRAVES, R. R., and HESSELTINE, C. W. Fungi in flour and refrigerated dough products. Mycopathol. Mycol. Appl. 29: 277–290 (1966).
15. PEPPER, E. H., and KIESLING, R. L. Differential media for the isolation of bacteria and fungi from plated barley kernels. Cereal Chem. 40: 191–193 (1963).
16. AMERICAN ASSOCIATION OF CEREAL CHEMISTS. Cereal laboratory methods (7th ed.). The Association: St. Paul, Minnesota (1962).
17. KIRSCH, R. H., BERRY, F. E., BALDWIN, C. L., and FOSTER, E. M. The bacteriology of refrigerated ground beef. Food Res. 17: 495–503 (1952).
18. HAYNES, W. C., WICKERHAM, L. J., and HESSELTINE, C. W. Maintenance of cultures of industrially important microorganisms. Appl. Microbiol. 3: 361–368 (1955).
19. PRIDHAM, T. G., ANDERSON, P., FOLEY, C., LINDENFELSER, L. A., HESSELTINE, C. W., and BENEDICT, R. G. A selection of media for maintenance and taxonomic study of *Streptomyces*. Antibiotics Annual, Medical Encyclopedia, Inc., New York (1956–1957).
20. SOCIETY OF AMERICAN BACTERIOLOGISTS. Manual of microbiological methods. McGraw-Hill: New York (1957).
21. BREED, R. S., MURRAY, E. G. D., and SMITH, N. R. Bergey's manual of determinative bacteriology (7th ed.). Williams & Wilkins: Baltimore (1957).
22. HUGH, R., and LEIFSON, E. The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various gram negative bacteria. J. Bacteriol. 66: 24–26 (1953).
23. VAUGHN, R. H., BALATSOURAS, G. D., YORK, G. K., II, and NAGEL, C. W. Media for detection of pectinolytic microorganisms associated with softening of cucumbers, olives, and other plant tissues. Food Res. 22: 597–603 (1957).
24. ALARIE, A. M., and GRAY, P. H. H. Aerobic bacteria that decompose cellulose, isolated from Quebec soils. I. Isolation and description of the species. Can. J. Res. 25C: 228–241 (1947).
25. SKERMAN, V. B. D. A guide to the identification of the genera of bacteria. Williams & Wilkins: Baltimore (1959).
26. SHIMWELL, J. L., CARR, J. G., and RHODES, MURIEL E. Differentiation of *Acetomonas* and *Pseudomonas*. J. Gen. Microbiol. 23: 283–286 (1960).
27. GIBSON, T., and ABD-EL-MALEK, Y. The formation of CO_2 by lactic acid bacteria and *Bacillus licheniformis* and a cultural method of detecting the process. J. Dairy Res. 14: 35–44 (1945).
28. HUCKER, G. J. Studies on the coccaceae. II. A study of the general characters of the micrococci. N. Y. State Exp. Sta. Tech. Bull. 100 (1924).
29. KING, ELIZABETH O., WARD, MARTHA K., and RANEY, D. E. Two simple media for the demonstration of pyocyanin and fluorescin. J. Lab. Clin. Med. 44: 301–307 (1954).
30. SMITH, N. R., GORDON, RUTH E., and CLARK, F. E. Aerobic sporeforming bacteria. U. S. Dept. Agr., Agr. Monograph 16 (1952).
31. LYONS, A. J., JR., and PRIDHAM, T. G. Proposal to designate strain ATCC 3004 (IMRU 3004) as the neotype strain of *Streptomyces albus* (Rossi-Doria) Waksman and Henrici. J. Bacteriol. 83: 370–380 (1962).
32. THOMAS, S. B., and HOBSON, PHYLLIS M. Coli-aerogenes bacteria isolated from ears and panicles of cereal crops. J. Appl. Bacteriol. 18: 1–8 (1955).
33. PRIDHAM, T. G., HESSELTINE, C. W., and BENEDICT, R. G. A guide for the classification of streptomycetes according to selected groups. Placement of strains in morphological sections. Appl. Microbiol. 6: 52–79 (1958).

[Received July 25, 1966. Accepted February 24, 1967]