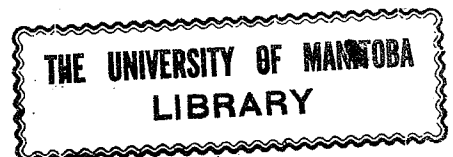


CERTAIN STUDIES ON THE MICROFLORA OF WHEAT

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A Major Thesis submitted to  
the Graduate Studies Committee of  
The University of Manitoba  
in candidacy for the  
Degree of Master of Science

1946



#### ACKNOWLEDGMENTS

The writer takes this opportunity of expressing her gratitude to Dr. Norman James, of the Department of Bacteriology and Animal Pathology, who gave liberally of his time and experience during the work reported and in the preparation of this paper.

Thanks are also due to Dr. H. J. Brodie, of the Department of Botany, for his advice concerning the mycological studies presented.

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## INTRODUCTION

Heating damage in stored grain presents an acute economic problem in Canada and the United States today. This has been accentuated by the necessity of storing large quantities of grain, for longer periods of time than was the case in prewar days. In May 31, 1943, the total storage in Canada had risen to 603 million bushels, an increase of 180 million bushels or approximately 43 percent of the storage capacity over 1939 (24). Formerly the view was held that this spoilage is due to increased respiration in wheat of high moisture content (1). In more recent years there has been some doubt as to the validity of this theory. Workers using various grains and grain products have brought forward presumptive evidence that the primary factor in this heat damage may be micro-organic activity. Many investigators (6, 14, 22) attribute the damage to fungal growth, or indicate that fungi are the primary cause of heating, with bacterial infection a contributing factor at higher temperatures; while some (15, 19, 21) merely state more generally that the increased carbon dioxide out-put in damp wheat is due to micro-organic activity. Due to the fact that the presence of bacteria is not readily apparent, the probable part played by bacteria in this spoilage has been given only passing mention.

Without information as to the numbers and types of organisms on normal wheat, it is difficult to assess the value of results obtained from reports on spoiled grain. Therefore much fundamental experimentation is necessary before the role of micro-organisms in heating damage to wheat can be properly evaluated. An investigation of the flora of normal wheat was undertaken as the first step towards a better understanding of the problem. The study was

carried out by the technique of the bacteriologist interested primarily in micro-organisms of spoilage.

#### HISTORICAL

Kent-Jones and Amos, 1930, (12) carried out preliminary studies on the bacteriology of wheat and flour. Counts of bacteria in six samples of low grade Manitoba wheat, plated in nutrient agar at 37°C. for 48 hours, ranged from 1,260,000 to 8,000,000 per gram. Fifteen samples from other countries gave counts between 8,000 and 219,000 per gram. The count on samples held under normal storage conditions became lower, whereas counts on samples that were conditioned in the laboratory increased. Counts on plates incubated at 20°C. were larger than those at 37°C. The Bacillus mesentericus group of bacteria was found to be most universally present on wheat and flour.

Greaney and Machacek, 1941, (8) carried out an extensive survey on the prevalence of seed-borne fungi on cereals from certain seed inspection districts in Canada. These investigators found species of Alternaria to be the most common fungi on wheat, oats, barley and rye; and species of Helminthosporium and Fusarium to be the most important disease-producing organisms.

Leach, 1944, (15), carrying out laboratory experiments under controlled conditions, found various fungi developing on heating wheat. They consisted chiefly of species of Penicillium, Aspergillus and Fusarium, with Cladosporium herbarum, types of Alternaria tenuis, various yeasts and species of Monilia occurring less frequently.

Gordon, 1944, (7) dealing specifically with the genus *Fusarium*, found that in the 1448 farm samples of seed of common wheat tested species of this fungus were present in approximately 39% of the samples; and of the 262 samples of durum wheat 38% showed species of *Fusarium* to be present. However, species of this fungus were isolated only from a small percentage of the seed (.66% of common wheat and .50% of durum wheat).

Investigation of other grains and various seeds has also been undertaken. Thom and Le Fevre, 1921, (25) studied the flora of cornmeal. They felt that it might be possible, by routine culturing, to determine the species represented and something of their relative abundance in the sample. The following bacteria were isolated: micrococci, mesentericus, the colon-aerogenes group and lactobacilli with the morphological and cultural characteristics of organisms of the pickle and sauerkraut form. Molds such as *Aspergillus repens*, *A. niger*, *A. flavus*, *Fusarium sp.*, various *Mucor spp.* and the occasional green species of *Penicillium* were found. A freshly milled sample of meal contained 1,000,000 bacteria per gram (60% of which were acid producers) and 100,000 molds per gram of meal.

Bakke and Noecker, 1933, (2) isolated *Aspergillus niger*, *A. flavus*, *Rhizopus sp.*, *Fusarium sp.*, *Penicillium sp.* and various species of bacteria from oats. Irregularities in oxygen consumption and temperature of oats packed in flasks were thought to be due to variation in the fungal flora of the different seed lots.

Ramstad and Geddes, 1942, (19) isolated a wide variety of fungi from soybeans: *Alternaria sp.*, *Fusarium sp.*, *Penicillium sp.*, the

Aspergillus repens group, the A. niger group, Chaetotheca sp.,  
Verticillium sp., the Aspergillus flavus-oryzae group, Mucorales,  
Rhizopus nigricans and Acrostalagmus sp.

A detailed investigation of the microflora of soybeans was undertaken by Ian W. Tervet (23). Soybeans were surface sterilized and then plated on potato dextrose agar. Species of Alternaria developed the most frequently, with other species in the following order:

Fusarium sp., Aspergillus sp., Rhizopus nigricans, Chaetomium sp.,  
Cephalothecium roseum and Trichoderma viride. Seeds stored at 45°C.  
showed a maximum degree of infection.

#### NUMBERS OF ORGANISMS IN WASHINGS FROM WHEAT

Two sets of samples were provided through the courtesy of the chemist-in-charge, Board of Grain Commissioners for Canada, Winnipeg. The first set was taken from individual box-cars shipped from various points in Western Canada during the period May 1, 1943 to January 15, 1944. It included 12 samples of No. 1, 12 of No. 2, 6 of No. 3 and 6 of No. 4 Manitoba Northern grades of red spring wheat. The second set will be referred to later.

In order to get reliable estimates of the populations, tests were run on six replicate portions of each sample. Each portion consisted of 10 grams, which was transferred aseptically to a 6 oz. glass screwtop bottle containing 90 ml. of sterile water and a small quantity of sterile gravel. The bottles were then shaken for 30 minutes by a mechanical shaking device. All plating was carried out in a room which was previously

sprayed with a disinfecting solution of chlorine.

Preliminary tests were made on each sample to determine the dilution which could be counted with the most accuracy. Two plates were prepared from the dilution, selected as above for bacteria, two from the dilution for yeasts and two from the dilution for fungi. This gave an estimate of bacteria on one sample representing the average from 12 plates and an estimate of yeasts and of fungi based on the same number of plates.

Nutrient agar medium was used as a substrate for bacterial growth and Czapek's agar, plus 0.5 ml. of 10% lactic acid per 100 ml. of medium, for yeasts and fungi. The addition of this amount of lactic acid appreciably inhibited bacterial growth, thereby encouraging a more rapid and luxuriant development of fungi.

In preliminary trials two temperatures of incubation were used, 25°C. and 37.5°C., the latter temperature being employed in the hope that organisms with high optimum growth temperatures might be isolated. However, since counts at 37.5°C. were much lower than those at 25°C., and differed widely in replicate plates from one sample, study at this temperature was discontinued.

The fungi and yeasts were counted throughout from the 1:100 and 1:1000 dilutions, respectively. Differences in bacterial counts were more varied and necessitated the use of different dilutions, depending on the grade of wheat under consideration. Estimates on grade 1 samples usually were based on plates from the 1:10,000 dilution; on grades 2 and 3 from either 1:10,000 or 1:100,000 dilutions; on grade 4 from the 1:100,000 dilution; and on grades 5 and 6 from dilutions as high as 1:1,000,000. The results are presented in tables 1a, 1b and 1c.

Table 1a. Bacteria washed from box-car samples of Manitoba  
Northern red spring wheat, Grades 1, 2, 3 and 4.  
(100 thousands per gm. expressed as logarithms,  
averages from 12 plates)

Replications	Grades			
	1	2	3	4
1	0.78	0.89	1.06	2.29
2	0.98	1.24	1.00	1.85
3	0.30	0.84	0.98	1.79
4	0.00	0.89	1.56	1.37
5	0.36	1.32	1.60	1.88
6	0.71	0.00	1.76	1.90
7	0.76	0.79		
8	0.42	1.51		
9	0.59	0.85		
10	1.00	0.97		
11	0.32	1.42		
12	0.00	1.50		
$\bar{x}$	0.52	1.02	1.33	1.85
$s_{\bar{x}}$	0.10	0.12	0.14	0.12
t	5.20	8.50	9.50	15.42
d.f.	11		5	
5% level for t	2.20		2.57	

Table 1b. Fungi washed from box-car samples of Manitoba  
Northern red spring wheat, Grades 1, 2, 3 and 4.  
(Hundreds per gm. expressed as logarithms,  
averages from 12 plates)

Replications	Grades			
	1	2	3	4
1	0.83	1.06	1.14	1.29
2	0.90	0.83	1.08	0.74
3	0.70	1.01	0.83	0.62
4	0.46	0.91	1.21	1.28
5	1.73	0.91	1.32	0.99
6	0.86	1.42	0.61	1.09
7	1.06	0.81		
8	1.47	1.33		
9	0.87	1.13		
10	0.88	0.72		
11	0.85	1.06		
12	0.72	1.16		
$\bar{x}$	0.94	1.03	1.03	1.00
$s_{\bar{x}}$	0.10	0.06	0.11	0.11
t	9.40	17.16	9.39	9.09
d.f.	11		5	
5% level for t	2.20		2.57	

Table 1c. Yeasts washed from box-car samples of Manitoba  
Northern red spring wheat, Grades 1, 2, 3 and 4.  
(Thousands per gm. expressed as logarithms,  
averages from 12 plates)

Replications	Grades			
	1	2	3	4
1	1.59	0.84	0.65	1.30
2	1.66	1.09	0.77	1.86
3	1.06	1.39	1.26	1.52
4	0.78	0.75	0.43	1.52
5	1.21	0.75	1.81	1.61
6	1.56	0.00	0.82	1.02
7	1.05	1.34		
8	0.96	1.59		
9	1.47	1.06		
10	1.39	1.60		
11	0.02	1.46		
12	0.24	1.64		
$\bar{x}$	1.08	1.13	0.96	1.47
$s_{\bar{x}}$	0.15	0.14	0.20	0.12
t	7.20	8.07	4.80	12.25
d.f.	11		5	
5% level for t	2.20		2.57	

It is apparent from the data presented in table 1a that Manitoba Northern red spring wheat harbours a large bacterial population and that, on the average, this population increases regularly from grade 1 to grade 4. The variation between samples of any grade does not exceed the generally accepted limit, as is shown by a t value for each set of replicates greater than that at the 5% level for the appropriate number of degrees of freedom.

The data for fungi on the same samples, presented in table 1b, on the contrary indicate that the numbers of fungi are relatively small and that, on the average, there is little difference between grades. Here too, the means provide reliable estimates of the populations in the different grades.

Yeasts, isolated on the medium used, represent many times the numbers of fungi and, as shown in table 1c, there is little evidence of a characteristic trend between numbers and grades.

The second set of samples, covering the same period and the same area as the former set, represented 4 Winnipeg averages of each of the 4 grades of Manitoba Northern red spring wheat and of No. 5 and No. 6 wheat. The Winnipeg average was a composite of all samples of one grade taken by the inspection staff from box-cars in a period of 2 weeks. The counts on these samples should represent the populations on wheat better than the counts on individual box-car samples. This is borne out in the data and statistics presented in tables 2a, 2b and 2c. Again the actual counts have been transformed to logarithms.

Table 2a.

Bacteria washed from Winnipeg average samples  
of Manitoba Northern red spring wheat, Grades  
1, 2, 3 and 4 and No. 5 and No. 6 wheat.

(100 thousands per gm. expressed as logarithms,  
averages from 12 plates)

Replications	Grades					
	1	2	3	4	5	6
1	0.81	1.03	1.59	2.48	3.05	3.22
2	0.54	0.94	1.72	2.09	2.63	2.88
3	0.45	0.76	1.55	2.16	2.51	2.82
4	0.52	0.87	1.56	2.06	2.69	2.94
$\bar{x}$	0.58	0.90	1.60	2.20	2.72	2.96
$s_{\bar{x}}$	0.07	0.06	0.04	0.10	0.11	0.09
t	8.28	15.00	40.00	22.00	24.73	32.89
d.f.	3					
5% level for t	3.18					

Table 2b.

Fungi washed from Winnipeg average samples of Manitoba Northern red spring wheat, Grades 1, 2, 3 and 4 and No. 5 and No. 6 wheat.

(Hundreds per gm. expressed as logarithms, averages from 12 plates)

Replications	Grades					
	1	2	3	4	5	6
1	1.08	1.23	0.97	1.25	1.27	1.09
2	0.96	1.11	1.18	0.82	0.92	0.72
3	0.62	1.01	1.40	0.91	1.01	1.22
4	1.09	1.03	0.93	0.98	0.93	1.06
$\bar{x}$	0.94	1.10	1.12	0.99	1.03	1.02
$s_{\bar{x}}$	0.11	0.05	0.11	0.10	0.08	0.10
t	8.54	22.00	10.28	9.90	12.87	10.20
d.f.	3					
5% level for t	3.18					

Table 2c.

Yeasts washed from Winnipeg average samples of  
Manitoba Northern red spring wheat, Grades 1,  
2, 3 and 4 and No. 5 and No. 6 wheat.

(Thousands per gm. expressed as logarithms,  
averages from 12 plates)

Replications	Grades					
	1	2	3	4	5	6
1	1.24	1.72	1.53	1.74	1.81	1.55
2	0.89	1.29	1.35	1.19	1.71	1.32
3	1.27	1.17	1.23	1.27	1.63	1.34
4	0.79	1.32	1.39	1.10	1.44	1.41
$\bar{x}$	1.05	1.38	1.38	1.32	1.65	1.40
$s_{\bar{x}}$	0.12	0.12	0.06	0.14	0.08	0.05
t	8.75	10.78	23.00	9.43	20.61	28.00
d.f.	3					
5% level for t	3.18					

The data on these samples are quite similar to those for the box-car samples, shown in tables 1a, 1b and 1c. They are presented graphically in Fig. 1. The analyses of variance follows in table 3. The bacterial population is large. The difference between grades is highly significant and there is a regular upward trend from grade 1 to grade 6. The fungal counts are low and uniform. Yeast counts show wide variability. The difference between grades and counts and between dates of sampling and counts are highly significant, although they fail to indicate trends that are explainable on the basis of the data available.

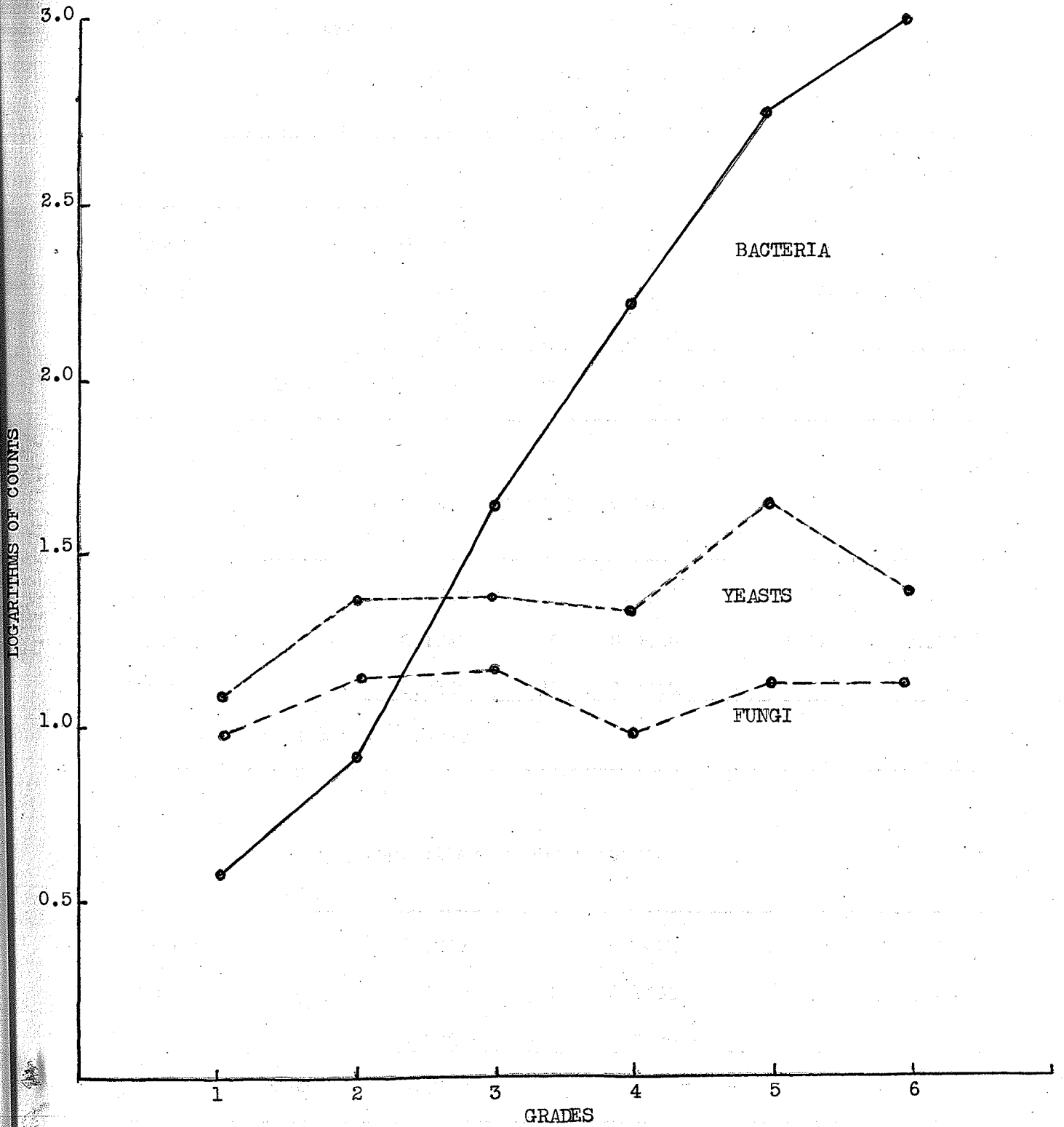


Fig. 1. Relation between grades of wheat and counts - bacteria X 100,000, yeasts X 1000 and fungi X 100.

Table 3. Analysis of variance - Winnipeg average samples.

## a. Logarithms of bacterial counts.

	SS	DF	MS	F	5% pt.
Grades	18.7735	5	3.7547	383.13	2.90
Dates	0.3534	3	0.1178	12.02	3.29
Error	0.1467	15	0.0098		
Total	19.2136	23			

## b. Logarithms of fungal counts.

Grades	0.0900	5	0.0180	0.53	2.90
Dates	0.1249	3	0.0416	1.22	3.29
Error	0.5100	15	0.0340		
Total	0.7249				

## c. Logarithms of yeast counts.

Grades	0.7357	5	0.1471	7.78	2.90
Dates	0.4630	3	0.1543	8.16	3.29
Error	0.2822	15	0.0189		
Total	1.4809				

## TYPES OF BACTERIA IN WASHINGS FROM WHEAT

When estimates were made on the microflora of wheat it was noted that the bacterial plates showed a large number of what appeared to be one type of organism. This observation prompted the idea that there was possibly a flora proliferating on wheat, with a few predominant bacterial species. To obtain information on this point, 44 colonies were picked at random from the bacterial plates referred to earlier. Twenty-two of these isolations represented one type of bacteria and it was estimated that this organism made up about 85% of all the bacteria removed from the grain by washing in sterile water.

An attempt was made to identify these organisms by recognized methods of culturing on various common media and on certain selective media.

The predominating species, not identified and referred to hereafter as Type 1, had many of the cultural and physiological characteristics of Phytomonas translucens var. undulosa (Smith, Jones and Reddy) Hagborg - a plant pathogen. Conceivably, wheat might harbour a parasitic species with many of the characteristics of a pathogen - a phenomenon that is accepted with respect to parasitic bacteria on the human skin. A description of Type 1 follows:

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Type ISource - wheatDate of isolation - May 16th, 1944Medium - Nutrient agar      Temperature - 26°C.      Age - 3 daysVegetative cells - aerobicSize - 0.6 - 0.8 μ by 1.2 - 1.6 μ      Ends - rounded, in some cases capsules were seenGram-negative rods in singles and pairsMotile in brothEndospores absentTemperature - 26°C.Agar Colony      Age - 3 daysAgar Slant      Age - 3 days

Form - circular

Growth - moderate

Surface - smooth

Form - beaded

Edge - entire

Lustre - glistening

Elevation of growth - flat

Chromogenesis - yellow

Optical character - opaque

Odor - none or slight

Consistency - butyrous

Medium - unchanged

Nutrient Broth      Age - 3 daysGelatin Stab      Age - 6 days

Surface growth - pellicle

Growth - best at top

Clouding - strong

Line of puncture - filiform

Odor - decided

Liquefaction - crateriform

Sediment - flaky

Medium - unchanged

Amount of sediment - moderate

Potato Slant

Growth - abundant

Elevation - flat

Form - spreading

Optical character - translucent

Lustre - glistening

Odor - decided, sweet

Consistency - butyrous

Chromogenesis - yellow

Medium - browned

Litmus Milk      Age - 5 days

Consistency - clear

Reaction - alkaline

Nitrates reduced to nitrites.

Ammonia produced from nitrate-peptone broth.

No indol produced from tryptophane broth.

No starch hydrolysis.

Acid, but no gas from dextrose, lactose and sucrose.

Type II was isolated five times. It closely resembled Phytomonas atrofaciens (McCulloch) Bergey et al., differing in that it did not produce indol, while Phytomonas atrofaciens produces slight amounts. A detailed description follows:

Type IISource - wheatDate of isolation - May 23rd, 1944.Medium - nutrient agarTemperature - 26°C.Age - 3 daysVegetative cells - aerobicSize - 0.5 - 0.8  $\mu$  by 1.2 - 2.7  $\mu$ Ends - rounded

Gram-negative rods in chains

Rapidly motile in broth

Endospores - absent

Agar colony

Age - 3 days

Agar Stroke

Age - 3 days

Form - circular

Growth - moderate

Surface - smooth

Form - echinulate

Edge - entire

Lustre - glistening

Elevation - flat

Chromogenesis - creamy

Optical character - translucent

Odor - slight

Consistency - butyrous

Medium - slightly yellowed

Nutrient Broth

Age - 3 days

Gelatin Stab

Age - 4 days

Surface - pellicle

Growth - best at top

Clouding - moderate

Line of puncture - filiform

Sediment - fine, granular

Liquefaction - infundibuliform

Amount of sediment - scant

Medium - unchanged

Odor - slight

Potato Slant

Growth - moderate

Odor - distinct

Form - spreading

Consistency - butyrous

Elevation - flat

Medium - greyed or browned

Lustre - glistening

Chromogenesis - white

Optical character - translucent

Litmus MilkAge - 4 days

Consistency - peptonization (serum zone); at 6 days complete peptonization.

Reaction - unchanged ; at 6 days, alkaline.

Nitrites not produced from nitrates.

No indol production.

No hydrolysis of starch.

Acid and no gas from dextrose and sucrose. Lactose turns alkaline with no gas.

Yellow-green fluorescent pigment produced in culture.

Three cultures did not appear to agree with any species described in Bergey's manual (3). The presence of swollen and irregular forms suggested the possibility that they might belong to the Rhizobiaceae, but growth on mannitol agar produced only a few small pinpoint colonies. They did not grow on a medium containing cellulose as the only source of energy, thereby eliminating another possibility.

Two *Serratia*-like organisms were isolated, which although they produced typical reactions in litmus milk, failed to liquefy blood serum. Also, the pink pigment which was produced was neither water nor alcohol soluble. The organism was a truncate rod, differing entirely from the typical cocco-bacteria of *Serratia marcescens* but possibly similar to *Serratia plymouthensis* in this respect.

Two cultures of *Flavobacterium radiatum* Bergey et al. were isolated. This organism was originally isolated from water.

Two of the isolates agreed with *Bacterium globiforme* Conn, with

one exception. This was that the nitrates were reduced to ammonia and not simply to nitrites. Bacterium globiforme is widely distributed in soils.

The only coccus isolated was Staphylococcus epidermidis Bergey et al. The usual habitat of this organism is the human skin but it is of fairly wide distribution and it is conceivable that it might occur on the seed coat of grain.

One of the cultures, on ordinary laboratory media showed the general characteristics of Phytomonas tritici (Hutchinson) Bergey et al., but unlike the latter it produced a slight infundibuliform liquefaction in gelatin. Undoubtedly it is a member of this genus.

Four other motile, gram-negative rods were isolated which produced limited amounts of acid on carbohydrate media but which did not agree with any previously named species.

#### TYPES OF FUNGI IN WASHINGS FROM WHEAT

Identification studies on the fungi were carried out on the plates used for obtaining estimates. A pure culture of each type was subcultured on a Czapek's agar slant and preserved for future reference. The following types were isolated; the most prevalent being species of Penicillium, Aspergillus, Alternaria, Fusarium and Cladosporium.

Acrostalagmus cinnabarinus Corda

Alternaria tenuis Nees

Aspergillus candidus Link

- A. flavus Link
- A. fumigatus Fres.
- A. glaucus Link
- A. niger van Tiegh
- A. oryzae (Ahlburg) Cohn
- A. versicolor (Vuill.) Tirab.
- Botrytis cinerea Pers.
- Cephalosporium curtipes Sacc.
- Cephalosporium spp.
- Cephalothecium roseum Corda
- Cladosporium herbarum (Pers.) Link
- Fusarium culmorum (W. G. Smith) Sacc.
- F. Poae (Peck) Wollenw.
- F. Scirpi var. acuminatum (Ell. and Ev.) Wollenw.
- F. semitectum Berk. and Rav. var. majus Wr.
- Helminthosporium sativum Pam., King, and Bakke
- Hormodendron pallidum Oudem
- H. viride (Fres.) Sacc.
- Monilia spp.
- Mucor circinelloides van Tiegh
- M. racemosus Fres.
- Paecilomyces varioti Bain.
- Penicillium chrysogenum Thom
- P. flavi-dorsum Biourge
- P. frequentans Westl.
- P. purpurogenum Stoll

P. rugulosum Thom

P. spinulosum Thom

P. terrestre Jensen

Phoma spp.

Rhizopus spp.

Scopulariopsis brevicaulis (Sacc.) Bain.

Scopulariopsis sp.

Septoria nodorum Berk.

Torula spp.

Trichoderma lignorum (Tode) Harz

Species of Penicillium appeared in abundance in practically all samples, with Penicillium chrysogenum, P. frequentans and P. terrestre occurring the most frequently.

#### ATTEMPTS TO FREE WHEAT FROM MICRO-ORGANISMS

In order to establish experimentally the direct relationship between micro-organisms and heating, it is necessary to control the flora on wheat and, in fact, to free the grain from micro-organisms in the check sample. This was first attempted by exposing the sample to repeated washings in sterile water. The procedure was the same as that followed for determining the populations on wheat, except that the shaking was repeated six times, for half hour intervals, instead of only once. After each shaking the rinse water was poured off and a fresh supply of sterile water added. Then the sample was rinsed in sterile water five times,

without the use of the mechanical shaker, and a final or twelfth time on the mechanical shaker for one half hour. The final rinse water was plated. The results for one of 3 replicates of this experiment, shown in Table 4, demonstrate clearly that relatively large populations of bacteria and yeasts remain on wheat even after repeated washings.

Table 4. Numbers of bacteria, fungi and yeasts in washings from wheat previously washed 11 times in sterile water.

Sample	Bacteria	Fungi	Yeasts
	Millions per gram	thousands per gram	
Pitlochry, Man.	0.10	0.0	0.08
Dysart, Sask.	0.25	0.01	0.18
Frankslake, Sask.	0.06	0.01	0.30

Disinfection of the grain was considered as the next method of procedure. Several reports deal with different chemicals on a variety of seeds. Larmour, Clayton and Wrenshall, 1935, (14) made an attempt to surface sterilize wheat by immersion in 0.1% mercuric chloride solution. From their data it was obvious that the treatment with mercuric chloride was ineffective as a sterilizer for fungi. Swanson, 1934, (22) used Ceresan, a substance composed of 2% ethyl mercury chloride in 98% finely powdered, dry, inert material. Traces of Ceresan, which could not be

removed, damaged the quality of the wheat, therefore, the investigator states that it is difficult to know how much value this substance had in preventing damage by inhibiting mold growth.

Hofer, 1936, (9) and Hofer and Hamilton, 1938, 1940, (10, 11), after a thorough investigation of a large number of reports on chemical disinfection, conducted a rather extensive study of seed sterilization. Finally these workers came to the conclusion that a 2% chlorine solution diluted 1:10, by adding 8 parts of water and 1 part of 4% benzoic acid in alcohol, was highly satisfactory for freeing legume seeds from contaminating bacteria. Accordingly the procedure outlined by Hofer was adopted as a basis for disinfecting wheat.

The grain used for these experiments was a high quality wheat provided through the courtesy of the Plant Science Department of the University of Manitoba. In preliminary trials varying amounts of a mixture of 2% available chlorine, from a stabilized chlorine product, and 4% benzoic acid in alcohol were allowed to act on the wheat for different periods of time. Satisfactory results were obtained when 50 grams of wheat were treated with 20 ml. of chlorine, 20 ml. of benzoic acid, and 60 ml. of water for 10 minutes. After this treatment the grain was rinsed six times with sterile water. Effectiveness of the disinfection was demonstrated by transferring 18 treated seeds to tubes of nutrient broth, one seed to a tube. In order to determine the presence of micro-organisms that might not be affected by treatment because of protection by dirt or otherwise, half of the seeds were removed after 30 minutes, while the other half were left in. Tubes were incubated at 26°C.

and readings were made after several days. The results of 10 replications of this experiment are presented in Table 5. They show that bacteria were washed from 9% of the treated seeds removed from the broth after 30 minutes and from 43% of the seeds left in the broth during the period of incubation. This is accepted as evidence that bacteria are protected in crevices of the seed-coat or probably under it.

Table 5. Sterility tests on wheat after disinfection.

(Number of seeds producing bacterial growth  
in nutrient broth)

Replications	Seeds removed after 30 minutes		Seeds left in broth	
	No. tested	No. positive	No. tested	No. positive
1	9	0	9	3
2	9	2	9	1
3	9	0	9	7
4	9	1	9	4
5	9	0	9	3
6	9	1	9	3
7	9	0	9	4
8	9	3	9	3
9	9	0	9	7
10	9	1	9	4
% of total positive:		9%	43%	

In order to ensure that the chemical treatment followed did not interfere with the normal metabolism of the grain, tests for viability were made by transferring 25 of the treated seeds to nutrient agar plates and incubating at 26°C. The experiment was repeated 10 times. Three check samples, treated with water, instead of the chlorine-benzoic acid mixture, were tested for germinating power during the course of the experiments. The results, shown in Table 6, indicate that the chemical treatment followed had no effect on germination.

Table 6. Viability tests on wheat after disinfection.

(Number of seeds germinating)

Replication	Treated		Not treated	
	No. tested	No. viable	No. tested	No. viable
1	25	22	25	21
2	25	25	25	23
3	25	25	25	25
4	25	24		
5	25	24		
6	25	23		
7	25	23		
8	25	20		
9	25	25		
10	25	22		
% viable seeds		93%	92%	

Again, to be certain that the chemical treatment did not affect the diastase in wheat, a simple test was used to determine whether or not diastatic activity was the same in treated and untreated samples. The remainder of the treated seeds were dried in the 37.5°C. incubator for two days. Fifty grams of finely ground flour from each sample were shaken with 200 ml. of water, after which the mixture was allowed to stand for 3 hours. The procedure followed for testing for diastase was the Wohlgenuth iodine method (26). Dilutions of 1.0, 0.5, 0.25, 0.125, 0.062, 0.031 and 0.016 were made from the supernatant liquid of the grain by the addition of sterile water. Five ml. of each of the above dilutions were added to tubes containing 5 ml. of 0.1% starch solution. To inhibit bacterial action 0.5 ml. of toluol was added to each tube. The experiment was carried out in duplicate and the tubes were incubated at 37.5°C. for 24 hours. A check sample was run. After the incubation period the presence of starch was demonstrated by the addition of a few drops of iodine. The presence of a blue red color, indicating the conversion of starch to dextrin, was detected in the 0.031 dilution in all samples, including the check. This is regarded as the limit of the activity of the enzyme. It may be concluded therefore that the chlorine-benzoic acid treatment did not interfere with diastatic activity.

#### TYPES OF FUNGI AND BACTERIA RESISTING TREATMENT

The types of bacteria and fungi resisting treatment were studied. Appropriate dilutions were made from the nutrient broth tubes which showed positive results and nutrient agar and Czapek's agar plates were prepared.

Sixty-eight types of bacteria were isolated, 64 of these being spore-formers, 2 non-sporeforming rods and 2 cocci. The following species were isolated:

Acrostalagmus cinnabarinus Corda

Alternaria spp.

Cladosporium spp.

Fusarium spp.

Paecilomyces varioti Bain.

Penicillium chrysogenum Thom

Penicillium purpurogenum Stoll

EFFECT OF VARIOUS CONCENTRATIONS OF CHLORINE ON GROWTH  
OF BACTERIA ISOLATED FROM GRAIN

The possibility that small amounts of residual chlorine might inhibit the growth of micro-organisms seemed worthy of consideration. If small amounts of active chlorine on the treated samples were transferred to the medium it is conceivable that a negative result might be due to inhibition of bacteria and fungi rather than to freedom from these organisms. In order to determine the amount of chlorine that might be transferred in this manner, seeds disinfected by the procedure of Milner, Washowsky, Tervet, and Geddes, 1943, (17) were tested for residual chlorine by the Hellige Color Disk method. Two hundred and fifty treated seeds in 100 ml. of water carried over sufficient chlorine to give a reading of 0.015 parts per million of available chlorine.

Tests were then made to determine whether chlorine in various concentrations would inhibit growth of bacteria. Eight species of bacteria, isolated from the normal wheat populations, were used. Calcium hypochlorite solutions were made up to give the following approximate concentrations of chlorine; 0.018, 0.037, 0.075, 0.10, 0.3, 0.5, 1.0, 1.8, 3.7, 7.5, 15.0, 30.0 parts per million. One tenth ml., 0.2 ml., and 0.3 ml. of each were added to tubes containing 5 ml. of dextrose broth to which a loop of a young culture of bacteria had just been added. Vigorous growth took place in all cases, with no indication of inhibition. This would suggest that the chlorine added is dissipated, probably by chemical union with some protein matter in the broth. Consequently, small traces of chlorine that might have been carried over to the broth on the seed, would not exert any inhibitory effect on micro-organisms that might have withstood the chemical treatment. In other words, negative results in the tests for sterility reported in a previous section of this paper can be accepted as indicative of freedom from bacteria and fungi capable of growing under the conditions provided.

#### DISCUSSION

Evidence that commercial wheat harbours large numbers of a wide variety of micro-organisms was not unexpected. During harvesting, threshing, transportation and storage grain is exposed to the elements and, consequently, might serve as a mechanical carrier of any of the many species of bacteria and fungi normally present in soil and dust. Most of

the species of fungi identified in this study are listed as having been isolated from Manitoba soils (4, 5). Six additional types were found on grain, namely: Cephalothecium roseum, Septoria nodorum, Fusarium semitectum, the Aspergillus glaucus group and A. oryzae. Evidently no one has prepared a list of the bacteria in soil in this area.

Too, one might expect to find pathogenic species of fungi on wheat, especially on samples representing many different areas of production. The following isolations in this study are recorded in Orton's bibliography, 1931, (18) on seed-borne parasites:

Acrostalagmus sp.

Alternaria tenuis Nees

Aspergillus niger van Tiegh.

Cephalothecium roseum Corda

Cladosporium herbarum (Pers.) Link

Fusarium culmorum (W. G. Smith) Sacc.

Helminthosporium sativum Pam., King and Bakke

Septoria nodorum Berk.

Two findings in this study present presumptive evidence of the existence of a bacterial flora that proliferates on wheat. Many of the low grades, undoubtedly, were exposed to weathering conditions for long periods of time. This exposure to dampness and warmth would favour the activity of this flora and could account for the significant rise in bacterial population from grade 1 to grade 6. The theory of the existence of a bacterial flora on the seed coat is supported also in the data on attempts to free wheat of organisms by repeated washings in sterile water

presented in table 4. The bacteria in washings from good quality wheat, previously exposed to several washings, average 0.14 millions per gram; or about one-third the average number in the first washings from the grade 1 samples shown in table 1a.

Likewise the presence of relatively large numbers of yeasts on all samples of wheat studied suggests the existence of a yeast flora that proliferates on wheat. This population is much larger than the yeast population in soil. In fact, only seldom are yeasts found developing on Czapek's medium when soil is plated at the dilution used in this study (4, 5).

The relatively small numbers of fungi found on all grades of wheat in this study, on the contrary, do not support the theory of a saprophytic fungal flora on the seed coat of wheat.

Although the investigation carried out here would not be sufficient to establish the existence of an internal microflora in wheat, this possibility cannot be ignored. Some investigators have considered this to be the case with reference to other grain and legume seeds. Robinson, 1910, (20), believed that the failure to sterilize peas and beans indicated either chance infections more resistant than those on other seeds or the possibility that sometimes the infection existed under the seed coat. In many attempts to sterilize seeds the conclusion seemed inevitable that fungi occurred under the seed coats rather frequently in some lots of seeds. Mische, 1930, (16) suggested the same possibility in a description of an experiment with pumpkin seeds. Although the experiment was carried out no less than four times, and every aseptic precaution was observed, the seeds showed bacterial infection. The incidence of

infection was so regular that there could be no question as to any fault in technique; but rather the unavoidable conclusion was that the interior of the pumpkin seed was not sterile.

Fungi have been found within the seed coat. Koehler, 1938, (13), in his study of fungus growth in shelled corn states that the "blue eye" condition of corn is caused by the growth of certain species of *Penicillium* between the germ and the seed coat.

The presence of bacteria on the outside and also in the interior of a large proportion of peas, soybeans, and alfalfa seeds has been demonstrated by Hofer and Hamilton, 1938, (10). Ramstad and Geddes, 1942, (19) state that surface sterilization techniques indicated that bacteria and fungi were present, not only on the surface of soybeans, but in the interior as well.

#### SUMMARY

1. Samples of wheat shipped from various points in Western Canada were found to harbour large bacterial populations. Numbers of bacteria washed from wheat of different grades show a regular and significant increase from grade 1 to grade 6.
2. Likewise, the yeast populations were found to be relatively large and to be subject to wide fluctuations.
3. On the contrary, fungi were present in small numbers, although isolated from all 10 gm. samples studied.

4. Presumptive evidence to support the theory of the existence of bacterial and yeast floras that proliferate on wheat is presented.
5. Eighty-five per cent of all bacteria removed from grain by washing with sterile water had most of the cultural and physiological characteristics of Phytomonas translucens var. undulosa (Smith, Jones and Reddy) Hagborg.
6. The fungi isolated from wheat were, for the most part, soil types and some species known to be wheat pathogens. The most prevalent were species of *Penicillium*, *Aspergillus*, *Fusarium*, *Alternaria* and *Cladosporium*.
7. Attempts to free wheat from micro-organisms by repeated washings proved unsuccessful.
8. Almost complete freedom from micro-organisms was obtained when 50 grams of wheat were treated with 20 ml. of chlorine, 20 ml. of benzoic acid and 60 ml. of water for 10 minutes.
9. Viability of the seeds was unharmed by this chemical treatment.
10. The chlorine-benzoic acid treatment did not interfere with diastatic activity.
11. The types of organisms resisting treatment were principally sporebearing bacteria and species of *Alternaria*, *Fusarium* and *Penicillium*.
12. Small amounts of chlorine that might have been carried over to the broth on the seed would not exert any inhibitory effect on micro-organisms that might have withstood the chemical treatment.

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