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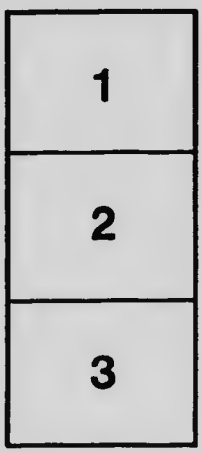

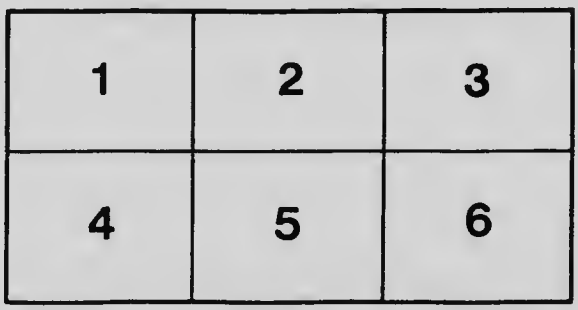

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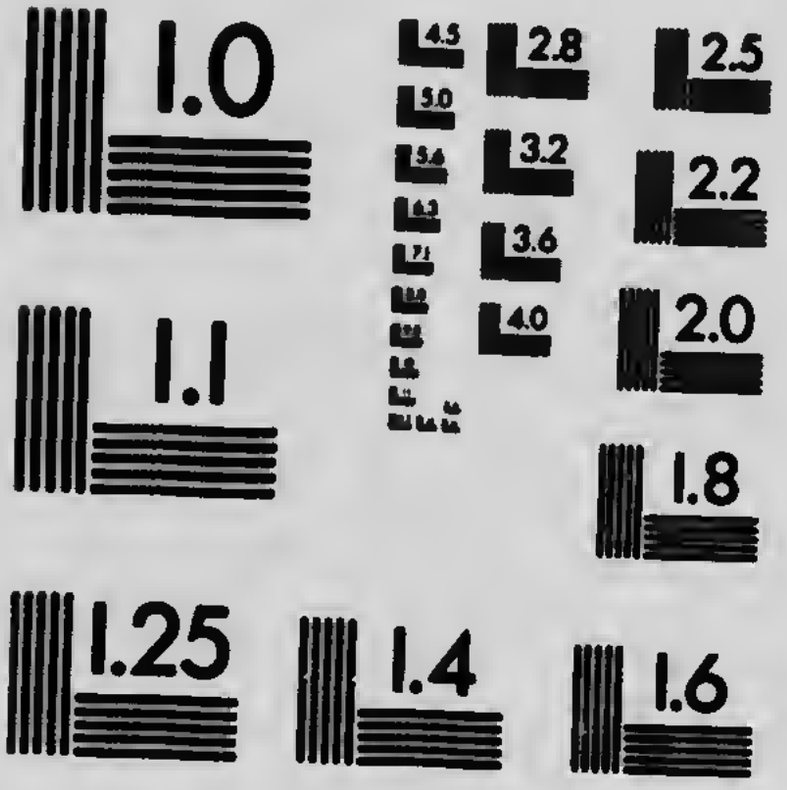

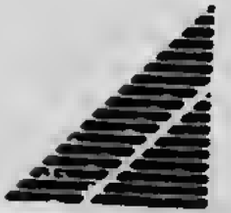

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BULLETIN 141 '09 AP...

ONTARIO AGRICULTURAL COLLEGE AND EXPERIMENTAL FARM

GAS-PRODUCING BACTERIA AND THEIR EFFECT ON MILK AND ITS PRODUCTS.

By F. C. Harrison, Professor of Bacteriology.

One of the commonest troubles in a cheese factory is the affection known to cheesemakers as "gassy" milk, which gives rise to off-flavors, and swelling and huffing of the cheese, and whilst it is impossible to give figures showing the financial loss by depreciation in the value of such tainted cheese, we know that such losses are frequently very serious.

Considering the importance of the bacteria which induce these changes in milk, very little attention has been given them by American bacteriologists, but in Europe, a number of valuable investigations have been made but under conditions which are very different from our own. On this account, a number of experiments were planned and carried out in the Bacteriological Laboratory of the College, aided by the facilities afforded by the College cheese factory.

Many of the details of these experiments are of a technical nature, dealing with the peculiarities of the shape, structure, and growth of the sixty-six varieties of gas-producing bacteria isolated from various sources. No mention will be made of these in this bulletin, but, in addition to the scientific data, a number of practical points were investigated which are now set forth in this bulletin.

The gas-producing bacteria were isolated from the milk supplied to the College Dairy by farmers in the vicinity. This milk compared favorably with the ordinary factory supply, as constant endeavors have been made to instruct the patrons in the most approved manner of handling their milk. In spite of this fact, the College cheesemaker is very often bothered with gassy fermentations in the curd and cheese, and this investigation was undertaken in order to find out the habitat, ascertain the number and study the effects of the various species of gas-producing bacteria present in milk and cheese.

Samples of milk were taken in sterile tubes from the mixed milk of each farmer, and immediately brought to the laboratory and analysed. This sample, after taking out the quantity that was used for the analysis, was kept for a day or two at blood temperature in order to ascertain if gas was produced. Occasionally, we found gas bubbles in the milk sample, but no gas-producing bacteria developed in the gelatine plates, a probable proof of the small number of this class of organisms in the sample at the time the examination was made.

The samples of milk from some farms always showed gas, whilst from others it was only occasionally present, and a few samples never showed a trace. On the hottest days the number of gas-producing bacteria was often very large, whilst on cooler days the number present was always very small.

From a number of samples of milk obtained from other sources, we isolated gas-producing bacteria, but these samples were not so fresh as those we collected at the Dairy. The following table shows the results of the examination of milk from the College Dairy herd and from the mixed milk of farmers supplying the factory. A perusal of this table shows that for the 27 examinations here recorded the percentage of gas-producing bacteria varied from a fraction of one per cent. to over 34 per cent., with an average of 4.67. This table by no means represents the total number of examinations made, as in nearly every case each farmer's milk was examined from three to seven times.

Source.	Total No. of Bacteria per cubic centimetre, (16 drops.)	No. of Gas-producing Bacteria per c c. (16 drops.)	Percentage.
Dairy Herd...	1,915,000	46,000	2.4
Farmer H.....	30,000,000	12,900	0.043
" "	7,000,000	123,000	1.7
" "	807,000	2,000	0.2
Farm Department.....	1,750,000	5,120	0.2
" "	208,000	16,000	7.6
Farmer G.....	40,600,000	96,000	0.2
" Gn.....	539,000	154,300	28.6
" H.....	49,000,000	112,000	0.2
" "	714,000	3,840	0.5
" D.....	360,000	1,200	0.3
" L.....	3,240,000	77,500	2.3
Dairy Herd...	7,737,000	6,240	[illegible]
" "	432,000	2,400	
" "	3,000,000	333,600	11
Farmer M.....	17,000,000	153,000	[illegible]
" "	29,000,000	3,200	
" "	101,000,000	43,000	0.04
" McD...	3,494,000	112,000	3.2
" "	9,885,000	3,397,000	34.2
" H.....	77,000,000	448,000	0.53
" P.....	4,170,000	794,440	19.0
" "	270,000	9,000	3.3
" "	446,000	2,880	0.6
" R. L.....	5,000,000	100,000	2.0
" "	5,654,000	260,000	4.5
" L.....	1,872,000	36,000	1.92
Average.	14,892,333	235,208	4.67

Having ascertained the fact that numerous gas-producing bacteria were present in the milk as delivered at the factory, the next step was to try to find out how the gas-producing organisms got into the milk.

Some investigators have shown that the milk before it leaves the udder may be contaminated with bacteria, and further that these bacteria were occasionally gas-producing organisms. There is also a well known fact that inflammation of the udder (mastitis) is, at times, caused by gas-producing bacteria. Taking every possible precaution to guard against the entrance of germs from the air, and from the hairy coat of the animal, we examined the milk from the 25 cows comprising the Dairy herd, and from the milk of two of them a number of gas-producing bacteria were isolated. The results of this experiment are important, because it explains why some of the factory inspectors have been able to trace gas production to a single cow in a herd.

Photograph of gelatin plate, made from a drop of milk, shewing colonies of gas-producing germs. Each white dot is called a colony, and is made up of huge numbers of individual germs, the result of the continued growth of a single germ that was in the drop of milk, and which was held in place when the gelatin solidified. Note the gas bubbles at G.

Thirteen analyses were made of the stable air, but this was remarkably pure and no gas-producing bacteria were found.

During the movements of milking, particles of skin, hair, etc., and with them bacteria, are dislodged from the animal's coat, and drop into the milk pail. We found that gas-producing bacteria were present upon the hairy coat of the animal. When the udder and flanks were wiped

with a wet cloth, these bacteria were prevented from falling into the pail, as germs are unable to leave a moist surface.

The cows drank from a wooden trough in the pasture field, and, on examination, this water was shown to contain gas-producing organisms which probably came from the soil, as the water obtained from the tap was remarkably pure, containing less than 20 bacteria per c.c. and none of these gas-producing forms.

By washing out clean, dry cans which had been cleaned in the ordinary manner with sterilized water, we obtained gas-producing bacteria.

Very many flies were present in the stable, and these frequently fell into the pail and added undesirable bacteria, which find in milk a good food for growth. A number of these flies were captured, and single flies were placed in test tubes containing a measured quantity of sterilized water and well shaken. This water on analysis was found to contain large numbers of gas-producing bacteria. Frequently, 50,000 bacteria were obtained from a single fly, and of these over 20,000 were gas-producing bacteria.

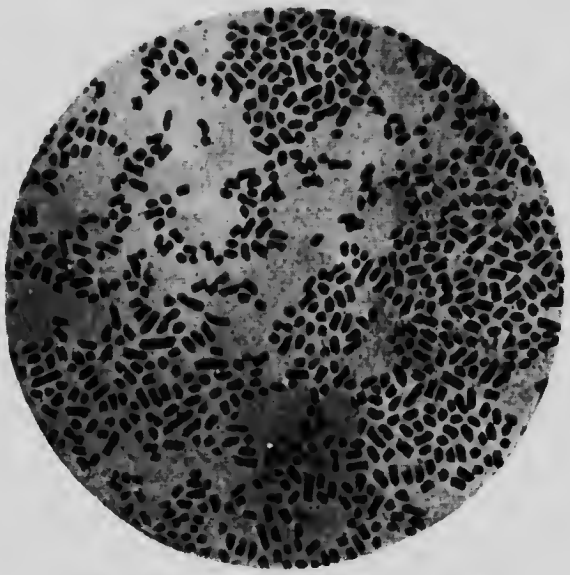
Large numbers of gas-producing bacteria were also obtained from manure. The ratio of gas-producing germs to other species in some 17 examinations was as 250 to 1. Cultures in sterilized milk made from some of these bacteria gave the peculiar odor known to cheesemakers as "gassy" milk, and others gave a characteristic "cowy" odor, although this peculiar smell has usually been ascribed to stable odors.

To summarize, gas-producing bacteria were found to be occasionally present (1) in the udders of certain cows, (2) on the hairy coat of the animal, (3) in clean, dry milk cans, (4) in the watering trough, (5) from flies, and (6) from manure. From these various locations, the gas-producing bacteria may contaminate the milk.

The gas-producing bacteria were readily killed by an exposure to temperatures ranging from 137 degrees to 146 degrees F. for 10 minutes. They were all killed by immersion in a 2 per cent. ammonia washing powder solution at 140 degrees F. and also in a 2 per cent. soda solution at 140 degrees F. for 10 minutes. These soda and ammonia washing powders are scarcely more effective than hot water for the destruction of these bacteria, but these substances aid in washing by helping to remove the dirt.

By continued growth in milk it was found that these gas-producing bacteria increased their power of fermenting the milk sugar. Thus, one variety, which originally produced 26 per cent. of gas, after growing for some time in milk produced 62 per cent.; hence those bacteria which were not killed by the hot water used in washing the cans would be more liable to produce larger quantities of gas than those which came in from other sources.

In dairy practice a starter or culture of a lactic acid bacillus is used to overcome the gassy fermentation of milk, and in order to quantitatively establish the working of this process, a number of experiments were instituted, in which gas-producing and lactic acid organisms were mixed together in order to study their antagonistic relations. The results of these experiments in general showed that the number of gas-producing germs decreased with the increase of lactic acid bacteria. Occasionally, however, some organisms were experimented with which were not so adversely influenced by the lactic acid germs.

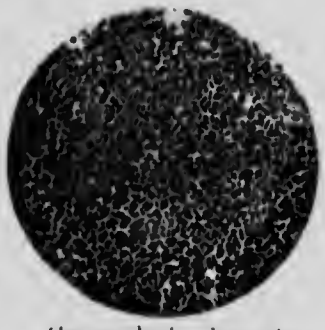


Gas-producing bacteria; magnified 1500 diameters.

CHEESE EXPERIMENTS. A number of cheese were made from milk to which various quantities of a culture of gas-producing bacteria was added. A few of these experiments my be cited:—A cheese was made on the 5th of October from 300 pounds of milk to which 2 pounds of a 24 hour old milk culture of a gas-producing variety was added. This culture was acid, very gassy, with a bitter, astringent taste. The cheese curd was also very gassy, floating on the top of the whey. After matting, the cheese was put into the curing-room with an average temperature of 55 degrees F., and bacteriological analyses were made from time to time. At the age of 21 days, the percentage of gas-producers was 76, at the end of 38 days, 71; and at the end of 52 days, 11; and at this stage, the cheese showed white and grey lines and spots, an appearance known amongst cheesemakers as "mottled." The cheese was scored, but no points could be given to it for flavor. The odor was something like rotten meat, and the mottled appearance was very striking.

A second cheese was made with a different culture of a gas-producing organism, and at the age of seven days was found to contain 15 per cent. of gas-producers. This number gradually declined, and at the age of 45 days only 2 per cent. of gas-producing bacteria were present. The cheese was examined at the end of 63 days, and was found to be slightly unpleasant in smell and taste, but was judged to be better than that made on the 5th of October, receiving 15 points out of 45 for flavor. On November 2nd, two cheeses were made. In the A cheese, ½ per cent. of a 24 hour old milk culture of a gas-producing germ was used, and in the B cheese ½ per cent. of the same culture and ½ per cent. of the lactic acid bacillus. Both curds were floating about three hours from setting; the flavor was gassy. The B curd was better than the A, although even B was very

Growth of gas-producing germs in test tubes containing sugar gelatin. Note the gas bubbles.

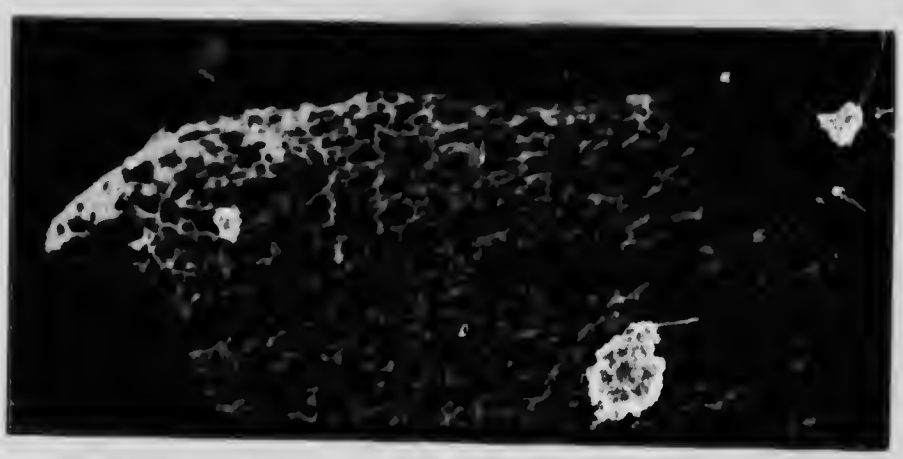


Gas-producing bacteria; magnified 1000 diameters.

gassy. The B cheese was placed in an ordinary curing-room, and the percentage of gas-producing germs at the age of 10 days was .5. The cheese was examined at the end of 63 days, and was found to be slightly mottled, with fair flavor. The curd for the A cheese was divided into equal portions, one of which was placed in an ordinary curing-room at a temperature of 55 degrees to 60 degrees F., and the other put into the refrigerator curing-room, the average temperature of which was 40 degrees F. The percentage of gas-producing germs in both of these cheeses was very high. They were judged at the end of 63 days, when the taste and odor of both were found to be bad. The appearance of the one in the ordinary curing-room was very mottled. The one in the refrigerator curing-room was also mottled, but to a lesser extent.

These experiments show that gas-producing germs are able to produce a bad odor and flavor in cheese, and cause a mottled appearance, which is probably brought about by the bleaching action of the gases generated by the organisms introduced in the culture. The good effect of

a lactic acid starter when these injurious bacteria were present very noticeable, and caused great improvement in the flavor and appearance of the cheese.



A piece of curd taken from a vat ripened with a starter containing gas-producing bacteria.

A floating curd caused by gas-producing bacteria.

BUTTER EXPERIMENTS. Pasteurized cream was inoculated with 5 per cent. of a gas-producing culture, ripened for 24 hours at 58 degrees, and then churned. The butter had a bitter, disagreeable, and slightly astringent flavor, and scored only 32 per cent. These experiments were subsequently repeated with other varieties of gas-producing bacteria, and with the same results, showing that these organisms were just as injurious to the flavor of the butter as they were to the flavor of cheese.