

BIOLOGICAL TREATMENT OF MOLASSES MEAL TO REDUCE STICKINESS

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Introduction

Molasses meal is a popular stock feed which is manufactured from two by-products of the sugar milling process, namely cane flour and molasses. Cane flour, or pulp, is prepared from bagasse by separating the pithy fraction and then drying and milling it. In this form it is capable of absorbing up to four times its own weight of molasses.

One such stock feed, produced in Natal, is made up of 75% molasses and 25% cane flour. The molasses used has the following approximate analysis: moisture 22-26%, protein 3-4%, carbohydrates 61.7% and ash 8.6%. The carbohydrates consist of 36.4% sucrose, 13.39% invert sugars, 0.63% starch, and 4.69% gums. The meal itself has the following average analysis: 19.0% moisture, 4.4% protein, 0.8% fat, 59.1% carbohydrates, 8.4% fibre, and 8.3% ash.

Molasses meals have gradually increased in popularity as a stock feed, although they suffer from one important drawback which detracts from their wider use, namely their stickiness. Thus, when bags of molasses meal are stacked and transported, the contents tend to become compact and lumpy and, as a result, it is difficult to mix it with other ingredients to prepare a balanced ration. This stickiness was thought to be due to the gums present in the molasses itself, but to clarify this point, the fate of the gums was examined in detail.

The Problem

The task set in this study was to determine the cause of stickiness in molasses meal, and to ascertain if it could be successfully eliminated. Fortunately, bulk samples of molasses meal sometimes lose their stickiness and become free flowing, due to fermentation induced by microbes. It was this feature that narrowed the field to one of a microbiological study.

The Study Programme

The first task was the isolation of micro-organisms associated with free flowing properties of molasses meal, and the identification of those actually capable of breaking down the gums present. This was done by isolating and culturing micro-organisms found in samples of free-flowing and sticky meals, selecting those associated with the former, then using these to infect natural gums.

The next stage was to test, in the laboratory, the effect of selected organisms on sticky meal. This was followed by small scale factory tests of the apparently useful organisms and finally the further selection of organisms for routine industrial use.

Material and Techniques Used in these Studies

Culture Media

The following media were used for plating:

1. Nutrient agar: Beef extract 2 g, yeast extract 2 g, peptone 5 g, sodium chloride 5 g, agar 15 g (pH 5.6 and 7.2).
2. $\frac{1}{3}$ -D-Nutrient agar: Beef extract 3 g, peptone 4 g, dextrose 4 g, sodium chloride 6 g, agar 15 g (pH 5.8 and 7.4).
3. Potato-dextrose agar: Extract of 300 g of potatoes, dextrose 20 g, agar 15 g (pH 5.6 and 7.5).
4. Malt extract-molasses agar: Malt extract 25 g, molasses 10 g, agar 15 g (pH 5.8).
5. Gum-nutrient agar: Gum arabic 5 g, beef extract 3 g, peptone 4 g, sodium chloride 7 g (pH 5.6 and 7.2).
6. Czapek's solution agar: Sodium nitrate 3 g, dipotassium hydrogen ortho-phosphate 1 g, magnesium sulphate 0.5 g, potassium chloride 0.5 g, ferrous sulphate 0.01 g, sucrose 30 g, agar 15 g (pH 6.8).
7. Steep liquor Czapek's agar: Czapek's agar plus 10 ml of concentrated corn steep liquor (pH 7.0).
8. Glycerol asparaginate agar: Glycerol 10 g, dipotassium hydrogen ortho-phosphate 1 g, sodium asparaginate 1 g, agar 15 g (pH adjusted to 7.0 using calcium carbonate).

Identification of organisms

Isolates of bacteria, actinomycetes, yeasts and fungi were all subjected to microscopic examination. Bacteria were stained by Gram's technique and by using methylene blue. Capsule formation was determined in indian ink preparations and by the Hiss technique¹⁶. Fungi were stained in Heidenhain's haematoxylin solution before examination under a microscope. The identification and classification of nineteen bacteria which showed promise in laboratory tests, either by decomposing gums or acting as antagonists, was carried out using recognised cultural, physiological and biochemical techniques^{3, 4, 6, 8, 9, 10, 11, 13, 14, 15, 16}. Established techniques were also used for the identification of fungi^{1, 2, 5, 7, 12, 16, 17, 18}.

Methods and Results

Molasses meal used in this study was derived from 11 different sources. Sub samples were taken at different depths in free flowing molasses meal; from the centre of bulk consignments on the verge of spontaneous combustion; from plastic bags (in which the meal is invariably sticky) at the edges of stacks, at different intervals of time after stacking; and from similar bags in the centre of stacks three weeks after stacking.

TABLE I
The average number and types of micro-organisms per gram in sub samples of a molasses meal
(f=free flowing sample; st=sticky sample)

Source	Sampling	Bacteria*			Actino- mycetes†	Yeasts‡	Fungi‡
		Aerobic		Anaerobic			
		mesophilic	thermophilic				
BULK LOT (normal (f))	Surface	5.1×10^6	7.9×10^2	4.7×10^2	2.1×10^3	4.3×10^3	9.6×10^4
(f)	24 in deep	6.8×10^6	1.5×10^3	5.3×10^3	1.9×10^2	3.9×10^3	7.5×10^4
(f)	48 in deep	2.8×10^6	7.8×10^5	8.8×10^5	9.4×10^1	2.5×10^1	3.9×10^3
(f)	72 in deep	9.6×10^5	1.7×10^6	2.9×10^6	4.5×10^1	7.0	7.3×10^3
BULK LOT (on verge of spontaneous combustion)	12 in from heated core	4.0	4.2×10^2	7.0×10^2	Nil	Nil	Nil
	36 in from core	3.7×10^2	6.9×10^5	7.6×10^5	1.3×10^1	Nil	Nil
STACKED BAGS							
Random sample (st)	Time interval						
	At bagging	9.3×10^2	8.2×10^3	5.9×10^2	2.2×10^1	6.1×10^1	1.4×10^1
Edge sample (st)	1 week later	8.7×10^2	9.6×10^3	1.2×10^3	1.7×10^1	4.2×10^1	5.2×10^2
Edge sample (st)	2 weeks later	1.4×10^3	5.9×10^3	9.1×10^2	1.2×10^1	1.8×10^2	7.5×10^3
Edge sample (st)	3 weeks later	2.8×10^5	4.2×10^4	2.6×10^3	3.1×10^1	9.4×10^1	4.8×10^3
Centre sample (st)	3 weeks later	6.4×10^3	6.9×10^6	1.7×10^6	4.8×10^1	Nil	6.9×10^1

* Grown on 1/3-Dextrose nutrient agar.

† Grown on glycerol asparaginate agar.

‡ Grown in malt-extract molasses agar.

The relative merits and importance of micro-organisms present in these samples was determined quantitatively and qualitatively by measuring populations of groups and by isolating and identifying individual species. Quantitative assessments were obtained by counting bacteria raised on $\frac{1}{3}$ -dextrose nutrient agar, actinomycetes raised on glycerol asparaginate agar, and yeasts and fungi by rearing them on malt extract molasses agar. Details are given in Table I.

Isolates were obtained by plating replicated samples of diluted meal, removing single cell samples of identified organisms, and then replating these as uncontaminated specimens. Anaerobic organisms were selected by plating the samples in media 1, 2, 3, 5 and 8, in an atmosphere of pure hydrogen.

Thermophilic organisms were obtained and isolated by adding meal samples to sterilised broth of media 1, 2, 3, 5 and 8, omitting the agar. These were then heated for 5 minutes at temperatures of 40, 50, 60, 70, 85 and 100 C, the last named temperature ensuring the death of even the spores of the thermophilic organisms. After this preliminary heating, the test tubes which had been heated to temperatures in excess of 56 C were cooled rapidly to this point, and retained overnight in a shaking incubator. These broth cultures were then plated using the same media, but adding 15 g agar per litre for the three lower temperature treatments or 15 g of silica gel per litre for the three higher temperature treatments. The agar plates were then incubated for 48 hours at 37, 45 and 50 C, and the silica gel plates for the same time at 60, 65 and 70 C. A final plating was carried out on $\frac{1}{3}$ -dextrose nutrient agar, and the isolated colonies then transferred to nutrient agar to create permanent cultures.

The total number of isolates obtained from the initial cultures comprised 63 different bacteria, 5 actinomycetes, 9 yeasts and 17 different genera of

fungi. Isolates derived from the sticky meal which were not present in the free flowing material are important only in relation to their population densities and their infection potential. The remainder, however, all of which were associated with the free flowing characteristics of molasses meal, were multiplied for further use.

Multiplication of selected isolates

Bacteria selected for multiplication were grown in Erlenmeyer flasks on a medium containing 0.1% blood meal, 0.1% molasses, 0.1% peptone and 0.1% beef extract. These ingredients were first mixed with water to yield a suspension, and then sterilised in the flask for 20 minutes at a pressure of 18 lb. Inoculation was carried out when the sterilised flasks were cooled.

Actinomycetes and fungi were multiplied using similar techniques but, because of their specific requirements for sporulation, different media had to be used. Thus, actinomycetes were reared in media 2 and 8, and the fungi were cultured in media 3, 4, 6 and 7. The latter were then transferred to a 9:1 bagasse-molasses mixture, in order to induce sporulation. Details of the preparation of this mixture are provided in Appendix 1.

Quantitative importance of micro-organisms

Counts of micro-organisms present in samples of meal are shown in Table I. It can be seen from these that the numbers of aerobic mesophilic bacteria decrease towards the centre of the bulk mass. In contrast, the thermophilic aerobic spore-forming bacteria, and the anaerobic bacteria, increase in numbers the deeper one penetrates the pile. Actinomycetes were present in relatively low concentrations both in the free flowing and in the sticky meals. Yeasts and fungi were found in relatively high numbers in free flowing meal to a depth of 24 inches, but decrease in number at greater depths.

Samples taken 12 inches from the centre of a bulk consignment on the verge of spontaneous combustion showed that only thermophilic and anaerobic bacteria were present, and only relatively small numbers of these. However, 36 inches from the core, the number of thermophilic bacteria totalled 690,000, while anaerobic bacteria numbered 760,000 per gram. Mesophilic bacteria, actinomycetes, yeasts and fungi were rare or absent in these samples.

In the bagged consignments, the initially sticky meal usually remained sticky. Samples taken immediately following bagging revealed that a remarkable number of mesophilic, thermophilic, and anaerobic bacteria were present, although the number of actinomycetes, yeasts and fungi were low. The highest population group proved to be the thermophilic spore-bearing organisms. It is obvious therefore, that despite the high temperature of the molasses when it is mixed with the bagasse, micro-organisms associated with the latter, or added by contamination during milling, are not all killed.

The microflora in bags of stock feed taken from the edge of the stacks, changed only slightly over the first two weeks. However, during the third week there was a much greater change and this was associated with the damp atmosphere in the store caused by rainy weather. By the end of the third week, the numbers of mesophilic, thermophilic and anaerobic bacteria increased respectively from the original 930, 8,200 and 590 per gram to 80,000, 42,000 and 2,600. This increase, most of which had occurred in the third week, was accompanied by a rapid increase in the temperature of the stacks themselves. Samples taken from bags in the centre of the stack during the third week yielded very high counts of thermophilic spore-bearing bacteria. 6.9 million of these were recorded per gram of meal together with a further 1.7 million of anaerobic bacteria. On the other hand, the number of mesophilic bacteria in the samples dropped to 6,900, while the populations of other groups of micro-organisms remained unchanged.

During the heating or fermentation process, some samples of molasses meal were found to have lost their stickiness. Furthermore, under the high temperatures prevailing in the centre of the stacks, there was a sour alcoholic odour, similar to that of butanol with acetic acid. In other cases there was no change in the stickiness of the meal and the stock meal became very compact.

The Effect of Selected Micro-Organisms on Natural Gums

Natural gums exuded by plants tend sometimes to disintegrate and disappear, presumably as a result of breakdown by micro-organisms. These, it was felt, might act as indicators of the gum metabolising properties of organisms which break down stickiness in molasses meal.

To test this hypothesis, gum acacia, gum dammara and bark exudates from citrus suffering from gummosis disease were secured as samples. They were then exposed to fumes of formaldehyde, chloroform and ethylene oxide for not less than five hours, in

order to kill any micro-organisms already present. To remove the toxic effect of these sterilant gases, the samples were then transferred to perforated test-tubes which were placed in a desiccator, and they were then aerated for 96 hours, the air used being passed through a cotton filter. Next, to rehumidify the samples, the test-tubes placed in a nylon stocking were suspended for an hour in a sterile container at 100% relative humidity. Finally, they were transferred to plastic bags containing free flowing molasses meal for a period of 48 hours, during which time the tubes were agitated periodically. The gum samples, now contaminated exclusively with micro-organisms from the molasses meal, were then removed from the plastic bags and placed in humidity chambers at 90, 95 and 100% relative humidity. One set of samples was kept at a temperature of 27 C while the other was retained at 32 C. Specimens from each set were examined twice a week.

Evidence of micro-organisms colonizing the gums were found 3-4 days after exposure in the test-tubes retained at relative humidities at 95 and 100%. It was seven days before similar evidence was found on samples retained at 90% relative humidity. Growth of the organisms was, in all cases, more rapid at the higher temperatures. Examples of this growth are shown in Fig. 1-10. Despite active growth of the micro-flora, no signs of gum disintegration were found until the third week after inoculation. Subsequent changes in the surface structures of the gums, leading to their disintegration, varied between samples, and in some cases there was no disintegration at all.

Isolations of micro-organisms responsible for breakdown of the gums showed that some were identical with those found in free flowing meal, while others were found only on the natural gums. The gum decomposing organisms in the free flowing meal include bacteria, fungi and three actinomycetes. One of the last named organisms was identified as a species of *Nocardia*, the two others, being species of *Streptomyces*.

Laboratory Tests of Selected Isolates

Isolates selected for their association with the free flowing characteristics of molasses meal, together with those selected for their ability to decompose natural gums, were multiplied for use in laboratory tests. Samples of the sticky meal were then sprayed independently with the pure cultures, while additional samples were treated with more than one culture. The treated samples were kept in polythene bags and stored under conditions simulating those prevailing in the factory store.

Bacteria

Twenty-six isolates were tested, seventeen of which were found to reduce stickiness to a level which, statistically, was significantly better than the untreated control. In addition, two species of *Pseudomonas*, one of which was identified as *P. aeruginosa* (Schr.) Mig., were selected, as they controlled the growth of other bacteria responsible for engendering spontaneous combustion. This attribute was checked by

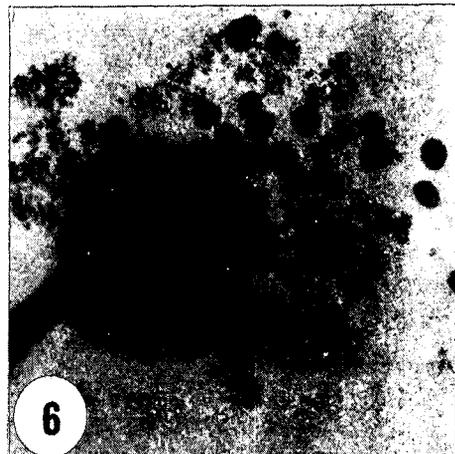
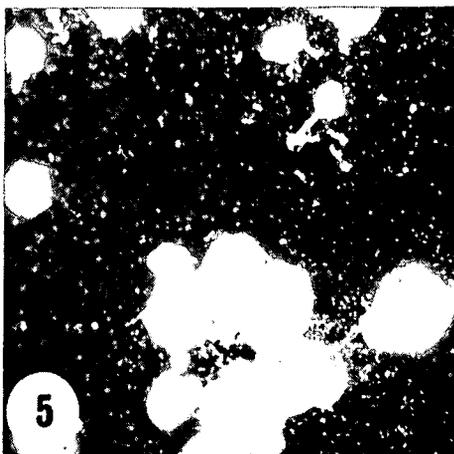
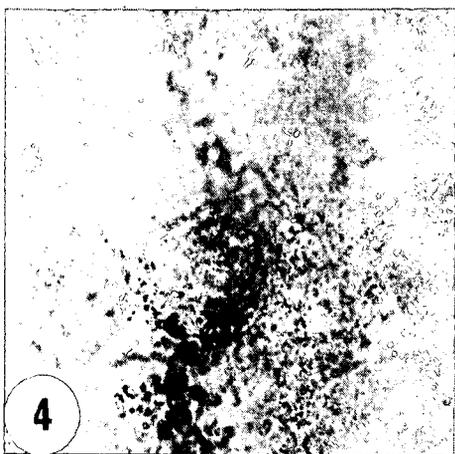
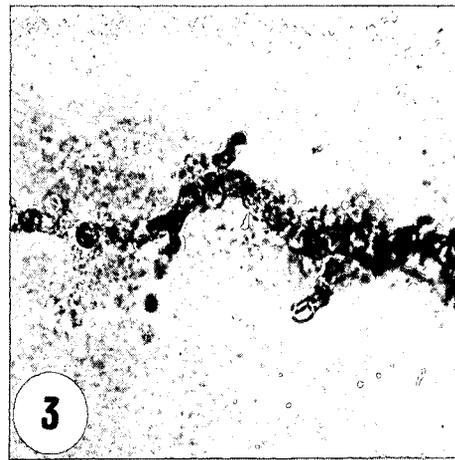
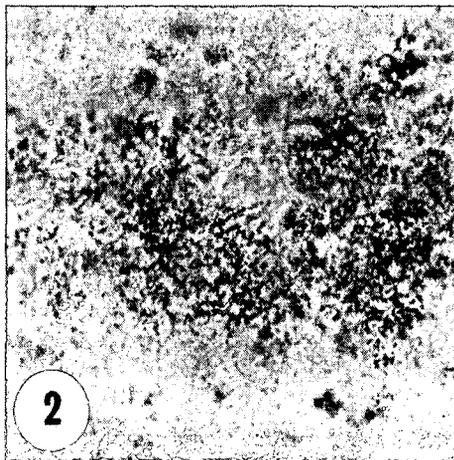
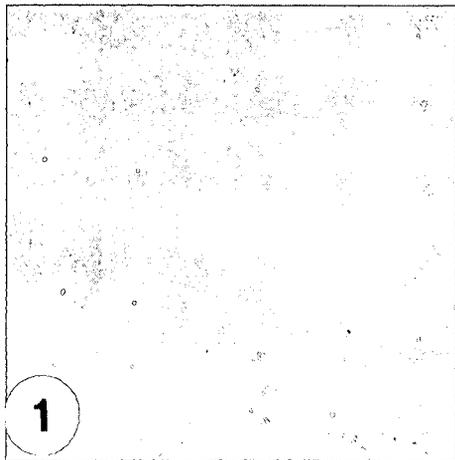


FIGURE 1: Uniform surface of an uninfected sample of a natural gum (gum acacia).

FIGURE 2: Development of bacteria which are breaking down natural gum under conditions of high atmospheric moisture. (x 650).

FIGURE 3: Development of bacteria and fungi on gum samples.

FIGURE 4: An advanced stage in the decomposition of gum acacia.

FIGURE 5: Development of conidiophores and conidia of *Aspergillus* sp. on natural gums.

FIGURE 6: Conidiophore and conidia of *Aspergillus* sp. on gum samples.

FIGURE 7: Deterioration of the surface of a gum sample caused by micro-organisms.

FIGURE 8: A mite carrying gum decomposing micro-organisms.

FIGURE 9: Surface of a deteriorating gum sample completely covered by micro-organisms. Note the hyphae of *Penicillium* species.

FIGURE 10: Different stages in the growth of micro-organisms on gum samples.

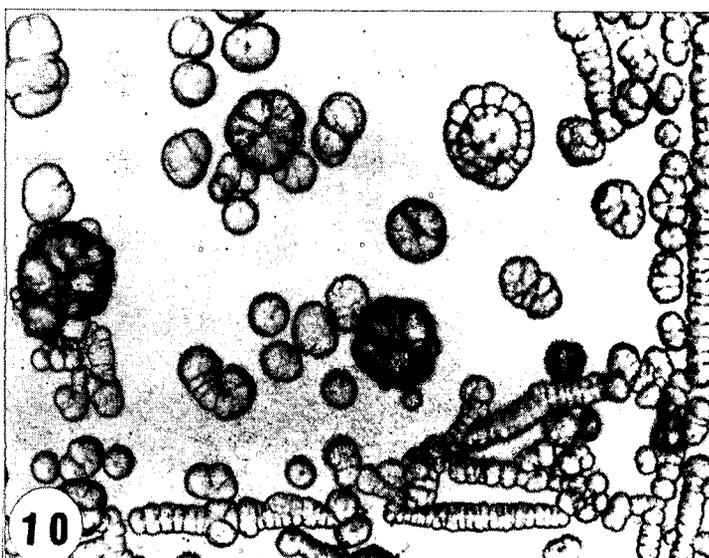
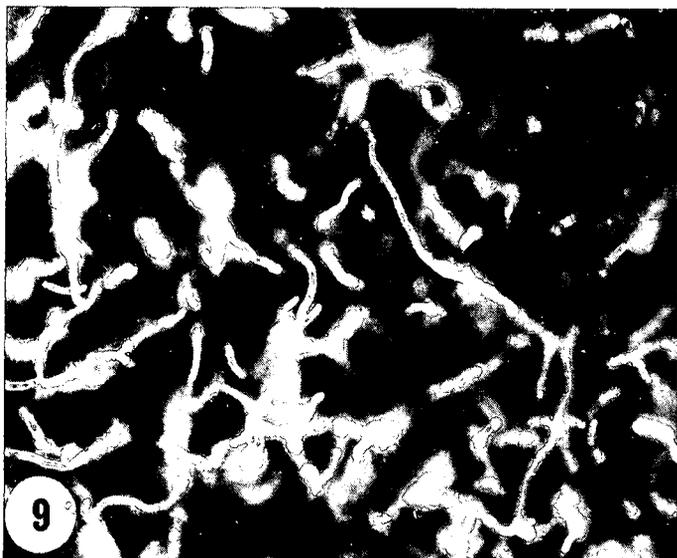
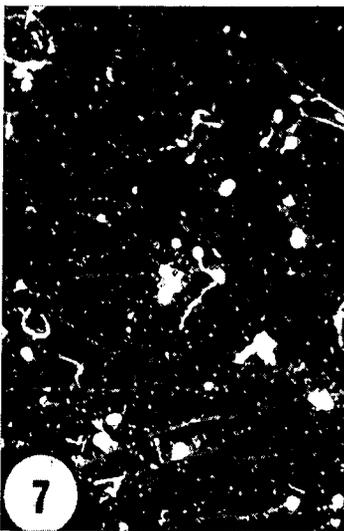


TABLE II
Identification of gum decomposing bacteria

Group	Genus and species	Sources				
		Free flow meal	Natural gums	Air	Area of spontaneous combustion	Centre of stacks
Non-sporing rods:	<i>Arthrobacter globiformis</i> (Eisenbg.) Bergey	+	—	+	—	—
	<i>Agrobacterium rubi</i> Hildebrand	+	—	—	—	—
	<i>Achromobacter</i> sp.	+	+	+	—	—
	<i>Beneckeia ureasophora</i> Campbell	—	+	—	—	—
	<i>Escherichia</i> sp.	+	—	+	—	—
Aerobic mesophilic spore bearing bacteria	<i>Bacillus cereus</i> var. <i>mycoides</i> (Flügge) Sm.	+	—	+	—	—
	<i>B. megaterium</i> de Bary	+	+	+	—	—
	<i>B. pantothenicus</i> Proom & Knight	+	+	—	—	—
	<i>Lactobacillus brevis</i> (Orla-Jensen) Bergey	+	+	—	—	—
Thermophilic facultative anaerobic spore bearing bacteria	<i>Bacillus coagulans</i> Hammer	—	—	+	+	+
	<i>B. stearothermophilus</i> Donk	+	—	+	+	+
	<i>Lactobacillus plantarum</i> (Orla-Jensen) Bergey	—	—	—	+	+
Anaerobic meso- and thermophilic spore bearing bacteria	<i>Clostridium acetobutylicum</i> McCoy et al.	—	—	+	—	+
	<i>Clostridium felsineum</i> (Carb. & Tomb.) Bergey	+	+	—	—	+
	<i>Clostridium flavum</i> McClung & McCoy	+	—	—	+	+
	<i>Clostridium thermoaceticum</i> Fontaine et al	+	—	+	—	+
	<i>Clostridium thermosaccharolyticum</i> McClung	—	—	—	+	+

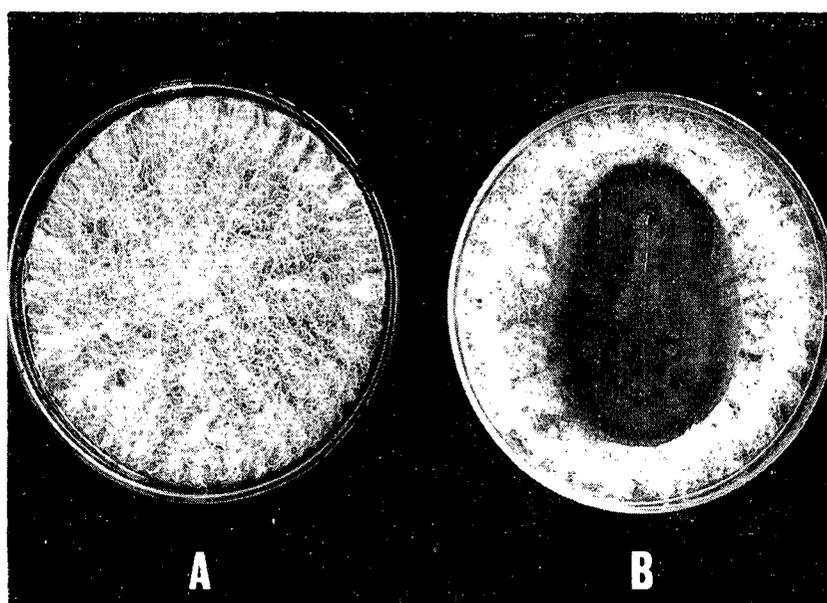


FIGURE 11: (A) *Bacillus cereus* var. *mycoides*, isolated from molasses meal and used as a test organism for selection of antagonistic micro-organisms.
(B) *Pseudomonas aeruginosa* showing typical antagonistic reaction against *B. cereus* var. *mycoides*, grown on an artificial medium.

testing the bacteria on *Bacillus cereus* var. *mycoides* reared on an artificial medium (Fig. 11).

Bacteria selected for their ability to reduce stickiness in these laboratory tests are listed in Table II. These, however, include five micro-organisms associated with spontaneous combustion, 3 from the group of thermophilic facultative anaerobic spore-bearing bacteria and two from the anaerobic meso and thermo-philic spore-bearing bacteria. These were discarded for further study as their value in

inducing free flowing characteristics is not restricted to gum decomposition.

Actinomycetes

The two species of *Streptomyces* and the *Nocardia* sp. which were found to attack natural gums, showed in laboratory tests that they were capable of reducing stickiness in molasses meal. They exhibited a very strong anti-bacterial and anti-fungal reaction affecting a wide range of micro-organisms, including some of the thermophilic spore formers.

Environmental conditions influence the activity of these organisms. A good supply of air increased their rate of activity, while acid conditions suppressed their growth. The pH of free flowing meal in bulk was 4.8 to 5.5 while the fresh sticky meal had a pH of 5.4 to 6.0, both of which are too low for these organisms to operate effectively. However, by adding NaCl, MgSO₄ and CaCl₂ to the sticky meal, to raise the pH to 7.5 or above, the activity of the *Streptomyces* spp. was effectively stimulated.

Fungi

Laboratory tests were carried out with a number of spore-forming fungi including selected species of the genera *Aspergillus* and *Penicillium*, and with different isolates of *Trichoderma lignorum*. These three genera turned out to be the most promising and a list of the different species used is provided in Table III. Some of the *Penicillium* species proved to be most successful in reducing the stickiness of molasses meal. However, as in the case of *Streptomyces* spp., their activity is influenced by environmental conditions. The growth of some of the selected *Penicillium* spp. is illustrated in Fig. 12 and 13.

Factory Tests

Micro-organisms which, in laboratory tests, were found to be most effective in reducing the stickiness of molasses meal, were used for larger scale pilot tests in the factory. Fifteen different isolates of bacteria and fungi were selected for this purpose. Good results were obtained with five bacteria, but by far the best results were obtained using strains of the fungi *Penicillium notatum* and *Penicillium chrysogenum*.

Penicillium notatum was selected for full scale

commercial tests. Several hundred tons of molasses meal were therefore treated with a spore solution of *P. notatum*. To each ton, 15 ml of a spore solution were added, the spore density of which varied between 6.0×10^5 and 1.4×10^5 per cubic centimetre. This spore density was checked using a haemocytometer, which consisted of a double cell counting chamber with improved Neubauer ratings. Meal treated with the fresh active spore solution invariably became less sticky than similar untreated materials, and changed very little in colour.

Difficulties were encountered when, under factory conditions the agar cultures were allowed to dry out or were contaminated by other microbes. Where such material was used to prepare the spore solution, the treated meal not only failed to lose its stickiness, but was often prone to suffer from spontaneous combustion. Studies show that this is due to contamination with the thermophilic bacteria listed in Table I. Further investigations designed to select organisms to suppress those responsible for spontaneous combustion need to be carried out.

Discussion

Studies of micro-organisms as tools which can be used to reduce the stickiness of molasses meal have been carried out with a substantial degree of success. Only one of a number of micro-organisms selected was used on a commercial scale. This was the fungus *Penicillium notatum*. There is, however, good reason to think that some of the other suitable organisms, used either alone or in combination, may yield a substantially improved molasses meal.

The eradication of stickiness was achieved by using micro-organisms to break down the gums present. To do this effectively with *Penicillium*

TABLE III
Identification of gum decomposing fungi

Genus and species	Source		
	Free flowing meal	Air	Natural gums
<i>Trichoderma lignorum</i> (Tode) Harz.	+	+	+
<i>Aspergillus flavus</i> group	+	—	+
<i>Aspergillus fumigatus</i> group	+	+	—
<i>Aspergillus glaucus</i> group	+	+	—
<i>Aspergillus nidulans</i> group	—	+	+
<i>Aspergillus niger</i> group	+	+	—
<i>Aspergillus terreus</i> group	—	+	+
<i>Aspergillus ustus</i> group	—	+	+
<i>Penicillium brevi-compactum</i> series	—	+	+
<i>Penicillium chrysogenum</i> series	+	+	+
<i>Penicillium citrinum</i> series	—	+	+
<i>Penicillium claviforme</i> series	+	—	—
<i>Penicillium commune</i> series	+	+	—
<i>Penicillium cyclopium</i> series	—	—	+
<i>Penicillium duclauxi</i> series	+	+	+
<i>Penicillium expansum</i> series	+	—	+
<i>Penicillium granulatum</i> series	+	+	—
<i>Penicillium luteum</i> series	+	+	+
<i>Penicillium notatum</i> series	+	+	+
<i>Penicillium purpurogenum</i> series	+	+	+
<i>Penicillium rugulosum</i> series	—	+	+
<i>Penicillium thomii</i>	+	+	—
<i>Penicillium viridicatum</i> series	—	—	+

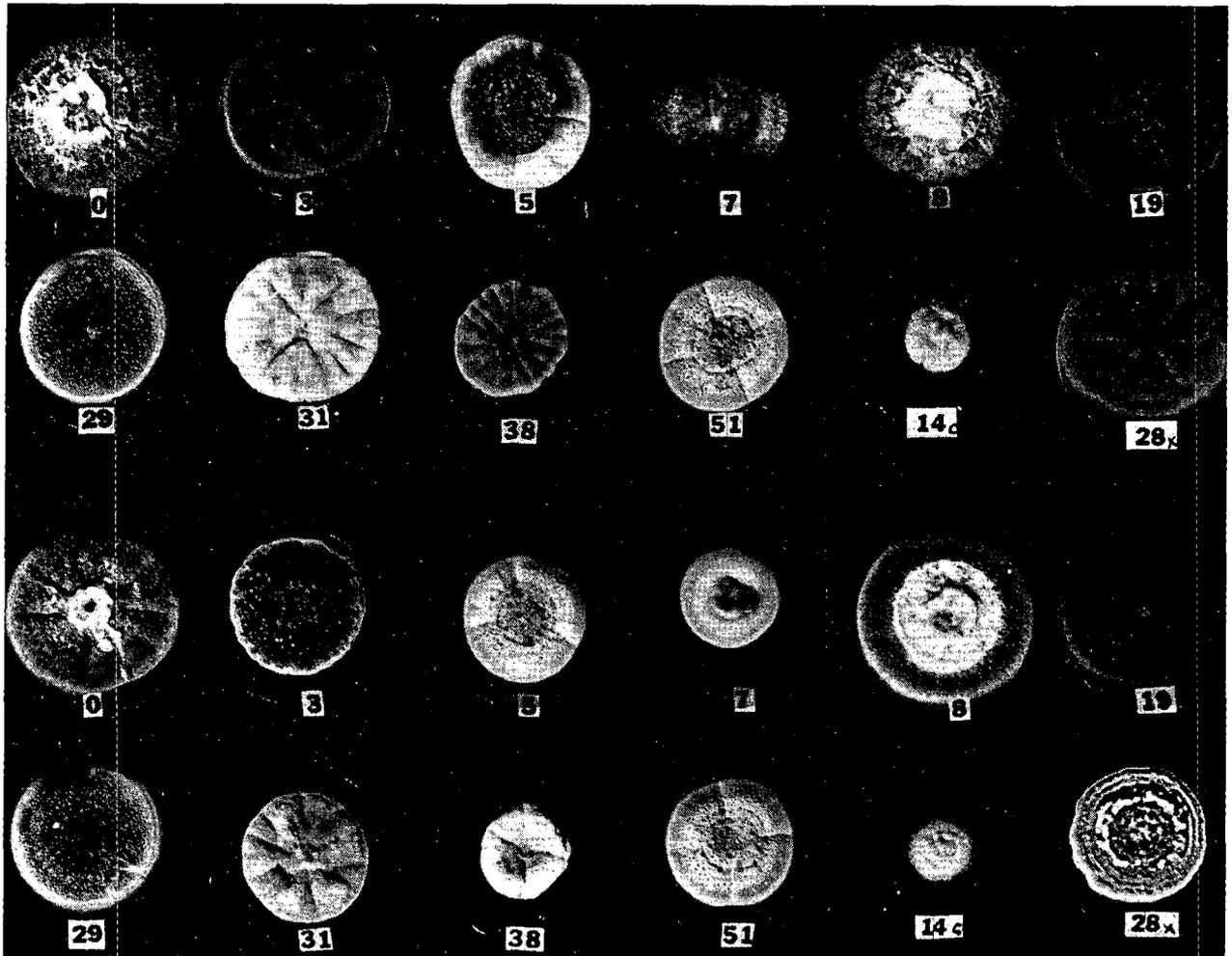


FIGURE: 12. Pattern of growth of some gum decomposing *Penicillium* species on steep liquor Czapek's agar (two upper rows) and Czapek's solution agar (two lower rows). The following series of *Penicillium* are illustrated: 0=*Penicillium notatum*, 3=*P. sclerotium*, 5=*P. chrysogenum*, 7=*P. granulatum*, 8=*P. paxilli*, 19=*P. notatum*, 29=*P. chrysogenum*, 31=*P. lanosum*, 38=*P. luteum*, 51=*P. chrysogenum*, 14c=*P. granulatum*, 28x=*P. claviforme*.

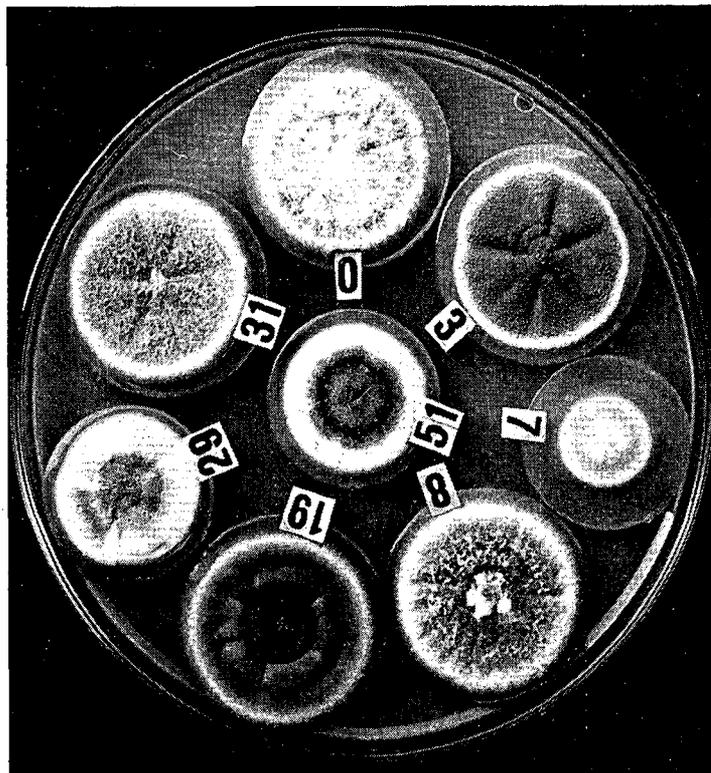


FIGURE 13. The growth on Czapek's solution agar of 8 different *Penicillium* spp., selected for their ability to reduce stickiness of molasses meal. No. 0, 19, 29 and 51 are better than the others, the best being No. 19.

notatum, or with any of the other selected micro-organisms, calls for a high standard of purity in the bulk rearing of the biological material. Contamination, especially by thermophilic organisms, can and does lead to complete failure, or even an increased risk of damage. Furthermore, the activity of the micro-organisms is affected by the environment, and particularly by acidity, aeration, humidity and temperature. Thus, suitable species of *Streptomyces* become effective only under alkaline conditions, while development of undesirable thermophilic and anaerobic bacteria is stimulated by high humidity.

It has been shown that the problem of thermophilic and anaerobic bacteria can be dealt with effectively by employing antagonistic organisms. But those so far selected operate only under relatively well aerated conditions. It is possible that others exist which would work under facultative anaerobic conditions, and this possibility justifies further exhaustive study.

The use of micro-organisms as a practical means of ensuring the free flowing characteristic of a molasses meal has been achieved. Refinements are called for, both in factory technique and by broadening the spectrum of micro-organisms selected for use under factory conditions.

Summary

Molasses meal, a popular stock feed manufactured from by-products of the extraction of sugar from sugarcane, suffers from stickiness. Stored in bulk, samples sometimes become free flowing, a characteristic which, it has been shown, is due to fermentation caused by various micro-organisms. The more important micro-organisms, capable of decomposing the gums which cause the stickiness, have been isolated and used to treat molasses meal. This study involved exhaustive examination of a wide range of micro-organisms. Of these, nineteen bacteria, five actinomycetes and seventeen genera of fungi were selected, either for their ability to reduce stickiness, or to combat organisms responsible for spontaneous combustion. Nine different species of yeast were also found.

Among the actinomycetes the genera *Nocardia* and *Streptomyces* were selected as the most useful for gum decomposition. Only three genera of fungi were selected for detailed studies, and of these the genus *Penicillium* was selected as being of greatest immediate use. Fifteen *Penicillium* species were identified, of which *Penicillium notatum* and *Penicillium chrysogenum* gave the best results. Isolates of *Trichoderma lignorum* and seven different groups of *Aspergilli* were also used successfully in laboratory trials.

On a commercial scale, only *Penicillium notatum* has been employed. This proved to be successful, substantially reducing the stickiness of molasses meal, so that within two weeks of treatment it became free flowing. There remain, however, a wide range of micro-organisms, selected in laboratory trials that have not as yet been used on a commercial scale. These may conceivably prove to be more useful or more effective than *P. notatum*. They

should be exhaustively studied in future factory trials.

Appendix 1

Preparation of Bagasse-Molasses Mixture for Multiplication of Fungi

Molasses meal is diluted with ordinary tap water, the water is drained off and the meal pressed out using a cheese cloth or medium weight calico bag as a filter. Erlenmeyer flasks of 1 litre capacity are then two-thirds filled with the pressed meal. Ten cc of 5% malt-extract solution are then added to each flask, and the flask and its contents sterilised in an autoclave for 20 minutes, at a pressure of 18 lb per square inch. After cooling, the flasks are injected with spores of the fungi taken from pure cultures, and the inoculated flasks kept in an incubator at a temperature of 30 C for at least one week. During this time the fungi grew through the meal and formed a tremendous number of spores.

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