THE MICRO-FLORA OF STILTON CHEESE.

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When well made a Stilton is usually admitted to be the finest of the English varieties of cheese, but like all choice dairy products it is exceedingly difficult to manufacture of uniformly good quality. Great uncertainty exists, even amongst the most experienced makers, in regard to the factors which govern the excellence of flavour and texture in this kind of cheese: the high moisture content favours the rapid growth of harmful as well as useful organisms and "off flavour," objectionable taints, and irregular consistence due to the activity of the former class are common.

Some five years ago the authors began the study of the micro-flora of Stilton cheese, in the hope that a more complete knowledge of the organisms present would assist in the elucidation of the ripening process, and be a step towards placing the manufacture of cheese of fine quality on a sound basis.

In the first instance cheeses made at the British Dairy Institute under the supervision of Mr Miles Benson were investigated; later examples from the best dairies of the Melton Mowbray district were chosen for examination. Our work has been directed towards the determination of the kinds of bacteria and fungi present in normally ripened cheeses: at the same time we have endeavoured to estimate the numbers of organisms in cheeses at different periods of ripening. The investigation of both these problems has proved of great complexity, and much remains to be done.

We have, however, thought it opportune to give an account of the work already accomplished, for in regard to the normal aerobic species of bacteria and fungi which are found in this variety of cheese we consider that the investigation is fairly complete. We have not attempted to deal with the parts which the several kinds of organisms

these problems, as well as the search for anaerobic organisms, must be left for future study.

Attention was first paid to a determination of the number of organisms in the cheese at various periods, from 24 hours old, up to complete ripeness, 80—100 days later.

Cylindrical pieces were withdrawn with a sterilised cheese-borer, driven into the cheese to approximately the same depth each time: one gram of the extracted core was rapidly weighed, and then rubbed up in water in a small sterilised glass mortar.

This was ultimately diluted to 1 in 1,000,000, and 1 c.c. of the dilution was added to a tube of the medium used for plating out. It was found that dilution to 1 in 100,000 was insufficient to allow of precise counting of the colonies, especially where very young cheeses were under examination.

Three media were used, namely lactose-gelatine (100 grams gelatine and 20 grams lactose per litre of medium, acidity 10+), a similarly acid lactose agar (1·5 % agar) and a neutral litmus lactose-gelatine.

After incubation of the gelatine plates at 22 deg. C. and the agar at 35 deg. C. for two days, the colonies were counted.

The distribution of the organisms in the cheese was studied by cutting thin slices of the latter with a razor and staining with eosin-methylene blue or thionine: examinations were also made of cover-glass impressions of the broken curd: both methods revealed an uneven grouping of organisms. Single isolated bacteria, mainly rods, occur fairly evenly distributed throughout the cheese, but the majority are found in irregularly arranged colonies of very variable size. On account of this irregularity in the disposition of the organisms, it is not possible to obtain strictly comparable samples from day to day from the same cheese, without taking a large number of separate borings on each occasion, a proceeding which would render the cheese useless for future investigation.

The results of our work indicate that so far as an estimation of the number is concerned, a gram of cheese taken from a single boring is too small a portion to be regarded as completely representative of the bulk of the core withdrawn. Moreover the numbers obtained differ with different media used for plating out: some grow better on one substance than on another, the numbers being considerably less on agar than on either of the gelatines employed. As indicated later, the presence of one kind of organisms more or less entirely prevents

the appearance of another species on the same plate. Extended trials have shown that a complete and precise estimate of the numbers of organisms in a cheese of the type of Stilton with its very moist and loosely packed curd cannot be attained by small samples obtained from single borings. However, repeated examination during the last four or five seasons of portions of single borings removed from the cheeses at short intervals has enabled us to obtain a general view of the rise and fall in the numbers of organisms and the changes which occur in the micro-flora as ripening proceeds.

In the first 48 hours there is an extraordinary development of organisms from 1000 to 3000 million per gram being frequently found, more than 90 °/₀ of which are cocci or short rods capable of producing lactic acid in milk. The numbers reach a maximum within four days, and decline slowly afterwards up to the time of complete ripeness, when the cheese contains from 50 to 100 millions per gram, mostly moulds and yeasts; concurrent with the diminution of bacteria there is a gradual rise in the development of fungi.

As mentioned later, a considerable number of species of bacteria and fungi are found in Stilton cheese, the greatest variety being found on plates from young specimens before the acidity has reached its maximum, and in the later periods of ripening when the acidity is lowest and open spaces due to shrinkage have begun to form: occasional moulds are met with throughout the whole period of ripening, but they make their appearance in increasing numbers when the cheese is 30—40 days old.

The great variety of organisms we have isolated may be classified into two groups, namely:—

(1) Bacteria and fungi, which from their constant occurrence in Stilton cheeses whenever and wherever they are made, may be regarded as normal constituents of the micro-flora, and concerned more or less directly with the ripening process of a good cheese.

(2) A miscellaneous group whose presence is accidental and whose influence is either harmless or detrimental to the quality.

Normal Bacteria. Three forms of bacteria are invariably present, namely, a coccus, a rod-shaped organism belonging to the lactic acid group, and a species of *Tyrothrix.*

1. *Coccus A* is a small roundish-oval organism, 0·4—0·5 μ in diameter (Fig. 1). It usually occurs in pairs, but occasionally may be found in short chains of three and four together. It grows slowly on solid media in the form of minute white colonies beneath the surface.

In milk it produces a dense uniform curd without gas bubbles or separation of whey. It curdles milk slowly, taking three days at 22 deg. C. to produce a solid curd.

We consider this typical *Streptococcus lacticus*. It is abundant in all cheeses we have examined.

A larger form is found in some cheeses with oval cells somewhat elongated—1·1 μ to 1·3 μ long. Just before or at the time of division, the organism resembles a short rod. In milk and in cheese 24 to 48 hours old it is found in well-developed chains, consisting of 6 to 12 or more cells. In cheeses a few days old, however, and on gelatine and agar media, the chain form is lost, the organism then appearing chiefly in pairs (Figs. 2 and 3). The colonies are white and very small —$\frac{1}{2}$ mm. or less in diameter: they grow beneath the surface of the medium. Milk is curdled by this bacterium in 24 hours or less when kept at 22 deg. C., the curd produced being dense and uniform, without gas bubbles or separation of whey.

The organism is a form of *Streptococcus lacticus*, and appears to be identical with the coccus present in most of the commercial starters so commonly used by butter makers and some makers of cheese: Stiltons containing it are of fair mild flavour, but too dry and acid. Possibly vigorous acid formers like this species may be useful in repressing the objectionable organisms found in dirty dairies, or where cheese is manufactured from doubtfully clean milk; we feel convinced, however, that they should find no place in a Stilton dairy where cheeses of the finest flavour and texture are desired.

2. *Bacterium A*. Another common characteristic bacterium in Stilton cheese is a short stumpy rod, 2—4 μ long and 0·7—0·8 μ thick (Fig. 4): sometimes it occurs in the form of slightly oval cells, and then resembles the large *Streptococcus* referred to above.

The colonies on the surface of the media are circular, from 1—2 mm. in diameter, white, moist and raised a little above the surface; in the substratum they are smaller and either round or spindle-shaped; old colonies become yellowish.

The organism is Gram positive and non-motile.

It curdles milk slowly, taking 6—8 days at 22 deg. C. to produce a dense curd, which very closely resembles that thrown down by *Streptococcus lacticus*; no gas bubbles appear, and there is little or no separation of whey.

When grown in broth it lengthens to 4 or 4·5 μ, but preserves its somewhat oval shape: it appears to be a variety of *Bs. acidi lactici*

of Hueppe, but unlike the latter it does not ferment glucose nor lactose: some forms of it give a slight Voges and Proskauer reaction, but none produce indole.

In fully ripe cheeses lactic acid organisms are comparatively few in number; many die off altogether in the ripening process, and those which remain possess diminished vitality and are only able to acidify milk very slowly.

3. A species of *Tyrothrix* is invariably present in Stilton cheeses. It is found in all stages of ripening after the third or fourth day, but is never abundant.

It is the chief and usually the only organism which appears on plates inoculated with a "dilution" of the cheese heated to boiling point. The organism is rod-shaped, and feebly motile in young cultures. The individual cells from agar colonies are 6—12 μ long, 1—1·25 μ broad. In milk they are longer and thinner and often united in the form of tangled threads, some of which may reach a length of 150 μ (Fig. 5).

It forms oval spores, which measure 2 $\mu \times 1$ μ.

Gelatine is liquefied rapidly by it, and milk is rendered alkaline and coagulated, the curd being soon digested: no gas is produced in glucose, saccharose, or lactose bouillon.

The surface colonies on agar are white, round, smooth and moist at first; later they spread over large areas and become wrinkled and dry, the margins of such colonies being fimbriated. Beneath the surface of the medium the colonies remain small and are more or less granular with characteristic mycelioid or floccose margins.

The organism is closely allied to, if not identical with, Duclaux's *Tyrothrix tenuis*.

4. *Miscellaneous Bacteria.* In addition to the four species of bacteria already mentioned, which we consider from their physiological activities and constant presence are directly concerned with the ripening of Stilton cheese, there are many others of accidental occurrence. In the latter class we include *Bacterium coli*, *B. lactis aerogenes*, and many chromogenic species of bacilli and cocci giving rise to violet, pink, yellow and orange colours.

The number of kinds are found to vary with the season when the cheese is made and the source from which the milk is derived. Of this miscellaneous group, *B. lactis aerogenes* is the most commonly found: typical *B. coli* is rare.

Fungi. In the course of our investigations we have observed a

considerable number of species of fungi, those of most frequent occurrence being *Oospora lactis*, *Mucor mucedo*, *Aspergillus glaucus*, *Cladosporium herbarum*, *Penicillium glaucum*, and several forms of *Torula*. The four first mentioned appear only on the coat of the cheese, rarely or never in the interior. They may be regarded as accidental or unavoidable in the ordinary course of manufacture of Stilton cheese, and apparently play little or no part in the ripening process.

Oospora is very abundant on the outside during the first 15 to 20 days, when the coat of the cheese is moist.

Mucor, *Aspergillus*, and *Cladosporium* are more casual in their occurrence, and may appear upon cheese of all ages in small irregular numbers.

The fungi which are undoubtedly of great importance in the ripening process of Stilton cheeses are *Penicillium glaucum* and one or two forms of *Torula*; these are found throughout the interior of the cheese.

When cut across a well-made ripe Stilton exhibits a characteristic mottling of blue veins most abundant in the softer centre, and radiating in an irregular manner towards the firmer outside. These veins are crevices more or less filled with the mycelium and conidiophores of *Penicillium glaucum*. We have observed conidia in small numbers in the fresh curd; they are, however, very sparsely distributed and difficult to find, and the fungus rarely occurs on plates inoculated from cheeses less than three weeks old. Either from want of air or from the presence of inhibiting substances the spores appear to be prevented from germinating in the closely packed curd in which they are imbedded. In the shrinking cheese cavities arise at the points where the separate pieces of curd first packed in the mould touch each other: it is in these cavities that the mycelium of the fungus makes its appearance and spreads over the surface of the crevices at a rapid rate without penetrating into the substance of the cheese more than a very small fraction of an inch.

The mycelium is colourless, and it is not till the formation of the conidiophores and the conidia that the blue-green tint of the "veins" in the cheese is developed.

In open spaces with plenty of room for growth the conidiophores are of the ordinary type, the hyphae being about 3 μ in diameter, with sterigmata 5·5—8 μ long, bearing round smooth conidia 2·8—4 μ in diameter. When seen in mass the conidia are of a bluish-green tint:

grown on agar or gelatine media the blue-green tint of the colonies ultimately changes to a greyish brown or mouse colour.

We found that in many of the crevices, especially those of small dimensions, the hyphae of the mycelium become much thickened, their diameter reaching 9—12 μ. The apex of such thickened hyphae may give rise to a much thinner hypha which develops into a conidiophore (Fig. 7 *b*). Sometimes sterigmata and conidia arise on a very short hypha placed laterally upon the thick mycelial hypha as in Fig. 7 *a*. Not infrequently we have observed the development of a single sterigma with its conidia within the individual cells formed by partitions across the thickened hypha: the protoplasm of the cell shrinks, leaving an enclosed space, into which the fructification develops later (Fig. 7, *c* and *d*), such remarkable phenomena not only occurring at the apex of a hypha but sometimes in cells along the hyphae at random. Ascocarps have not been found.

We find that *Penicillium glaucum* does not grow on agar lactose media when the latter is inoculated at the same time with the Stilton *Tyrothrix*, although it grows luxuriantly enough in the presence of lactic acid organisms. The conidia germinate but the hyphae produced remain very short where *Tyrothrix* is growing freely; later, after the formation of spores by the *Tyrothrix*, the fungus develops rapidly.

We hope to further study the influence of these organisms upon each other.

Yeasts. One of the most frequent constituents of the micro-flora of Stilton cheese is a species of *Torula*. It occurs in cheeses of all ages, being abundant in those 24 hours old, as well as in those which are completely ripe.

The fungus is not only found on the coat, but is distributed throughout the interior of the cheese.

Plates inoculated with dilutions prepared from ripe cheeses exhibit colonies of the *Torula* in abundance, along with those of *Penicillium* and a few bacteria.

The cells of the Stilton *Torula* are round, 3·5—5 μ in diameter (Fig. 6).

Growth takes place more freely upon acid media than upon alkaline substrata. The colonies are round, white, and opaque, with smooth shining surfaces.

Less frequently met with is another form of yeast-like fungus, with oval cells 5·5—6 μ long and 3·5—4 μ broad. The colonies are white and opaque, with dull matt surfaces, which ultimately become wrinkled.

Fig. 1.

Fig. 5.

Fig. 2.

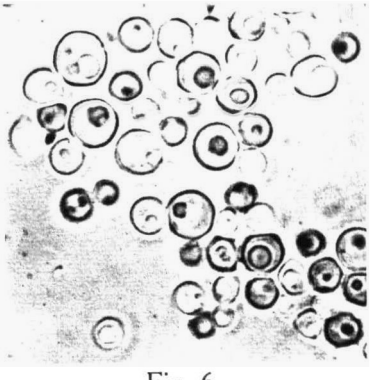

Fig. 6.

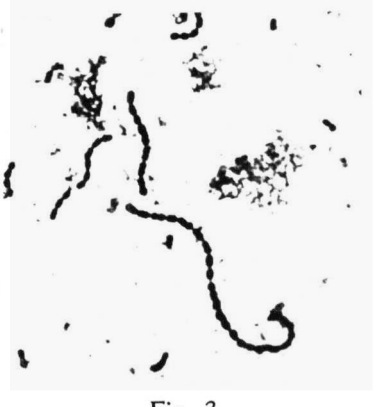

Fig. 3.

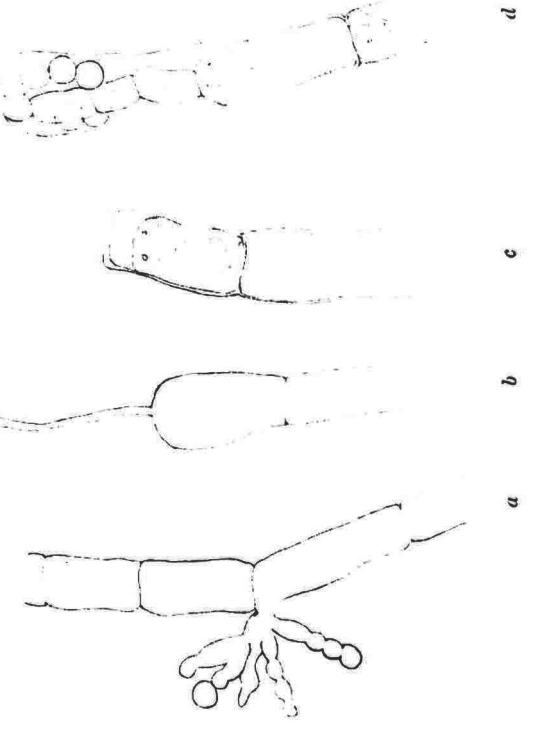

Fig. 7.

Fig. 4.

We have not been able to induce the formation of endospores in either of these "yeasts."

Summary.

1. The numbers of bacteria and fungi in a newly-made Stilton may rise to the enormous number of 1000 to 3000 millions per gram in the first week.

2. There is a gradual fall in the numbers up to the time of ripeness (100 to 150 days old), when 50 to 100 millions only are found.

3. In the early stages lactic acid bacteria are most abundant. When the cheese is ripe the lactic acid bacteria are few and weakened in physiological power; *Penicillium glaucum* and a form of *Torula* are then abundant.

4. Five characteristic organisms are found in all Stilton cheeses examined, viz.:—(1) *Streptococcus lacticus*, (2) a short rod-shaped form of *Bs. acidi lactici*, (3) a species of *Tyrothrix*, (4) *Penicillium glaucum*, and (5) a round form of *Torula*, sometimes accompanied or replaced by an oval form.

In cheeses where starters have been used we find a large celled form of *Streptococcus lacticus* present.

5. *Penicillium glaucum* is checked in its growth by the *Tyrothrix*.

We wish to acknowledge our indebtedness in this investigation to a grant made from the Development Fund by the Board of Agriculture and Fisheries.

EXPLANATION OF FIGURES IN PLATE VI.

The photomicrographs were taken with a 2 mm. Zeiss apochromatic objective and No. 8 eye-piece by Mr F. O. Mosley, University College, Reading.

Fig. 1. Small common form of *Streptococcus lacticus* from agar, × 900.
,, 2. Large form of *Streptococcus lacticus* from agar, × 900.
,, 3. ,, ,, ,, ,, milk, × 900.
,, 4. Short-rod lactic organism from agar (*Bs. acidi lactici* Hueppe), × 900.
,, 5. Stilton cheese *Tyrothrix* from agar, × 900.
,, 6. *Torula* from Stilton cheese from agar culture, × 900.
,, 7. Abnormal forms of hyphæ and conidiophores of *Penicillium glaucum* from interior of Stilton cheese.