MICRO-ORGANISMS FROM THE BRISBANE AIR.

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During the past eighteen months I have made a series of observations on the bacterial flora of the air of Brisbane, and I propose to give a short account of the results so far obtained, reserving fuller details for a subsequent occasion, when my observations shall have been more complete.

The method employed was simple. It was not my object to estimate the number of micro-organisms present in the air, but to obtain pure cultures of the different species, and to examine their morphological and cultural differences. The ordinary nutrient agar jelly (2 per cent. agar, 1 per cent. peptone, 0.5 per cent. common salt in beef broth) was poured into a sterilised Petri dish and allowed to cool. The plates so obtained were usually kept covered for a few days in order to allow the superfluous moisture to evaporate, and were then exposed by removing the cover for from two to five minutes. Some of these exposures were made in the laboratory, but the greater number in a small paddock outside, the dish resting on the top of a six-foot post. These agar-plates I have found preferable to gelatine plates for this purpose, inasmuch as they can be kept longer; whereas the latter rapidly liquefy, so that slowly-growing organisms may be lost. Further, on the old plates the colours of the pigment-producing organisms become far better marked than on recent plates.

The desiccated condition of the organisms deposited from the air is unfavourable to rapid development, and colonies are not visible to the naked eye before the second day. A few barely visible specks then can be usually detected by careful

observation. Watching these from day to day they will be seen to increase in size ; new specks will appear in other parts of the plate ; and differences between the colonies soon manifest themselves. Every day increases their diversity, and at the end of a week or longer the plate displays the variegated condition of those now exhibited. Every germ, where it has fallen, has given rise to its own characteristic growth, and we get as a result a small garden of various lowly organised vegetable forms, as diversified as the weeds which spring up in a fallow field.

The larger growths on these plates are readily recognised as those of the filamentous fungi commonly known as moulds. These occur in considerable variety, but as they are not the object of the present research, no attempt was made to study them minutely, although they are certainly well worthy of investigation.

We will now turn our attention to smaller growths on our plate which, even by the naked eye, are readily distinguishable from those of the mould fungi. These are circular, sometimes irregularly lobed or crenated at the circumference, more or less raised from the surface, and very variable in colour. They are colonies, each consisting of an immense number of a particular species of bacterium developed from parent organisms which have fallen on to our plate from the air. Some of these growths are whitish, some yellow, some more or less vivid orange, some pink. We can readily infer that they are of various species. But for the investigation of these special care is necessary, for we are here dealing with the most minute forms of life known to exist, and it is quite impossible to differentiate the species by morphological characters only. So simple are they in structure that it will need the most thorough examination by various methods of cultivation to decide whether any two colonies we may pick out belong to the same or different species. Our first object must be to obtain a pure culture of each sort. For this it might seem sufficient to at once inoculate our various culture media from the different growths. But, though this is sometimes successful, I have found it a very uncertain and untrustworthy method. It not unfrequently happens that two or more parent bacteria of different species

have fallen close to each other on the plate, and that what appears to be a single colony is really a mixed growth. In the case of most of the organisms first cultivated from apparently single colonies, I have had to avail myself sooner or later of the method of isolation by gelatine plates; and I now always adopt this in the first instance. Though tedious, no trouble is superfluous that assures the observer of the purity of his cultures. The original colony is touched with a sterile platinum wire, which is rinsed in a tube containing sterile bouillon. A very minute platinum loopful of the bouillon is then transferred to a tube of melted gelatine, which, after gentle agitation, is poured out into a sterilised Petri dish, where the separate colonies develop within two or three days and are utilised as the starting-point of pure cultures.

I have here a number of pure cultures secured from the Brisbane air of sixteen distinct species or varieties. It must not be supposed that these are anywhere near the total number of distinct forms that may be obtained in this locality by the method I have described. I have preferred to concentrate my attention in the first instance on a few forms, and more particularly on those presenting some distinguishing feature, such as the development of pigment.

The first culture to which I draw attention is not a bacterium at all, though indistinguishable in its method of growth. It belongs to the group of torulæ or yeasts, and consists of comparatively large oval, often vacuolated, cells which multiply by budding. The growths produced by it are of a beautiful pink colour, and do not liquefy gelatine. It resembles, and is probably identical with, a species of yeast organism commonly found in air cultivations in Europe. It has developed rather frequently on my plates, where its conspicuous colour readily attracts observation.

The bacteria proper are represented in the first place by eight different forms of cocci. These smallest forms of life are exclusively found in the form of excessively minute spheres. In the process of growth they divide into pairs, which, clinging together, form what are known as diplococci. Three or five cocci are not infrequently observed clinging together in chains. Longer chains were not observed in the species shown. Some-

times the diplococci divide transversely, forming tetrads. This, with a slight variability in the size of the individual cocci, exhausts the morphology of these simple organisms. After careful and repeated examinations, I was unable to find any ground of distinction from microscopical appearance in the eight varieties. None of them exhibited any independent motility, though Brownian movements were, of course, very marked. The distinctions between them depend entirely on their mode of growth on various media. In describing them it will be convenient to denote the varieties by letters of the Greek alphabet, as it would not be justifiable to give them distinct names until they have been compared with cocci previously described. In two, Coccus γ and Coccus μ, the growths are pink; in Coccus ζ, vivid orange; in Coccus η and Coccus λ, bright yellow; in Coccus θ and Coccus ν, whitish; in Coccus ξ, brownish. Another point of distinction is the presence or absence of the power of liquefying gelatine. Two, coccus ζ and coccus λ, never liquefy the gelatine, however old the growth may be. On the other hand, coccus θ liquefies the gelatine rapidly; coccus η, coccus ν, and coccus ξ slowly; coccus γ and coccus μ, after growing for a long period without any sign of liquefaction, slowly give rise to this after the lapse of several weeks. The slow occurrence of liquefaction is best determined by streak cultures on the surface of gelatine which has set in an obliquely inclined tube. The first sign of liquefaction is the formation of a groove in the surface of the gelatine occupied by the growth; at a later period the growth slides down to the bottom of the tube. By noting the date on which the groove is first distinct, the comparative rapidity of liquefaction can be estimated. For instance, in one experiment, during which the temperature ranged between 16 deg. C. and 22 deg. C., coccus θ and coccus ν formed a groove on the second day, coccus η on the fifth day, coccus ξ on the nineteenth day, coccus γ on the twenty-fourth day, coccus μ on the forty-eighth day.

Growths of these cocci on potato show no peculiarity except in their coloration and rapidity of growth. Growths in liquid broth show no peculiarity of any kind—merely a turbidity with a fine deposit formed by the organisms. Coccus θ is an exception. Broth cultures and liquefied gelatine cultures of

this organism contain a ropy substance like mucus, evidently a product due to some chemical fermentation. Agar cultures too of this coccus, form a very coherent film, which sticks glutinously together when distnrbed by the platinum needle.

The bacilli, or rod-shaped bacteria, show much greater morphological variety than the cocci we have been considering, and it will be convenient to describe the seven forms I have isolated from the air separately. Bacillus α, when examined under the microscope in a hanging drop of fluid, presents a very lively appearance, for each rodlet or sphere is actively motile, swimming about the field of view with great agility. It is a stout bacillus, usually short, but varying greatly in length, for very long motile rods are sometimes seen. On the other hand, the great majority may be so short as to be round and indistinguishable from cocci and diplococci. But some oval forms are always distinguishable. These roundish forms appear to be commoner in agar cultures, the longest rods in gelatine cultures. At first I suspected that I might be dealing with a mixture of organisms, but by making gelatine plates and examining individual colonies this was negatived. The growth on agar is rapid and of a pale orange or yellowish colour. Gelatine stab cultures show rapid liquefaction, the gelatine being in time completely liquefied, the growth sinking to the bottom of the tube. Gelatine plate cultures show rapidly spreading liquefaction of the individual colonies. I have not observed any spore formation in this bacillus.

In its morphological characters, bacillus β is extremely distinct from the preceding. It is an excessively minute, very slender bacillus, varying in length from roundish and oval forms to short rods. I have never observed it form rods of any considerable length. It shows no independent movements. On agar it forms a deep, somewhat greenish, yellow growth, provided the temperature at which it is grown is not too high. A streak culture on gelatine forms a narrow yellow streak which shows no sign of liquefaction for the first two weeks, but slowly liquefies at a later date.

Bacillus δ resembles the preceding in forming a yellow growth on agar and potato. But the colour, though well marked, is not so deep and has no greenish tinge. Cultures on

the surface of gelatine show a wider streak than the preceding, and stab cultures spread more widely at the surface. Liquefaction occurs tardily after the first week. It is a moderately stout bacillus, occurring in roundish, oval, and short rod-like forms, but does not appear to form long rods and is non-motile.

Bacillus κ is a moderate-sized, fairly stout bacillus, very similar to the preceding in morphological characters, but somewhat larger. It has no power of movement. Growths on agar develop rapidly, are whitish, with a faint yellowish tinge, which is better developed on potato cultures. Grown in gelatine it causes rapid liquefaction.

Bacillus π is a large, stout bacillus with rounded ends, very variable in length, but never forms long filaments. In young cultures many individuals show well marked, but sluggish, independent movements. Like all the preceding it does not appear to form spores. Its growth on agar is white, the centre of the various colonies being opaque, the periphery thin and transparent. It has a very feeble power of liquefying gelatine in old cultures. A stab culture develops slightly along the track of the needle, and spreads for a moderate distance at the point of entry. Occasionally feathery whorls spread out from the upper part of the stab into the substance of the jelly.

Bacillus σ is a very interesting organism. Its growths on agar spread at the edge in looped skeins of filaments, closely resembling the well-known growths of *Bac. subtilis*. If an old colony is examined, it will be found to consist at the edge of these filaments, which readily break up into rods, but the central portions consist almost entirely of a mass of spores. Its growth on gelatine at once differentiates it from *Bac. subtilis*. There is a slight growth along the track of the needle. From the point of entrance, a fine feathery growth spreads all over the surface, and the gelatine never liquefies. On a gelatine plate the colonies develop much more slowly than those of *Bac. subtilis*, the early stage of which they resemble, but differ in the entire absence of the circular liquefaction and rapid growth characteristic of the latter. On potato, bacillus σ develops slowly and scantily. The best method of observing its growth and spore formation is in a hanging drop of bouillon. When

such a drop, on a hollow slide carefully sealed with vaseline, has been in the incubator for twelve hours, it is observed to be full of actively motile rods, similar to those of the hay bacillus, but more slender. Filaments are not developed. After twenty-four hours most of the rods are unaltered, but a considerable number have lost their motility and show commencement of spore formation. The first change observable is a fusiform thickening near one extremity of the rod. This is at first devoid of structure, but soon is observed to contain a highly refractile particle, which gradually increases in size to form a mature spore. The spore-bearing rods have somewhat of a drumstick form, but the enlargement is seldom quite at the extremity of the rod, and has not the circular form displayed by the spore-bearing rods of *Bac. tetani*. The spores and rods can be doubly stained by suitable methods. For the formation of spores, a plentiful supply of oxygen appears to be necessary, When grown in broth this bacillus does not form any scum, but a deposit is formed at the bottom, which, when disturbed by the platinum needle, appears to be of a ropy consistence like mucus. A hanging drop of this ropy deposit shows immotile rods, some of which show fusiform enlargements, but none contain spores. If the hanging-drop preparation be kept a day or two in the incubator, the ordinary motile and spore-bearing rods are developed. The spores, are, however, only formed in scanty numbers, and most of the rods grow into long filaments, which are not observed in primary hanging-drop cultures.

Bacillus ϵ is a large, stout, non-motile bacillus occurring in straight rods with rounded ends, and in long, curved, jointed filaments. Grown in a hanging-drop of bouillon in the incubator, it forms within twelve hours a massive growth of tangled filaments; after twenty-four hours these break up into rods, many of which contain spores. On agar, and on potato, it forms a copious thick white growth. In an early stage this is seen under a low power to consist of convoluted bands or skeins like those of *Bac. subtilis*, but not throwing out isolated loops at the periphery. The growths, unlike those of the preceding, rapidly increase in thickness, so as to disguise their structure. Stab cultures in gelatine undergo rapid liquefaction, and small superficial floating flakes form at the periphery of the liquefied portion.

The question now arises, How far do these distinct strains of organisms represent distinct species ?

Their individual differences are for the most part constant ; that is to say, they breed true. I have cultivated most of the forms through a succession of generations during twelve months, and find them still true in minute detail to my original descriptions. But it is important to note that one of their characteristics, development of pigment, is much affected by temperature. To test this point I have made simultaneous agar cultures of all the coloured forms, growing one tube of each in the incubator at 36 deg. to 38 deg. C., and another at the temperature of the room, which at the time of the experiment varied from 16 deg. to 22 deg. C. In six cases the result was identical. The higher temperature stimulated the early growth, which was more rapid, sometimes much more rapid in the incubated tube during the first forty-eight hours, but the growth remained whitish and never developed the characteristic coloration. This was true of coccus γ, ζ, μ, and ξ, and bacillus α and β. During the hot summer months these bacteria lost their power of producing pigment, to regain it again the next winter ; all except coccus ζ, in which the vivid orange growth was never regained. It now develops only a pale orange. On the other hand, in four cases the coloration was equally well, or even slightly better developed in the incubated culture. This held good of coccus η and λ, and bacillus δ and κ. All these were yellow growths. On the other hand, bacillus β, which has a yellow growth, was decolorised in the incubator, as were all those which had a pink or orange growth.

We see, therefore, that one of the most prominent distinctions between our varieties varies with the temperature. This does not impair its value as a real distinction ; but the fact that in one form—coccus ζ—the power of colour formation has diminished, irrespective of temperature, certainly is of significance. But when we take a general view of the differences between the various forms, we find that some are as distinct as—for example—roses from brambles ; while others present but minor differences, with a close general resemblance, like the various kinds of roses and brambles respectively, as to whose specific value no two botanists are in agreement. For instance, the distinction

between the various bacilli is wide and patent. But among the cocci, coccus η differs from coccus λ only in the one point that it has a feeble power of liquefying gelatine, while the latter has no such power Again, coccus γ is very close to coccus μ, and it needs close observation to distinguish that the former has a more luxuriant growth on agar, that its growth is at first whitish but very gradually attains a pink colour ; while the growth of the latter is distinctly pink from the first. These differences, so far as I have observed, are constant, but they are slight, and of doubtful specific value. Again, among the bacilli, I have lately isolated a culture which appears to exactly resemble bacillus α, except that it possesses a less active power of liquefying gelatine. A larger experience will probably multiply the number of these closely similar varieties ; and their occurrence raises a doubt as to whether they may not in some cases be merely different forms of the same species, and may not actually be transformed by different conditions of growth from one form to the other.

In one instance I have actually succeeded in breeding two sub-varieties from a single species. In an agar culture of bacillus α, I observed two different kinds of colonies—one a very pale ochreous yellow, the other of an orange tint. The parent agar culture had been kept through several hot months, and was descended from a gelatine-plate colony. My first impression was that the originally pure culture had become contaminated. Microscopical examination of hanging-drop preparations showed that both kinds of colony consisted of morphologically indistinguishable actively motile bacilli of the form I have described as characteristic of bacillus α. From each growth I made a gelatine plate, and from this again gelatine and agar cultures. The gelatine growths of the two forms were absolutely indistinguishable and quite characteristic ; the agar growths presented the same differences of coloration that I have mentioned. The difference is not a very great one, but so far it breeds true, and the evidence seems conclusive that the two varieties have a common origin.