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COLONIZATION OF THE KRAKATAUS BY BACTERIA AND THE DEVELOPMENT OF ANTIBIOTIC RESISTANCE

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CONTENTS

Our work on the bacteria of the Krakataus reported in the preceding four papers in this series involves two habitats: the soil, and the guts of vertebrates. These investigations were made in the pursuit of two main interests: bacterial colonization ability and resistance to antibiotics.

1. Relative concentrations and diversity of microbiota as an indication of colonization ability

The first aim was to try to discover how the concentrations and diversity of microorganisms in the soils of the Krakataus, about a century after the explosive eruption of 1883, compare with those in the soils of a relatively unaffected wilderness area on Java some 50 km away. The interest here rests on the premise that the sterilization in August 1883 of the islands Sertung, Panjang and Rakata included microorganisms as well as macrobiota. If this is accepted, then such a comparison will give an indication of the ability of bacteria to colonize the Krakataus from Java and Sumatra over some 44–50 km of sea in the course of a century.

The assumption of sterility has not gone unchallenged. In the 1930s the botanist Bakker (1929), with some support, argued vociferously for incomplete sterilization as far as plants were concerned, let alone microorganisms. Defenders of the sterilization theory, however, led by Ernst (1934) and Docters van Leeuwen (1936) and supported by other botanists analysing later phases of the plant succession, have convinced most students of the Krakataus that the macrobiota was extirpated in 1883. The reader is referred to Thornton (1984) and to the introductory paper of this series (Thornton & Rosengren 1988) and references to this topic therein for summaries of the arguments presented by both sides. Although eradication of the macrobiota seems to have been generally accepted, the discovery of incompletely carbonized logs up to 30 cm in diameter beneath 4 m of apparently undisturbed pumice deposits on Rakata in the 1980s (Thornton & Rosengren 1988) creates at least some doubt. In the case of microorganisms the doubt must be greater, for they have not been surveyed anything like as extensively or frequently as the macrobiota, and in 1919, when the forest was beginning to expand and the first survey of soil microbiota was made, the results indicated that bacterial concentrations were similar to those of the soil at Bogor in west Java. This was confirmed by the analysis of another sample in 1928 (Graves *et al.* 1988*a*). Whether the similar concentrations were the result of over-sea colonization or the recovery of remnant populations on the Krakataus is an open question because of the uncertainty concerning microbiotic sterilization in 1883. Frederickson & Hicks (1987) recently reported a diversity of bacteria in cores taken in subsurface aquifer systems at depths (300 m) well below the known limit for microbial communities. Extirpation in 1883 is accepted here only as a working hypothesis.

The emergence of Anak Krakatau in 1930 and the development of the mobile, ever youthful spit on the north of Sertung provided opportunities for comparisons between the microbiota of young and older soils within the archipelago, comparisons that could have implications for the question of microbiotic sterilization of the soils of the archipelago in 1883. Unfortunately, these opportunities were not taken until the 1980s. There is, of course, no question about the initial sterility of Anak Krakatau as far as the terrestrial microbiota is concerned, for it emerged from the sea after building up from Krakatau's caldera some 170 m below the surface. The Sertung spit was also built up of deposits transported to the surface from the sea bottom (Rosengren 1985).

Comparisons of relative diversity and concentrations of microbiota, both between the archipelago and Java (Graves *et al.* 1988*a*), and between soils of different ages within the archipelago (Graves *et al.* 1988*b*), must be interpreted with caution. This is because the composition of the microbiota and concentrations of bacteria were determined by a method (the only one available to us: plate counts following culture on horse blood agar and MacConkey agar) that can show no more than relative trends, and only for those microorganisms capable of growing on these media rather than for the microbiotic communities as a whole. Moreover, the large differences in concentrations between samples at some sites are disturbing, although average concentrations within sites of about the same age are reasonably consistent.

Results from the sampling of sites on Java and the archipelago (Rakata) in 1984 suggest that Gram-negative rods (GNR) have colonized Rakata, and this suggestion is particularly evident in the comparisons of microflora between sites at higher altitudes in the two areas.

Differences in concentration between forested sites on Rakata and the Ujung Kulon peninsula of Java were not significant. However, comparison of samples taken in 1985 from young and old soils within the archipelago show concentrations lower in the young soils, and, moreover, that only in young soils where vegetation has become established do concentrations approach those of older soils. The results of the studies of relative concentrations thus tend to support assumptions of sterilization in 1883.

The results of the analyses of enteric bacteria of vertebrates on the Krakatau Islands (Graves *et al.* 1988*c*, *d*) suggest differences in the microbiotic flora between mammals and reptiles, for example in the frequency of *Escherichia coli* (higher in mammals) and *Citrobacter* (higher in reptiles), as well as between taxa within these groups, for example, between the two species of rats, between the genera of bats and between the geckos and other reptiles. It is suggested that such differences may be related to differences in mode of life. The geckos, for example, are arboreal insectivores and spend less time on the ground than the skink, snake and monitor (Graves *et al.* 1988*d*), and bats of the genera *Cynopterus* and *Myotis* have different foraging modes (Graves *et al.* 1988*c*), the former being an arboreal frugivore, the latter an aerial insectivore. The *Rattus* species are arboreal or terrestrial scavengers but also eat fruit, both fallen and on the trees. Thus, although rats may ingest faecal bacteria directly and indirectly, the bats would take them in indirectly, *Myotis* (which may eat contaminated insects) being more likely than *Cynopterus* to do so. Thus rats would be more likely to harbour *Streptococcus faecalis* (one of the commonest enteric bacteria of humans) than *Myotis* species, and *Myotis* species more likely than *Cynopterus* species.

S. faecalis was detected in 4 of the 12 *Rattus rattus* individuals and in two of the three individuals of the insectivorous bat *Myotis muricola* from Carita (a village on the coast of west Java) but not detected in any of the three individuals of *M. muricola* and four *R. rattus* sampled on the archipelago. Thus two mammals with different foraging modes show the same phenomenon: an absence of *S. faecalis* in samples from the archipelago, and its presence in samples from the mainland. Although this bacterium was also absent from 50 other individual vertebrates (16 *Rattus tiomanicus*, 18 *Cynopterus* and 16 reptiles) sampled on the archipelago, more sampling of *R. rattus* and *M. muricola* is needed to confirm its suggested absence from the archipelago. Graves *et al.* (1988*c*) suggest that inadequate sewerage facilities at Carita have permitted the transfer of this human enteric bacterium to the mammals of the area.

2. The incidence and extent of antibiotic resistance

The second interest of our studies, a comparison of the range of antibiotic resistance between GNR from the above areas, derives from the contrast between the exposure of the soils of the Ujung Kulon peninsula and of the Krakataus to human activity. As with the study of relative concentrations, an assumption is involved that cannot be supported by hard data. It is that on the archipelago such exposure was negligible because of the much less frequent and intense human activity there. The extent of human influence on the islands is summarized by Thornton & Rosengren (1988) and must at the least be considered small in comparison with the regular human activity on the Ujung Kulon peninsula before 1883 and the more frequent and numerous human presence there since then (two permanent posts for park rangers, and regular patrols) than on the archipelago.

The results show, however, that in general the range of antibiotic resistance (as determined by the number of resistotypes present) of soil bacteria on Rakata is of the same order as that of soil bacteria at Ujung Kulon, and do not support the hypothesis that antibiotic resistance is related to human activity. Nevertheless, fairly consistent differences were observed in this respect between GNR from the younger and older soils on the Krakataus, soils on Anak Krakatau and the Sertung spit showing less resistance than those both from the older, main part of Sertung island that is now covered in mixed forest and from Rakata, which is also forested. Were human activity a significant factor, the opposite of these results would have been expected; Anak Krakatau and the Sertung spit are among the archipelago sites most exposed to humans. Moreover, comparisons between individual sites on the young soils show a fairly good correlation between range of resistance and the presence of vegetation.

All the indications from these limited soil studies point to antibiotic resistance (and relative microbiotic concentrations, see the previous section) being related to the presence of vegetation rather than exposure to human activity. For example, GNR from soils on Zwarte Hoek, a well-vegetated but frequently visited anchorage on Rakata, show no greater range of antibiotic resistance than do those from Rakata's summit area, which is also well vegetated but visited very rarely. Comparisons between young soils on the archipelago also suggest that range of resistance is related to the presence of vegetation rather than to the frequency of visitors in the area sampled.

In contrast to the studies on soil bacteria, there are two indications from the study of mammalian (but not reptilian) enteric bacteria of the effect of exposure to human populations. The first, differences in the incidence of *Streptococcus faecalis* in the guts of wild mammals between the Krakataus and Carita has been mentioned above.

The second indication arises from the examination of antibiotic resistance of the enteric GNR of vertebrates to tetracycline, an antibiotic in widespread use without prescription in Java. Resistance of *E. coli* (in 2 of 20 strains isolated) and *Klebsiella* (3 out of 11) was restricted to samples from (4 of 12) *R. rattus* from Carita. In none of the 21 strains of *E. coli* and 25 of *Klebsiella* isolated from rats on the Krakataus did these bacteria show tetracycline resistance. Notwithstanding the expected differences in the frequency of contact with antibiotics and faecal bacteria between mammals because of their different modes of feeding, antibiotic resistance has been demonstrated on Java but not on the Krakataus: three different groups of mammals, with different foraging habits, show the same phenomenon. Some strains of *Enterobacter* isolated from Krakatau rats were resistant, although this may well be an inherent feature of this genus rather than an acquired characteristic as appears to be the case with *E. coli* and *Klebsiella*.

Thus considering the soil bacteria our results do not support the hypothesis that the development of antibiotic resistance is related to human activity, whereas for the enteric bacteria of vertebrates there are some indications that this is so. It is quite possible that these apparently conflicting results reflect a real situation, and that the development of antibiotic resistance in soil bacteria has little to do with human activity, being rather a by-product of plant growth and competition, whereas the development of resistance in the enteric bacteria of vertebrates may be more closely dependent on indiscriminate antibiotic usage by humans combined with inadequate sanitation. Alternatively, there may be a time-lag between the development of antibiotic resistance by soil bacteria and the ingestion and establishment of the resistant bacteria in the guts of mammals. In the case of tetracycline-resistant *E. coli* and *Klebsiella* on the Krakataus, we may be observing a stage before the latter process has been completed.

3. Future work

The indications from these preliminary studies allow the erection of several hypotheses.

1. Both the development of a microbiota and the concentration of bacteria in the soil of a primary xerosere are to a large extent dependent on the establishment of plants on the substrate.

This could be tested on Anak Krakatau by sampling a barren area that botanists predict will soon become colonized by plants, and resampling the same area a decade or more later when colonization by plants has occurred, using more extensive methods than those available to us. Above and below-ground components of well-isolated individual plants on Anak Krakatau could also be sampled, in addition to the adjacent soil, and the results compared with samples of soil of the same age in adjacent barren sites.

2. The development of antibiotic resistance in soil bacteria is also largely associated with the development of a plant cover, and is a natural concommitant of the successional process rather than being the result of exposure to antibiotics as a result of human activity.

Comparisons between sites on the islands that are fairly exposed to human traffic but differ markedly in plant cover would be one possible test of this. Samples from a vegetated site adjacent to the track at the western (inland) edge of the vegetated area on Anak Krakatau's eastern foreland could be compared with samples from barren ash near the concrete marker on the eastern rim of the island's outer cone, to which almost all visitors climb to take photographs and rest. Another test would be a comparison between sites that have similar plant cover and are at about the same successional stage but which differ markedly in their exposure to humans. In a few years' time, samples from the forest near the newly established (1987) Park Ranger post on the northeast coast of Sertung could be compared with samples from the forest in the adjacent hinterland of northern Sertung, which is rarely if ever visited.

3. The enteric microbiota of vertebrate groups is to some degree characteristic of the group.

This could be tested simply by a more extensive sampling programme involving larger numbers and a greater variety of vertebrate species.

4. Wild vertebrates incorporate into their enteric flora human enteric bacteria, some at least of which have developed antibiotic resistance as a result of the widespread use of antibiotics by humans and inadequate sanitation.

A well-planned intensive study comparing the enteric flora of vertebrates in a heavily populated area of west Java (e.g. the port of Labuan, near Carita) with the enteric flora of the same species of, for example, rats, bats, skinks or geckos on a little-visited area of the Krakataus, such as the hinterlands of southern Sertung, southern Panjang or southern Rakata may support or refute the hypothesis, which is based on our limited data on the incidence of *Streptococcus faecalis* and tetracycline resistance of *E. coli* and *Klebsiella* strains.

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