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Age and evolution of bacteria[1]

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Summary. Age and evolution of bacteria can be estimated, including facts and hypotheses belonging to morphology, biochemistry, paleontology, ecology and pathogenicity. The corresponding dates are summarized in the following.

About 3.5×10^9 years: Origin of heterotrophic eobiontes. – About 3.0×10^9 years: The increasing lack of prebiogenic substances is due to the evolution of the respiratory pathway, that is due to the evolution of the photoautotrophy and now released O_2 is due to the evolution of strictly aerobic cells. There is, simultaneously, a transition of spheres to long forms, development of an amoebalike motility, the evolution of spirochetes and the substitution of cholesterol for cardiolipin in the more evolved cells (i.e. strictly aerobic cells etc.). – About $2.0–1.0 \times 10^9$ years: Evolution of the eucyte by symbiosis of a great, primitive, anaerobic, cholesterol-containing cell with a little, strictly aerobic, cardiolipin-containing cell, with a spirochete and in some extent also with photoautotrophic cell. – About 1.0×10^9 years (maximum: $1.8–1.5 \times 10^9$ years, minimum: 7×10^8 years): Evolution of metazoa and begin of cell differentiation. – About $2.0–1.0 \times 10^9$ years: Evolution of the bacterial murein sacculus and then development of flagella mediated motility. – About 6×10^8 years (maximum: 1.0×10^9 years, minimum: 4.5×10^8 years): Evolution of the gram-negative cell wall. – About 4.0×10^8 years: Evolution of the gram-positive cell wall. – About 5.0×10^8 years: Gram-negative, strictly anaerobic bacteria become the first enteric bacteria in coelenterates. About 4.0×10^8 years: gram-negative, microaerophilic bacteria become Enterobacteriaceae in vertebrates in addition to the strictly anaerobic organisms. – About $3.0–2.0 \times 10^8$ years: Consolidation of the Salmonella in reptiles. – About $2.0–1.5 \times 10^8$ years: Consolidation of Escherichia and other coliform species in mammals. – About 10^6 years: Evolution of typically human pathogenic organisms, transmitted in homogeneous-homonomous infection ways, i.e. N. gonorrhoeae, S. typhi, T. pallidum, etc.

The history of the theories regarding the evolution and the age of bacteria reflects the actual knowledge at any time of biology. The old hypothesis of Orla-Jensen[3], according to which autotrophic microorganisms are considered as the oldest forms, and all conceptions based on it have been annulled or have even been reversed by the new theory dealing with the genesis of life on the earth.

Especially the epoch-making experiments of Miller, followed by those of many other research authors, make it probable today that heterotrophic organisms having relatively small enzyme components, which however are unspecific and of wide action, must have been at the beginning of the development[4–6].

In the following discussion we shall try a time classification of some important characteristics and groups of bacteria based on their physiological and morphological characteristics.

Primitive original forms

As a consequence of geological-palaeontological studies, especially of Barghoorn, Pflug and Schopf, it may now be considered as sure that the first organisms evolved $4–3 \times 10^9$ years ago[4–10]. Subsequently to the metabolizable substances of abiogenous genesis which

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[2] Acknowledgment. I thank Prof. F. Müller, Hamburg, and Prof. P. Sitte, Freiburg i. Br., for stimulating discussions.

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waned at that time, there came about approximately 3×10^9 years ago, to begin with, the respiratory pathway the use of which enabled a better utilisation of the still existing resources. The photogenesis did not evolve until then, and thus brought about the self-sufficiency of the biosphere[11].

Strictly anaerobic organisms should therefore have been at the beginning of evolution by which anaerobic organisms received their energy only through intramolecular processes, i.a. also the Stickland reaction and through the substrate-stage phosphorylation[12]. They were probably spherical, relatively large, approximately 20–50 μm, immotile and had a primitive cholesterol-containing membrane, as we know it still at present in various mycoplasms[13]. The recently described microorganism Anaeroplasma abactoclasticum, an indispensable anaerobic cholesterol-dependent mycoplasm, might, quite apart from the size, approach particularly the hypothetical original form[14,15].

Amoeboid motility and spirobacteria

But the shortage of food has very probably not only promoted the evolution of the metabolism up to the fully developed respiratory pathway and to photosynthesis, but it has also influenced the morphological evolution of these early cells which are similar to mycoplasms. The reduction of the cell volume, and the transition from the round to the long form, increased the ratio: Surface to cell volume, and thus improved also the absorption of substrate.

Simultaneously and in close causal relation with the modification of the cell form, these cells probably abandoned their immobility at that time. As the most primitive and, therefore, probably also as the earliest form of the locomotion, amoeboid motility may be considered. We know it still nowadays also in the case of mycoplasms[16]. To which extent there exists a correspondent locomotion for amoeba and amoeboid cells of higher organisms, remains doubtful. In any case, however, the motility was the prerequisite for the maintenance of heterotrophic metabolism. At the latest together with the disappearance of the abiogenous substrates, a rapacious way of life was indispensable for the species which had not yet progressed up to autotrophy. Evolution led here to increasing motile and graceful forms. The spiral motility of the spirochetes developed from the amoeboid motility of still relatively clumsy, mycoplasm-like cells.

An intermediary position between mycoplasms and real spirochetes is occupied still at present by the genus Spiroplasma, e.g. Spiroplasma citri[17] or the 277 F agent[18].

The existence of motile, heterotrophic and rapacious spirobacteria already in the Proterozoic formation, certainly more than 2×10^9 years ago, has not yet been proved, it is true, by direct microfossile findings, but it can undoubtedly be postulated from the symbiosis theory of the eucaryotes cell[19-21], as according to Margulis the flagella-bearing eucyte was created by the symbiosis of rapacious spirochetes with bigger, mycoplasm-like, target cells which originally had acted as the prey.

As a consequence of the oxygen set free in the photosynthesis, already at that time also strictly aerobic cells had evolved, presumably in close vicinity to photoautotrophous species. They must have been fairly small, as only in this way could they have formed parallelly in time to the genesis of flagella from spirobacteria equally by way of symbiotic absorption in the strictly anaerobic large cells the heterotrophic eucaryotes cell and there have taken over the function of mitochondria.

The time required for this process may be limited. It could certainly not have been performed until the development of the photosynthesis, i.e. after 3×10^9 years, and prior to the appearance of differentiated metazoans. Now there were encountered, in the Amelia dolomite of the McArthur River in North Australia, relicts of a metazoic, differentiated organism of 1.8–1.5×10^9 years of age. In the Nonsuch formation of Michigan there were evidenced mushroom formations of approximately 10^9 years of age, and with certainty the petalonameae of the Ediacara fauna in the neolithic Precambrian approximately 6.8×10^8 years ago were real animals. The estimated age resulting from it roughly amounts to approximately $1\text{–}2 \times 10^9$ years for the first eucaryotes. Schopf and Zeller-Oehler indicate for the latter an age of $1.4 \pm 0.1 \times 10^9$ years, Tomofeev and co-workers an age of 1.00 ± 0.05 years[10].

Cholesterol and cardiolipin

The structure of the cell wall is very interesting in this context. The early and primitive cell, i.e. the so-called eobiotes, should have contained cholesterol as lipid component of its cell membrane which presumably had a prebiotic genesis. Until today the cholesterol has been maintained as a component of the cytoplasm membrane in the mycoplasms[13] and, furthermore, in the heterotrophic eucaryotic cells.

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The eobiotes should additionally have contained phosphatides. This group of substances had at that time certainly 2 different functions: It played with great probability a part in the electron transportation system and even at present phosphatides are integral components of mitochondria equally as of photosynthetizing bacteria[22]. The phospholipides stabilized, furthermore, the cell wall in a similar way, together with the cholesterol.

But already prior to the genesis of the eucaryotes, the cholesterol seems to have lost in the more highly evolved procaryotes, its function for the stabilisation of the cell wall. The following possible reasons come into question for this development: Impoverishment as regards prebiogenous cholesterol in the environment. Especially the more highly evolved cells involving a more considerable metabolism and a shorter generation period were concerned by this fact. – The successful synthesis of functionally better, new phosphatides. – The transition to a less considerable size of the cells made the cholesterol dispensable.

It remains to be seen whether, and to which of these points, the decisive importance for the substitution of the phosphatides for cholesterol was exclusively due. In any case the phosphatidylethanolamine found until that time in all bacteria, should have been importance even then. It is presumable that already at that time the cardiolipin evolved from this or a similar structure. As an intermediate solution and a transition substance possibly has served, to begin with, at that time the plasmalogen (alk-1-enyl glyceryl ether), as it is still found at present in Anaeroplasma and strictly anaerobic bacteria to which a phylogenetically higher age may be attributed than to most of the other organisms[14,15]. In accordance with the symbiosis theory, we must assume that the small strictly aerobic procaryotes changed into mitochondria owned a cardiolipin membrane, whereas the large host cells absorbing them still contained cholesterol. Only in this way is it explicable that even at present the mitochondria membrane is composed of cardiolipin. The cholesterol layer overlying it must be correspondingly interpreted as an invagination of the cell membrane of the host cell, as came about in the past in the symbiosis. No other hypothesis meets the findings of the membrane chemistry to so fully as the symbiosis theory[23].

Because, however, the cardiolipin contrary to the other phospholipides is synthetized by the mitochondriae themselves, it is justified to conclude that this way of synthesis existed already prior to the genesis of the eucaryotic cell. At present still many bacteria synthetize the cardiolipin as a component of their cell wall[22,24]. After all, the unspecific complement binding reaction on the suspicion of lues well-known since Wassermann is based on this assumption.

The euraryotes must, however, shortly after their genesis have succeeded in bringing about the synthesis of the cholesterol. Its high value as a construction and structure substance especially for the heterotrophic metazoae, is even up to now shown by the biochemical fact that for this substance only insignificant ways of decomposition have been developed. Even the highly differentiated human organism is not able to dispose of its excessive cholesterol to a sufficient extent. It must deposit it and pays for it by an arteriosclerotic degeneration. On the contrary, the dependence on cholesterol of all mycoplasms and their inability to synthetize this substance, points to the fact that here the oldest parasites are concerned. Immediately after the total consumption of the prebiotically evolved cholesterol, the mycoplasms may have laid themselves out for an obligatorily parasitic life in or at the eucaryotic protozoae or metazoae which now themselves synthetized cholesterol.

That, it is true, also retroevolution processes, in the spirit of Horowitz[25] and Lewis[26], may have led to the same final result, especially in the case of the at present existing cholesterol-independent Acholeplasma spec., can be contested as little as the theory presented here. But our comprehension of the evolution of life from primitive heterotrophics cannot put any general significance to the retroevolution theory, however justified it may be in some individual cases.

That is why it is very improbable that all mycoplasms have been created by retroevolution. On the contrary, the hypothesis presented here gains more plausibility by virtue of the assumption that this early obligatory parasitic life of way for the mycoplasms did not only offer the advantage of a never-failing source of nourishment, but beyond that also a protection against all its enemies.

Murein sacculus

Certainly not until the evolution of the eucytes, and probably also after the adoption of a parasitic way of life by the mycoplasms, was there found specific construction of the murein sacculus, as we know it at present for the spirobacteria[27,28] as well as for the gram-positive and gram-negative eubacteria. It is very probable thus that the murein sacculus appeared about or after $2–1 \times 10^9$ years. 2 factors may have become decisive for its genesis for teleonomic reasons:

a) The murein sacculus became vital as an osmosis protection, as since the genesis of life in the primeval

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sea there had developed ever greater differences between the salt concentrations in the sea water and in cell-owned ion milieu. From this aspect, the evolution of a murein sacculus may have become indispensable in order to guarantee the physiological cell functions.
b) Possibly the murein sacculus was also developed as a protection against rapacious spirobacteria. The rigid cell wall may have offered here a first protection. It is true that also the spirochetes have soon provided themselves with such a murein protection that the predacious animal-prey ratio between the motile spirobacteria and the target cells was not altered in the long run.

The same kind of fighting strategy has in any case been maintained up to now, while the morphology has undergone a change. We find them at the attack of spirillum-like organisms[29,30] against Scenedesmus algae, equally in the case of Bdellovibrio. Even the bacteriolysis by corresponding phages follows this pattern.

Bacterial flagella

Whatever advantage may have offered the evolution of the murein sacculus for the cruder coccal and bacillus-shaped bacteria, a considerable disadvantage was undoubtedly the loss involved of the previously potentially existing amoeboid motility. Therefore the assumption appears to be plausible that the evolution of the bacterial flagella did not take place until after the evolution of the murein sacculus.

As regards the origin of the bacteria flagella, it is possible to consider 2 alternate hypotheses:
a) Also here the symbiosis of a small spirobacteria with the larger immotile murein sacculus-armoured cell can be imagined.
b) More probable, it is true, is the autonomous evolution of this motility organella, e.g. of pili, fimbriae or other cell-appendix textures. For this assumption, the primitive molecular composition equally as the pure rotating form of motility of the bacterial flagella seems more probable than the motility of spirobacteria and eucaryotic flagella.

Secondarily, is the question whether the flagella evolved in the first place in cocci or bacilli, as in the long form, is more favourable than the sphere for reasons of flow conditions.

Thus there are only very few flagella-provided cocci such as e.g. Methanococcus, Planococcus, Sporosarcina or enterococci. Most of the spherical bacteria, thereagainst, are immotile.

We can, therefore, suppose with some certainty that the first flagella were formed for water bacteria and that they were probably polar flagellae, monoflagellae or lophotrichous disposed flagellae. The peritrichous provision with flagella represents in contrast a better adaptation to life in and on semi-solid media. It should, therefore, have evolved at a later date and might be seen in relation with the conquest of the mainland in the Devonian formation.

Cell wall differentiation

Still more decisive than for the formation of the peritrichous flagella which might perhaps have taken place already a little earlier, the conquest of the mainland must have been performed approximately 4×10^8 years ago for the development of the cell wall. Also the procaryotes adapted to life on the mainland should have acquired at that time a thick mucopeptide cell wall further evolved from the murein sacculus as a protection against drying out. They became gram-positive germs. In this respect it is possible to interprete 2 facts:
a) Gram-positive bacteria are distinctly more resistant to dryness than gram-negative ones.
b) Reversely there are amongst the gram-positive bacteria only a few pronounced water germs in contrast to the gram-negative ones. Accordingly, there are, of the 48 genera of gram-positive bacteria, described in Bergey's Manual, 8th Ed., after all 24 immotile, 14 peritrichous and only 10 genera provided with flagella at the poles. But even these forms provided with flagella at the poles are exclusively land bacteria. One of the quite few sea-water germs is Planococcus.

Also the astonishing salt tolerancy of the Micrococcaceae, up to 15% NaCl, may also be seen in this context. There evolved in the Paleozoic formation, soon after the mainland had been conquered by plants and animals, a dry, arid climate with an increase of the land areas and a decrease of the sea areas during the Permic and Triassic formations. Flat seas were formed in general and the salt concentrations increased considerably, often up to the saturation limit, as is proved by numerous salt deposits of these epochs of the earth. Such meteorological conditions, continuing for approximately 10^8 years, might be responsible for the fact that from primitive prestages at that time the present halophilic and dryness-resistant staphylococci evolved with their thick mucopeptide cell wall. It is, however, true that also the sometimes high NaCl content on the skin of warm-blood animals is taken into consideration as a normal place, especially of the staphylococci for the formation of this feature.

For the evolution of the gram-negative cell wall, no equal distinct geological happening exists. We must assume that it was performed in the water, and it is not excluded that it progressed gradually in accordance with the complicated composition of the gram-negative cell wall. The formation of the gram-negative cell wall began probably already in the Precambrian or the

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Cambrian formations. It must, however, have taken place and have finished not later than during the Silurian formation. An indication of the end of the evolution of the gram-negative cell wall is given by the existence of Limulus polyphemus, as these fossile limuli still live at present and contain a hematocyte protein which reacts selectively with the lipopolysaccharides of gram-negative bacteria e.g. Bacteroidaceae, Enterobacteriaceae or Pseudomonadaceae, as it is utilized nowadays in the limulus test to prove the existence of appropriate bacterial endotoxins. It may be assumed that here a prevention system of these animals is concerned which originates from the period of their phylogenesis in the Silurian formation. Much seems to indicate the probability that the gram-negative cell wall is of the same age as the Limulus polyphemus, although it is older. Already at that time the gram-negative bacteria was probably of significance as potential agents for the provocation of infections of these sea scorpions.

Other lectins of fossile coelenterates, which equally react selectively with various surface structures of bacteria, probably originate equally from this epoch at the earliest. We may, therefore, in general start from the assumption that the bacteria, as we know them at present, have remained morphologically constant since the Devonian formation.

Ecological niche

Another access to the question of the age of bacteria is gained by the inclusion of their natural location and their ecological niche in consideration of their evolution. A prerequisite for it are the following assumptions:

- The spectrum of the biochemical characteristics of a species of bacteria is closely correlated with the factors which form the natural location and the ecological niche of this species.
- That means that the genetic determination of biochemical features of a species of bacteria is dictated by the conditions of the ecological niche.
- Any newly formed ecological niche induces, therefore, also the genesis of a new species of bacteria which occur in such a way that in virtue of well-known gene transfer mechanisms and perhaps also of selection criteria which are not yet clearly perceivable, for various genes as reveal themselves in the well-known, discrete G/C relations, as an anular chromosome is consolidated, the enzyme spectrum of which has adapted itself in the best possible way to the new environments.

According to our present knowledge of gene transfer, the genesis of a new species in a new ecological niche should take place in a period which is very short as compared with geological changes or the phylogenesis of higher organisms.

As criteria for the definition of the normal location and the ecological niche of a species of bacteria, especially when it is relevant from the points of view of medicine or veterinary medicine, the following items may be considered:

a) Regularity and frequency of the occurrence.
b) Correlation of biochemical features of the bacteria species with those of the location or the host.
c) Missing pathogenicity of a species toward the host may be considered, according to Burnet[31], as a proof of good adaptation and accordingly long saprobiosis or symbiosis.

In virtue of these items there may result, however, also well defined statements regarding the age of corresponding bacteria from the phylogenetic age of natural location, possibly for man, animals or plants. 2 factors, which in their turn are closely connected with each other, gain under this aspect for our question as to age and evolution of bacteria a particular interest. They are the evolution of the O_2 atmosphere and the phylogenesis of the higher animals.

Enterobacteria

After the development of the photoautotrophia, the oxygen concentration of the atmosphere had continuously increased. As a consequence, on the one hand the colonisation of the mainland, the phylogenesis of differentated big vertebrates and finally their homeothermy had become possible; on the other hand the sphere of life, to begin with of the strictly anaerobic organisms, followed by that of the microaerophilic bacteria, had shrunk to the same extent.

Only the strictly aerobic bacteria, which, it is true, have existed for a long time, but so far had not had any large possibility of expansion, found from that time better living conditions.

So they resulted, since the appearance of the first bigger animals in the Devonian and Carbon formations, new ecological niches also for anaerobic and microaerophilic bacteria. Presumably the strictly anaerobic, gram-negative germs were the first to adapt themselves to the intestine milieu. They became here Bacteroides. The next to follow them were microaerophilic, gram-negative bacteria, from which was evolved, after the adaptation to the intestine, the original form of the Enterobacteriaceae. That gram-positive germs did not become intestine bacteria until much later, results from the low age of the gram-positive cell wall equally as from the retroevolution processes just for some species. But in all cases the bile resistance became a typical feature of the intestinal flora. After all, bile salts appear already in primitive cyclostomata and decapoda; and, in accordance with old experience of bacteriologists, all typical enterobacteria are, therefore, independently of their taxonomic position possibly, in contrast to the inhabitants of the respiratory tract, also bile resistant.

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Escherichia and coliform bacteria

Contrary to all other oligosaccharides, the lactose is an 'invention' of the mammals. Therefore, also the lactose-splitting enzyme β-galactosidase, and hence all lactose-splitting bacteria, should not be older than the mammals themselves which evolved in the Triassic or Jurassic formation.

Escherichia coli and the coliform bacteria, such as Enterobacter, Klebsiella and in part also Citrobacter, are accordingly also the typical inhabitants of the mammalian intestine. They are regularly founded here and have no or a relatively negligible pathogenetic importance. Possibly also the extensive varieties of Escherichia coli types and affiliated species correspond with the riches in species and the present predominance of the mammals on earth.

The fact especially the lactose-positive behaviour of Enterobacteriaceae indicate the harmlessness and relative absence of danger for their hosts, whereas amongst the lactose-negative species the indispensable pathogenic bacteria such as Salmonella, Shigella and also Yersinia are encountered, is an old bacteriological experience the interpretation of which was not yet possible. It became understandable through the hypothesis that bacteria which are lactose-positive have adapted themselves simultaneously and for the same reason to the intestine of the mammal.

Salmonella

The other extreme is probably represented by the Salmonella. Their normal location seems to be the intestine of reptiles. They appear there with striking frequency and are not of pathogenic significance, as becomes distinctively evident from infection experiments, particularly with serpents and turtles[32]. Salmonella are, however, more or less pathogenic for birds and mammals. They do not form here part of the normal flora and, after infection, they are regularly reeliminated from the intestine earlier or later. Conversely, their inability to split lactose corresponds very well to their normal habitat in reptiles and to their insignificant adaptation to the intestine of mammals. From these facts it may be concluded that Salmonella might have be of about the same age as the reptiles, as the ecological niche 'intestine of reptiles' has probably not changed much more since the evolution of reptiles in the Permic and Triassic formations. A phylogenetic ancient ecological niche presumes, however, equally old inhabitants. As a whole the Salmonellae probably still represent an inheritance of the dinosaurs and are approximately $3.0–2.0 \times 10^8$ years of age.

This summary picture can be backed by detailed investigations: Within the group of reptiles, the lizards, which according to their morphology are the most conservative of their class, contain comparatively most frequently Salmonella, whereas in the further developed serpents, in addition to Salmonella, frequently Arizona, in the equally more progressive turtles, in addition to Salmonella, preferably Edwardsiella is encountered[33]. From this fact may be derived that – as serpents and turtles represent a little more recent evolutions within the group of saurians – also Arizona and Edwardsiella may be considered as more recent lines within the Salmonella group. The possibly irritating fact that in the intestine of reptiles, in addition to Salmonella and Salmonella-affiliated germs, nowadays regularly also E. coli and coliform bacteria are found, points to the considerable dominance of mammals and hence also of their lactose-positive intestine flora.

The evolution of the lactose-positive Arizona in serpents must possibly be interpreted in the same direction, as serpents live exclusively on animal food, and they are largely dependent on little mammals as prey. In this way it is, however, also possible to interprete the accumulated occurrence of the lactose-negative Edwardsiella in turtles, as mammals almost never come into consideration as prey of turtles.

Summarily, there results for the family of Enterobacteriaceae a fairly clear order of evolution: Starting from an original form of Enterobacteriaceae which might perhaps have many a likeness with Proteus, the genus Salmonella evolved for reptiles. Its high age is distinctly underlined by the numerous serotypes. Starting from the Salmonella, it seems that Arizona has evolved for the serpents and Edwardsiella for the turtles. Citrobacter should occupy an intermediate position which leads over to the E. coli and coliform Enterobacter-Klebsiella-group predominant in mammals.

Human pathogenic organisms

The most recent group is undoubtedly that of the human pathogenic organisms which is transferred in homogeneous-homonomous routes of infection from man to man. None of these bacteria is older than man himself, most of them are even essentially more recent than 10^6 years.

That applies possibly to C. diphtheriae, N. gonorrhoeae, S. typhi, T. pallidum and some similar infective agents. But from their spectrum of characteristics, it becomes obvious that they have diverged through few mutation steps from groups of bacteria which have occupied for essentially older ecological niches in the vicinity of man, the age of which, as a rule, is therefore also much greater.

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