In the past few years, a remarkable number of structurally unique and highly active metabolites has been published from marine bacteria, and especially marine actinomycetes have shown an impressive metabolic capacity. If these bacterial isolates are really indigenous to the marine environment is, however, often not proven. After decades of research mainly on terrestrial microbes, the yearly output of the marine research has passed now its terrestrial counterpart. This chapter summarizes the development of the marine microbial research since the year 2000.

In spite of the long and successful history of antibiotics of terrestrial origin, the search for marine microbial metabolites is even today a nearly untouched subject. The very first antibiotic – mycophenolic acid (1) from a Penicillium spp. (Bentley, 2000) – was described as early as 1896 and has even found medical application long before the discovery of penicillin; the first marine antibiotic, pentabromopseudilin (2), was described more than half a century later (Burkholder *et al.*, 1966; Hanessian *et al.*, 1966; Lovel, 1966; Andersen *et al.*, 1974). The reason for this unexpected fact is among others certainly the assumption of the past decades, that sea water is nearly sterile and that even microbes due to terrestrial pollutions cannot survive for long in the sea. It is now a fact, however, that the water column of the oceans contains on the average $10^4 - 10^6$ bacteria/ml, that is an estimated global weight of at least $10^{12}$ tons at a weight of 1 pg/bacterium and a volume of $1.35 \times 10^9$ km$^3$ of the oceans!

Due to the often poor physiological status of sea water bacteria and/or to the cultivation methods used, only a very small bacterial fraction of about 1% is usually cultured. However, during the last few years this percentage could be raised considerably by the development of fascinating novel strategies for cultivation and detection (Zengler 2002).

In spite (or because?) of the tremendous success of the past secondary metabolite research, the number of terrestrial antibiotics seems currently to approach a saturation curve with an apparent limit in the near future. The increasing number of duplications and the urgent demand for new leading structures in pharmacology has enforced the search for metabolites in so far untouched habitats like deserts, caves, hot springs, the polar ice and – the oceans. And although most of the so far investigated bacteria from the sea may not be truly marine, the number of newly discovered metabolites from these resources is indeed growing exponentially (Fig. 1). This will certainly continue, as not more than 12 % of the bacteria in the marine habitat seem to be known so far (Bull *et al.*, 1992). Since 2000, nearly 250 marine bacterial metabolites have been described. It should be stated, that in the same period the number of metabolites from terrestrial bacteria was less than 150!
The definition of a truly marine bacterium is somewhat difficult. Certain species and even genera have not been found in terrestrial surroundings so far, e.g. the Roseobacter clade or the genus *Pseudoalteromonas* (see below). Sea water and/or sodium requirements are unequivocal physiological characteristics of autochthonous marine bacteria, however, bacteria with broad salt tolerances may also be active members in some marine environments. Many marine bacteria use a respiratory chain-linked sodium dependent NADH:quinone reductase instead of the common proton-coupled enzyme e.g. *Salinospora* spp.), and korormycin (3) (Yoshikawa *et al.*, 1997) from a marine *Vibrio alginolyticus*, a specific inhibitor of this enzyme (Yoshikawa *et al.*, 1999), may be a tool to identify such sodium-dependent bacteria.

Only a few metabolites have been described from marine bacteria with sodium requirement. Not all bacteria in the sea have, however, the same surrounding and certainly also not the same requirements. A restriction to sodium-dependent bacteria would therefore be too narrow for practical use. Also the production of bromine-containing compounds is not sufficient, as also terrestrial bacteria were shown to synthesize bromoorganics in the presence of bromide (Hochlowski *et al.*, 1997). For the statistics of Table 2, a broader definition was used therefore, which was firstly given by Fenical (*Jensen et al.*, 1996): Marine bacteria are microorganisms which were isolated from the marine habitat and which are functionally reproductive under typical marine conditions. It is obvious that this description may also include terrestrial contaminants if they tolerate the 3.4 % salt concentration of the oceans. Most streptomycetes do so, and according to K. Schaumann from the Alfred-Wegener Institute in Bremerhaven, it might be advisable to name most marine microorganisms better as "isolates from a marine habitat".
There are obviously differences between the physical, chemical and biological characteristics of the marine and the terrestrial environment (Scheuer, 1990). One of the most intriguing factors is the strong mutual, epibiotic and even symbiotic interaction of microbes with higher forms of life in the sea, the majority of which is uniquely marine: In the oceans, nearly every surface is covered by microorganisms, and for a few cases, chemical interactions have been proven. One of the earliest examples is probably the presence of an Alteromonas sp. on the embryos of the shrimp Palaemon macrodactylus: These bacteria are producing isatine (4), a long-known synthetic product, which showed now a reasonable antifungal activity and protects the eggs successfully against the pathogenic fungus Lagenidium callinectes (Gil-Turnes et al., 1989).

\[
\text{\begin{picture}(50,50)
  \put(25,25){\line(0,1){15}}
  \put(25,25){\line(1,0){15}}
  \put(25,40){\line(1,0){15}}
  \put(40,25){\line(0,1){15}}
  \put(40,25){\line(-1,0){15}}
  \put(40,40){\line(-1,0){15}}
  \put(25,25){\line(0,-1){15}}
  \put(40,40){\line(0,-1){15}}
\end{picture}}\]

It was argued that ecological pressure and selection can rapidly produce specially adapted strains under marine conditions; the development of resistance against medically used antibiotics is a related well-known parallel. The reverse process is, unfortunately, also frequently observed. Meant is not the loss of biodiversity, which certainly also could happen on the microbial level, e.g. if the host of certain symbiotic bacteria is extinguished. More frequently observed is the loss of certain chemotypes inside a bacterial cluster under laboratory conditions: Artificial nutrient compositions are obviously lacking certain trigger substances or stress factors, and it is a common experience that bacterial cultures are rapidly loosing their metabolic capabilities under laboratory conditions if they are too often sub-cultured. The process itself is due to a loss of plasmids or just a selection of clones without needless capabilities and well understood. What we do not know, however, is a reliable technique to avoid this. We are also still at the beginning to understand mutual interactions between bacteria and their hosts or among each others, especially in the sea.

The structures of metabolites published in the past years have been summarized most comprehensively by Fenical (1993), in the annual reviews on marine natural products by Faulkner (2000), and recently by Blunt et al. (2003, 2004). This article is focussed therefore on some technical aspects and on the chemistry of marine bacteria of the past four years only.

**Isolation and Fermentation of Marine Bacteria**

The isolation of bacteria with special capabilities is generally rather an art than a technique. Samples are treated with heat or antibiotics and cultures are kept on different carbon and nitrogen sources, and especially a selection for slowly growing organisms is made. General techniques are summarized in ”The Prokaryotes” (Dworkin et al., 2005) and other handbooks of systematics (Wagenitz 2003, Berger et al. 1997).

While the isolation of pure cultures is predominantly a task of microbiologists, their bulk fermentation is already part of their chemical investigation. The search for metabolites with useful properties, the screening, goes parallel with the search for optimal conditions of their
production. The genom of e.g. a streptomycete has a size of up to 8 mega-base pairs (Mbps). As only approximately 4 Mbps are needed for the basic life processes and up to 100 kbps are needed to synthesize a simple polyketide antibiotic like 12, at least 40 products can be expected from each of the expected 20,000 streptomycetes (Omura et al., 2001). Usually, the number of isolated metabolites is, however, much lower. So it is obvious that we could make much more use of the metabolic capacities of the organisms under investigation, if silent genes could be switched on by added trigger substances or other influences. A few of these factors are known, however, due to their selectivity, they cannot be applied generally.

In most experiments, the search for optimal growth conditions is a matter of trial and error, starting with a standard broth composition and some (often secret) additives. In our laboratory, M$_2^+$ medium (Maskey et al., 2002e), a composition of glucose, yeast extract and malt extract in diluted (50%) artificial sea water containing trace elements (Biabani et al., 1998) gave good results with marine streptomycetes and bacteria of the free water column. Other compositions are on the basis of oatmeal, peptone, fish flour or chitin (crab shell powder). It should be mentioned that natural seawater contains already a high background of various biological activities and is not suitable if biological tests will be applied on the extracts.

The optimisation of chemical properties concerns usually an increase of the yield and sometimes also the number of metabolites and requires the search for suitable growth factors, the feeding of biosynthetic precursors or the generation and selection of mutants and high-production clones, respectively. Variation of pH and temperature, certain inorganic salts and a variety of carbon and nitrogen sources are commonly used, among others. Typical marine additives are algal extracts: The borine-containing aplasmomycin was formed by *Streptomyces griseus* ss-20 (FERM-p 3448) only in the presence of "Kobu cha", a commercial Japanese algal product (Fenical, 1993). Recently, however, we were able to isolate various aplasmomycins from the marine *Streptomyces* isolate Mei22 also without any additives. This indicates that general rules do not exist.

**Screening**

Published results show that in a screening for drug development by interactions with special receptors, the hit rate is as low as 10,000:1 $\approx$ 20,000:1, leading to the industrial high throughput screening as a logical consequence. Even very high sample numbers can be handled now easily, however, this advantage goes in parallel with an increasing number of disadvantages: The flood of data is getting more and more anonymous and only yes/no decisions are obtained, individual observations are impossible and side effects are getting lost.

In contrast to this expensive industrial „vertical screening“, at universities a broad „horizontal screening“ makes more sense: Tests just with one or two fungi, a yeast, a Gram-positive and a Gram-negative bacterium, a microalga and the brine shrimp test are supplying sufficient information about antibiotic, antifungal, phyto- and cytotoxic activity to decide if more detailed tests should follow or not.
Optimisation of Culture Conditions by Genetic Algorithms

A random variation of culture conditions will seldomly reach the best nutrient broth compositions, and a systematic variation of all parameters ends up in an unrealistic number of experiments. As especially about marine bacteria more information with respect to the carbon and nitrogen sources and many other factors is needed, mathematical approaches are making sense. A most suitable method has been developed years ago and has found many technical applications, however, is not often used in fermentations: the optimisation by genetic algorithms.

The rapid development of computers gave rise for a new definition of information as a function of self-organisation: The whole evolution of living beings can be understood as a tendency to optimise storage and processing of relevant information (Stonier, 1991; Hosp, 1994). If a system has no way for variations, there is also no way for development (Grzeganek, 2003).

The natural evolution is an ongoing experiment where the genetic information is continuously optimised. The progress of this evolutionary optimisation is achieved by mutation and recombination and controlled by a steady selection, resulting in the best fit of a population. Evolutionary algorithms (EA) are using these biological mechanisms of evolution to solve nonlinear-polynomial problems for technical applications (Coveney et al., 1995; Merkl et al. 2003).

The mathematical basis of EA has a strong correlation to evolutionary biology: The genetic pattern of an organism is defined by its genotype, and the phenotype is the sum of the resulting visible properties (caterpillar and butterfly are having the same genes). If in EA the genotype corresponds to the encoding of parameters responsible for the fitness, the phenotype is then the decoded fitness value of the solution. The "genetic information" of a cultivation experiment (e.g. the broth composition) is digitised as a matrix, changes are achieved by bit manipulations (Adolf, 2001), similar as in artificial neuronal networks (McNeill et al., 1994). Within the various EAs, genetic algorithms (GA) are among the most stable optimisation routines and deliver a solution, even if all other statistical methods fail due to a high number of parameters (Albertz, 1989; Bäck, 1996).

By copying the biological evolution process of selection, mutation and crossover, the GA will find a solution, which is better than the starting condition. Repetition of this procedure will approach to the global (!) optimum by stepwise improvements until a termination condition is fulfilled (Bäck, 1996).
In the case of a fermentation optimisation, a set of plausible nutrient compositions is defined as starting conditions. Each composition is encoded as a binary string, the “gene”. The sum of these genes are forming the gene pool of the respective experiment. After the parallel fermentation of different compositions in a small scale, the biochemical result (phenotype, e.g. the activity, the concentration of certain compounds or the cell mass) is validated. The binary strings (genes) of selected positive experiments are now subjected to "genetic manipulations" resulting in a first daughter generation. A second fermentation is performed, the phenotypes are again determined, and so on.

The number of repetitions (generations), the probability of mutations, recombinations, the number of individuals and their mortality are speed and quality determining factors. They do not obey general rules and have to be experimentally adopted to the problem in question (Clemens et al., 1996; Adolf, 2001).

For the optimisation of culture conditions, we used the program GALOP (Genetic Algorithm for the Optimisation of Processes, Möllney et al., 1998), where a complex influence on the selection and optimisation procedure is possible. For the example of a Streptomyecete GW27/1179, a nutrient broth consisting of glucose, maltose and yeast extract in artificial sea water was found to produce chartreusin (5) in low yield. As a stress factor, calcium chloride was added, which has an influence on the morphogenesis of microorganisms (Goodwin et al., 1979, 1985, 1992), and also effects many other cellular functions. To optimise the concentration of these four constituents, in each generation 15 individuals (15 inoculated Erlenmeyer flasks each with 50 ml nutrient broth) were fermented for 3 days and extracted using a standard protocol. The 5 concentration of each extract was determined by HPLC.
An increase of the CaCl₂ concentration and reduction of the other constituents afforded a fourfold increase in the production of 5. As in the third generation, the CaCl₂ concentration is again decreasing, obviously only a local maximum was reached (Table 1), and after the fifth generation, an increase of more than 700 % was obtained (Fig. 3). It should be stated that the number of parameters is theoretically not limited so that e.g. various carbon and nitrogen sources or stress factors can be tested simultaneously.

Table 1. The best individuals of five subsequent generations, according to the 5 concentration.

<table>
<thead>
<tr>
<th>Generation</th>
<th>Maltose [g/l]</th>
<th>Glucose [g/l]</th>
<th>Yeast extr. [g/l]</th>
<th>CaCl₂ [g/l]</th>
<th>concentration of 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8.1</td>
<td>7.9</td>
<td>1.9</td>
<td>0.3</td>
<td>5.7</td>
</tr>
<tr>
<td>2</td>
<td>3.1</td>
<td>5.8</td>
<td>0.6</td>
<td>1.7</td>
<td>22.8</td>
</tr>
<tr>
<td>3</td>
<td>9.0</td>
<td>8.8</td>
<td>7.5</td>
<td>0.4</td>
<td>31.5</td>
</tr>
<tr>
<td>4</td>
<td>5.8</td>
<td>8.2</td>
<td>4.1</td>
<td>1.3</td>
<td>38.9</td>
</tr>
<tr>
<td>5</td>
<td>1.6</td>
<td>8.5</td>
<td>3.9</td>
<td>2.0</td>
<td>42.1</td>
</tr>
</tbody>
</table>

Figure 3. HPLC chromatograms of the best individuals of S. sp. GW27/1179 for the production of chartreusin (5) in a) generation II and b) generation V, registered at λ = 260 nm.
Isolation and Separation Techniques

There is certainly no principal difference between the work-up procedures of terrestrial and marine bacteria, and therefore the following technical hints concern both groups.

For most medical applications a certain polarity profile is needed which can be described by the octanol/water coefficient, i.e. by the extractability with organic solvents and the Nernst equation. Many bioactive compounds can be extracted with ethyl acetate or at least butanol, however, highly polar compounds like sugars, certain polyhydroxy acids, amino acids and many peptides remain in the water phase. There are two ways also to extract at least some of these compounds: 1. the solid phase extraction with e.g. activated charcoal, lipophilised silica gel or special adsorber resins (mostly Amberlite® XAD-16 or Mitsubishi DIAION® HP20), or 2. the lyophilisation of the whole culture broth and then the extraction with a solvent of higher polarity like water-saturated ethyl acetate or even methanol.

In a few examples the metabolite in question can be precipitated directly from the crude extract; in all other cases, a sequence of separation steps is necessary. If a solid-phase extraction had been used, an elution by a methanol/water gradient of decreasing polarity can be used for a pre-separation. If a solvent-extraction was applied and larger amounts have to be handled, defatting is advisable as the first step. For this purpose, the extract is distributed between methanol and cyclohexane. The latter solvent is more suitable than hexane or petrol ether, which are also in use. Hexane is toxic and expensive, and both solvents contain contaminations of higher boiling alkanes, which are later on difficult to remove from the extract. Cyclohexane is not causing these problems.

For compounds of low or moderate polarity, the further separation steps may include column chromatography on silica gel, size-exclusion chromatography on Sephadex LH-20, HPLC with a wide variety of phases, and solvent/solvent distributions like high speed counter-current chromatography (HSCCC) and other techniques. For highly polar compounds, ion exchange chromatography, preparative electrophoresis and hydrophilic Sephadex are suitable. It is obvious that the separation processes have to be monitored, e.g. by the biological activity, by spectroscopic methods (mostly used are UV, MS, NMR, CD), TLC in combination with spray reagents (anisaldehyde/sulphuric acid for a wide range of compounds, Ehrlich’s reagent for indole derivatives, the chlorine/o,o´-dianisidine reaction for amides, especially peptides, ninhydrin for amino acids and peptides, aniline phthalate for carbohydrates, etc.).

Depending on the source organism and its origin and the investigated structures, 90 % or more of all isolated metabolites may already be known. It is extremely important therefore to eliminate these doublettes as early as possible, a process, which is called dereplication. The earliest possible stage to detect known metabolites is the investigation of the producing organisms itself by MS methods, e.g. by MALDI/TOF (Erhard et al., 1998, 2001). If high resolution is applied and MS/MS fragmentation patterns are known from reference measurements, this technique gains some reliability.

Extracts can be investigated by the same method, which is further enhanced by including additional dimensions, e.g. the HPLC retention time and UV data. Corresponding HPLC/UV databases are widely used and even commercially available (Fiedler, 1993), an HPLC/MS-
MS/UV database is applied for dereplication in our group (Oka et al., 2004). While investigations by means of MS daughter ions or the spectral fitting of UV data require the availability of reference compounds to measure the basic data set at least once under identical conditions, NMR measurements are delivering ab initio results which can be compared directly with the literature even prior to a full structure elucidation. Proton and carbon NMR spectra can be easily translated into a set of substructures, which can be used for a fragment search in suitable databases. It is even more important that in structure-based data collections also those structures can be searched, which are obviously not present in the compound under investigation, to reduce the size of the answer set. The Chemical Abstracts are in practice not applicable for this purpose, for a search with simple fragments will usually result in a system overflow. More suitable are the Dictionary of Natural Products on CD ROM (2005), which covers more than 170,000 entries and includes mainly terrestrial metabolites, and Marinlit (Blunt et al., 2005), a database of solely marine natural products including those from sponges and higher forms of life (presently ~15,000 entries). AntiBase (Laatsch, 1994-2005) (31,000 entries) was developed in our laboratory and is specially designed for the dereplication of terrestrial and marine microbial products (including those from fungi and algae) by means of structural features, UV and NMR data. The Umezawa Database (2005) is a related product, however, without NMR data and a smaller data set. Berdy’s BNPD was worldwide the first electronic inhouse database, however, was limited to the DOS operation system and had therefore restricted search capacities; it is not further supported. Some of these databases have been reviewed recently (Buckingham et al., 1997; Gringard et al., 1995).

A dereplication with AntiBase may proceed in the following way: The \(^1\)H and \(^13\)C NMR data of a yellow, green fluorescent compound showed 3 methine signals between \(\delta 95-100\) which were interpreted as acetal functions, 2 aliphatic ketone signals beyond \(\delta 200\) and 3 methoxy groups (3H signals between 3.6-4.0), and gave signals of an aromatic system. The substructure search in AntiBase successively step by step with 3 O-CH-O groups, 2 acetone fragments (aliphatic ketone substructure), 3 methoxy groups and a benzene ring afforded only 15 hits out of ~31,000 entries. As there was no typical colour reaction with sodium hydroxide, peri-hydroxyquinones were excluded, which left five trioxacarcin derivatives and one member of the hibarimicin group. Only one of them, trioxacarcin A (47a) had the observed mass of \(m/z = 876\).

After the dereplication step and the final purification, structure elucidation is performed usually on the basis of 2D NMR data and simple chemical derivatisation. It should be stated that completely new skeletons are extremely rare and certainly do not occur worldwide more than a few times per year among bacterial constituents. The result of a structure elucidation should be re-examined carefully therefore, if no related skeletons can be found in the literature. Databases like AntiBase can assist also this process:

A low-molecular weight compound (probably \(m/z = 342\)) from a streptomycete showed benzene signals of three protons in a row, a C\(_q\)-methyl, a methoxy and an isolated methylene group. The colour reaction with sodium hydroxide and the 1H NMR spectrum indicated a quinone with two chelated peri-hydroxy groups. Only two compounds were found with these data, but mass and expected spectra were not fitting, and so the metabolite was probably new. The compound was finally elucidated as the quinone 6 and named fuchurmycin B (Barckhausen et al., 1999).
Recently Described Metabolites from Marine Bacteria

Most marine bacterial metabolites have been isolated from species of the genus *Streptomyces* and *Alteromonas/Pseudoalteromonas* (the genus *Alteromonas* was revised 1995 by Gauthier et al., and most of the previous *Alteromonas* species were transferred to the new genus *Pseudoalteromonas*) (Table 2). However, also the search for further hot spots in other taxonomic branches is making sense. In the following part, recent results from the literature and our own work are summarised.

Table 2. Number M of published metabolites from marine bacteria according to their taxonomic origin since 1966 (Laatsch, 1994-2005)

<table>
<thead>
<tr>
<th>Genus</th>
<th>M</th>
<th>Genus</th>
<th>M</th>
<th>Genus</th>
<th>M</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Streptomyces</em></td>
<td>241</td>
<td><em>Janibacter</em></td>
<td>9</td>
<td><em>Brevibacterium</em></td>
<td>2</td>
</tr>
<tr>
<td>unidentified bacteria</td>
<td>65</td>
<td><em>Microbacterium</em></td>
<td>9</td>
<td><em>Chrysobacter</em></td>
<td>2</td>
</tr>
<tr>
<td><em>Alteromonas</em></td>
<td>47</td>
<td><em>Actinomadura</em></td>
<td>8</td>
<td><em>Enterobacter</em></td>
<td>2</td>
</tr>
<tr>
<td><em>Bacillus</em></td>
<td>37</td>
<td><em>Marinobacter</em></td>
<td>7</td>
<td><em>Pelagibacter</em></td>
<td>2</td>
</tr>
<tr>
<td><em>Vibrio</em></td>
<td>29</td>
<td><em>Salinospora</em></td>
<td>7</td>
<td><em>Blastobacter</em></td>
<td>1</td>
</tr>
<tr>
<td><em>Pseudomonas</em></td>
<td>28</td>
<td><em>Flavobacterium</em></td>
<td>6</td>
<td><em>Chainia</em></td>
<td>1</td>
</tr>
<tr>
<td><em>Actinomyces</em></td>
<td>25</td>
<td><em>Micrococcus</em></td>
<td>6</td>
<td><em>Cyclobacterium</em></td>
<td>1</td>
</tr>
<tr>
<td><em>Pseudoalteromonas</em></td>
<td>25</td>
<td><em>Halomonas</em></td>
<td>5</td>
<td><em>Deleya</em></td>
<td>1</td>
</tr>
<tr>
<td><em>Cytophaga</em></td>
<td>19</td>
<td><em>Ruegeria</em></td>
<td>4</td>
<td><em>Enterococcus</em></td>
<td>1</td>
</tr>
<tr>
<td><em>Micromonospora</em></td>
<td>19</td>
<td><em>Halobacillus</em></td>
<td>3</td>
<td><em>Erythrobacter</em></td>
<td>1</td>
</tr>
<tr>
<td><em>Myxobacteria</em></td>
<td>17</td>
<td><em>Nocardiosis</em></td>
<td>3</td>
<td><em>Flexibacter</em></td>
<td>1</td>
</tr>
<tr>
<td><em>Chromobacterium</em></td>
<td>15</td>
<td><em>Oceanibulbus</em></td>
<td>3</td>
<td><em>Maduramyces</em></td>
<td>1</td>
</tr>
<tr>
<td><em>Agrobacterium</em></td>
<td>14</td>
<td><em>Alcaligenes</em></td>
<td>2</td>
<td><em>Photobacterium</em></td>
<td>1</td>
</tr>
</tbody>
</table>

Metabolites from *Streptomyces*

*Quinones*

*Streptomyces* are filamentous actinomycetes and members of the Gram-positive eubacteria. Actinomycetes isolated from soil are the most important source of terrestrial metabolites, and the same situation is found in the marine habitat.
For a long time actinomycetes were considered to be rare or even non-existent in the sea. They are rare, indeed, in the free water column but they could be proven regularly in marine sediments even remote from land (Weyland 1969) although actinomycetes are not as abundant in marine as in terrestrial samples. Due to the work of Okami and co-workers at the Institute of Microbial Chemistry in Tokyo in Japan and the pioneering work of Weyland at the AWI in Bremerhaven (Berg et al., 1981, Weyland 1981, Weyland 1984, Weyland 1986, Helmke et al., 1984, Weyland et al. 1988) and others (e.g. Jensen et al., 1991), we have some knowledge about the occurrence of the different actinomycete types and their possible role in the marine environment. We know that the density of streptomycetes is decreasing with increasing distance from the shore. It cannot be excluded therefore, that at least some Streptomyces isolates may be terrestrial contaminants. This may explain that no obvious differences in the structures of metabolites from marine and terrestrial streptomycetes were found and that the percentage of halogen compounds is even less than in terrestrial bacteria! Representatives of the actinomycete genus Salinospora occur also preferably in coastal waters, nevertheless, these types are unequivocally indigenous marine organisms indicated by their sea water dependence (Pathirana et al. 1992). An active role of the actinomycete Rhodococcus marinonascens in offshore sediments is also unquestioned. However, Rhodococci strains are in general less attractive with respect to secondary metabolite formation but to bioremediation.

The density of rare actinomycetes e.g. Actinoplanes, Rhodococcus or Actinomadura, is obviously increasing in sediments of the open sea, which may be understood as an indication of a truly marine origin, so that also differences in their metabolism can be expected. And indeed, the marine Salinosporae have been delivering a number of fascinating structures in the recent past.

Streptomycetes are especially rich in biologically highly active quinones, and as these compounds are easily detected, it is not surprising that also many marine quinones have been described. Recent examples are the complex C-glycosides himalaymecins (Maskey et al., 2003a) A (7) and B (8), anthraquinones with the rare fridamycin E chromophor, a precursor of the anthracycline antibiotics. They were isolated from Streptomyces sp. B6921 isolated from a litoral sample from Mauritius and are showing strong antibacterial activity.

From the same strain, vineomycin C, an inhibitor of the inducible nitric oxide synthase (Alvi et al., 2000), and vineomycin B2 (9) were isolated. The lipophilic part of the extract consisted interestingly to 80 % of the ethyl glycoside 10a/b (K. Vossler, H. Laatsch, unpublished results), which were volatile enough for GC/MS investigations (Stritzke, 2003). It is still unknown if 10a/b were formed during the isolation, or originated already during fermentation.
Komodoquinone A (11) and its chromophor, komodoquinone B (12), are belonging to the anthracycline antibiotics, a large group with nearly 500 members. They have been isolated from *Streptomyces* sp. KS3 using a test for neuritogenic activity (Itoh *et al.*, 2003).

With nearly 500 members, the anthracyclines are forming one of the largest groups of antibiotics from actinomycetes. Most of them are highly glycosylated and exhibit strong antitumor activity doxorubicin (adriamycin) being a well-known example. Phipps *et al.* (2004) described recently four new rhodosaminosides 1-hydroxyauramycin T (13), 1-hydroxsulfurmycin T (14) and the diastereomeric (7S*9S*10R*)- and (7R*9R*10R*)-pyrromycins (15a, 15b), which showed the expected cytotoxicity against a P-388 cell line. It is remarkable, that the streptomycete produced monoglycosides with several variations in the aglycon; the previously described 'normal' pyrromycin had the (7S,9R,10R)-configuration (Brockmann, 1959, 1963).

At least 40 simple quinones with the skeleton of aloesaponarin II have been obtained from streptomycetes. New from a streptomycete from the Yellow Sea are the hydroxymethyl-quinone 16 (Laatsch, unpublished results) and the flavomarins A (17a) and B (17b), which were
isolated from \textit{S. sp. B1108} together with bafilomycins, feigrisolides, lactoquinomycin and the insect quinone deoxyerythrolaccin (Abdelfattah, 2003).

![Chemical structures of isolated compounds](image)

From \textit{Streptomyces} sp. B5543, the new espicufolins A and B (18) (Abdelfattah, 2003) and several related but known indomycinones were isolated.

A \textit{Streptomyces} sp. Mei 6-1,2 from the German North Sea delivered among others a series of simple anthra- and tetracenequinones 19a/b - 20; 19a was already known from synthesis (Laatsch, unpublished results).

![Chemical structures of 19a/b and 20](image)

Also anthracyclines are not a domain of terrestrial bacteria, cinerubin K (21) (Laatsch, unpublished results) from \textit{Streptomyces} sp. B9054, cinerubin M (22), 135 and 136 from \textit{S. sp. B8904} (Shaaban, 2004) being recent examples. The unusual glycocarbaminate fragments in 135 and 136 are typical for the bleomycin group, however, rare in other antibiotics; the only related quinones are rubomycin H and F (Fomicheva \textit{et al.}, 1992).

![Chemical structures of 21 and 22](image)
Only three benzopyrene quinones have been isolated from nature so far, resistomycin (23) (Brockmann et al., 1954), resistoflavine (24a) (Eckhardt et al., 1970) and its temptatively assigned methyl ether 24b (Laatsch, unpublished results); the former two have been isolated from terrestrial as well as marine streptomycetes. 1-Hydroxy-1-norresistomycin (25) (Kock, 2005) has now been obtained as a trace component from an isolate B8005. The same compound was recently isolated from a genetically modified streptomycete (Hertweck, 2004).

The renieramycins A-D (A = 26) are complex heterocyclic quinones from the marine sponges Haliclona sp., Cribrochalina sp., and Reniera sp., which differ in the ether residues at the quinone rings. It is highly fascinating that the closely related compounds 27 - 31 have now been isolated from a marine streptomycete isolated from a sponge (Saito et al., 2000). They differ from 26 in the substitution at the central bicyclus and also in the (preliminarily assigned) stereochemistry. It can be speculated that the renieramycins are formed by a symbiotic microorganism and perhaps just only modified by the sponge.

The isoquinolinequinones aclidinomycin B (= 12c-hydroxyaclidinomycin A (32a), C (33) and D (32b) (Cang et al., 2001) were isolated by Thorwest (Thorwest et al., 2001) from a marine streptomycete; they resemble the antibacterial and cytotoxic metabolites naphthyrimycin A (34) (Kluepfel et al., 1975) and the bioxalomycins, e.g. β1 (35). The latter was
isolated from the marine Streptomyces viridostaticus subsp. littus (LL-31F508) (Zaccardi et al., 1994; Bernan et al., 1994).

From various marine streptomycetes, we have isolated recently the mansouramycins 36 – 41 (Speitling, 1998; Fotso et al., submitted 2005). These isoquinolinequinones resemble the spongal cribrostatins (Pettit et al., 1992; 2000; Sandoval et al., 2004) from the blue marine sponge Cribrochalina sp. (e.g. cribrostatin 6, 43), the caulibugulones (Milanowski et al., 2004) from the marine bryozoon Caulibugula intermis (caulibugulone A, 44) and the related renierones (Sandoval et al., 2004; Kitahara et al., 1985) from the sponges Reniera, Petrosia, and Haliclona sp. All these isoquinolinequinones are showing a pronounced cytotoxicity with IC₅₀ values down to 30 ng/ml and are active against viruses, bacteria and malaria as well. From S. sp. B1848, also 6-hydroxyisatin (42) was obtained.
The 12 members of the LL-D49194 complex (e.g. LL-D49194\(\eta\), 45) are highly oxygenated quinone derivatives, which have been isolated from the terrestrial \textit{Streptomyces vinaceus-drappus} (Maiese \textit{et al.}, 1990). It is very surprising that the trioxacarcins 47a - 47f from the marine \textit{S. sp.} B8652 are derivatives of the same aglycone where, however, a sugar residue and one of the acetal methoxyls have changed places. Some of these trioxacarcins had been obtained previously from a terrestrial streptomycete (Shirahata \textit{et al.}, 1981). We observed now a so far unknown strong antiplasmodial activity of the trioxacarcins (Maskey \textit{et al.}, 2004a). Gutingimycin (47g), the trioxacarcins (47a - 47f) and also the previously described chromophor 46 (parimycin) (Maskey \textit{et al.}, 2002a) are displaying a characteristic green UV fluorescence on TLC, which is due to the 2,3-dihydroquinizarin skeleton.

![Chemical structures of compounds 45 and 46](image)

\(47a: \quad R^1 = \text{COCH}_3; R^2-R^3 = R^4-R^5 = O; R^6 = a; R = O\)

\(47b: \quad R^1 = \text{COCH}_3; R^2 = R^3 = \text{OH}; R^4-R^5 = O; R^6 = a; R = O\)

\(47c: \quad R^1 = \text{COCH}_3; R^2-R^3 = R^4-R^5 = O; R^6 = a; R = \text{OH}, H\)

\(47d: \quad R^1 = H; R^2-R^3 = R^4-R^5 = O; R^6 = a; R = O\)

\(47e: \quad R^1 = \text{COCH}_3; R^2 = R^3 = R^4 = R^5 = \text{OH}; R^6 = H\)

\(47f: \quad R^1 = \text{COCH}_3; R^2 = R^3 = R^4 = R^5 = \text{OH}; R^6 = a; R = O\)

\(47g: \quad R^1 = \text{COCH}_3; R^2 = b; R^3 = \text{OH}; R^4-R^5 = O; R^6 = a; R = O\)

The high cytotoxicity of trioxacarcin A (47a) is due to the cleavage of DNA: 47a forms a MS-detectable complex at guanine containing DNA/RNA positions, which decays under opening of the N-glycosidic bond and liberation of the conjugate 47g. This compound, gutingimycin, was isolated as a major component from B8652.
Compound 48a from the actinomycete ACT 7617 is an unusual quinone derivative as well (Laatsch, unpublished results); it is a hydroxy-luisol A (48b), related with nanaomycin aE and has also some similarity with granaticin C (49).

**Polyenes**

Polyene macrolides are a typical domain of streptomycetes and rare actinomycetes, all compounds of this type were isolated from these organisms. Halichoblelilde (50), a new member of the efomycin/elaiohylin group from *Streptomyces hygroscopicus* isolated from fish, is a further marine example, which shows, however, potent cytotoxicity instead of the antifungal activity of the larger polyenes (Yamada et al., 2002).
The antifungal oxopentaen-macrolide dhanyabadomycin (51) (Maskey, 2001) from *S.* strain B 8905 had been isolated previously as TG-488 from terrestrial streptomycetes, however, was not published in detail (Takahashi *et al.*, 1995).

Aureoverticillactam (52a) (Mitchell *et al.*, 2004), a novel 22-membered macyclic lactam from the marine actinomycete *Streptomyces aureoverticillatus*, is a demethyl-BE-14106 (52b) from a terrestrial streptomycete and is also related with GT-32B (52c). All these polyene macrolactams exhibited a strong cytotoxicity.

A streptomycete M045 from the sediment of Jiaozhou Bay in China produced the chinikomycins (53a) and B (53b) (Li *et al.*, 2005) A, unusual chlorinated rearrangement products of manumycin with some structural similarity with 64-p-ABA-2 (53c), which was obtained by precursor-directed biosynthesis (Zeeck *et al.*, 1993). They exhibited antitumor activity against...
different human cancer cell lines (IC\textsubscript{50} = 5.6-5.9 µg/ml), however, were inactive in antiviral, antimicrobial, and phytotoxicity tests.

A very unusual polyen is nitrated β-lactone lajollamycin (54) from a marine \textit{Streptomyces nodosus} strain NPS007994 (Manam \textit{et al.}, 2005), which is nearly an isomer of 16-methyloxazolomycin (55) from a terrestrial streptomyecete (Ryu \textit{et al.}, 1999).

\textit{Macrolides and other Lactones}

A similar intensive greenish fluorescence as in the trioxacarcins is also displayed by chartreusin (5), a compound which is not only antibacterially active, but shows also a very promising antitumor-activity against different human cell lines (McGovren \textit{et al.}, 1977), a fact that has stimulated the search for related compounds. The marine streptomyecete isolates B5525 and B5342 produced chartreusin (5) in high yields of 10-90 mg/l, in addition to 2-methyl-3-hexen-2,5-diole (58), phenylacetic acid, streptazolin (Drautz \textit{et al.}, 1981), and 5-hydroxy-5-methylhex-3-en-2-one (58b) (Speitling, 1998). Two trace components were identified as the monoacetates 56 and 57. While one of the monoacetates showed a normal acetate methyl signal at \(\delta = 1.97\), the corresponding 2"-signal in the minor component 56 was extremely upfield shifted to \(\delta = 1.39\), an effect which was accounted to the influence of the aromatic ring system. Even stronger upfield shifts with signals at \(\delta = 0.81\) and 0.71, respectively, were observed for the 2"-acetate groups in the tetra- and pentaacetates (Maskey \textit{et al.}, 2002b).
Complex aliphatic lactones are very common among streptomycetes: examples are the uncounted natural and synthetically modified erythromycins, many of which are having clinical applications. A 16- instead of a 14-membered lacton is found in chalcomycin B (47b) (Maskey et al., 2002c) from *Streptomyces* sp. B7064, which shows a high antibiotic and moderate phycotoxic activity, similar to that of chalcomycin A (59a).

Smaller lactones occur very frequently in terrestrial streptomycetes. Recent examples are the feigrisolides A (60a), B (60b), and C (61) from *Streptomyces griseus* (Tang et al., 2000), which were also found in many marine streptomycetes, together with further minor metabolites, e.g. feigrisolide E (62) from *S*. sp. B5375 (Laatsch, unpublished results). Attempts to synthesize two of the feigrisolides have been published recently: The stereochemistry of 60b is obviously wrong, as synthetic and natural product were not identical; the correct stereochemistry is still unknown (Sharma et al., 2004). For feigrisolide C (61), even an open acid structure 63 was suggested based on the synthesis (Kim et al., 2005a,b), so that the planar structure of the chiral feigrisolide C is now identical with that of the racemic bonactin (Schumacher et al., 2003). As the authors of the old structure 61 claimed, however, to be able to distinguish a lactone and a free acid, also the structure of feigrisolide C is still under debate.
On ESI MS measurements, some of the feigrisolide fractions are showing indeed a typical molecular weight distributions as of a polymer, and it is plausible that lactones as 60a can yield a mixture of oligomers on work-up. The antifungal and antibacterial bonactin (64) (Schumacher et al., 2003) from S. sp. BD21-2 is such a linear dimer of nonactic and homononactic cid, and the isolation of higher isomers would not be surprising.

Nonactic and homononactic acids are building blocks of more than 50 antibiotics like pamamycins, feigrisolides C (61) and E (62), macrotetrolides and others. Borolactin A (65) Shin et al., 2001) is a new monomeric lactone of homononactic acid, which we recently re-isolated from the marine S. sp. B5375.

The antibiotically inactive N-formylantimycic acid methyl ester (66) (Seo et al., 2001) is the major constituent of the bislactones of the antimycin and urauchimycin series and was now isolated for the first time as a natural product from S. sp. M03033. It is also part of the new urauchimycin C (67) from S. sp. B175 (Schiebel et al., 2005).

Polyhydroxybutyric acid (sPHB, 69a) is a very common energy storage material of bacteria (and also often found in marine streptomycetes), which is of commercial interest as biodegradable plastic material. It was surprising now to find for the first time low-molecular weight polyhydroxybutyric acids 69b (Maskey et al., 2002d) with 8-20 repetition units and a main component 69b with n = 13. According to Seebach's hypothesis (Seebach et al., 1995), such oligomers may be channel-forming (cPHB), in contrast to the high-polymeric storage sPHB.
From most strains, sPHB or cPHB were isolated, and only in a few cases both were found together.

Crude PHBs are obtained easily as insoluble residues, if ethyl acetate extracts of fermentations are dissolved in methanol. It should be mentioned that EI mass spectra of both PHBs are displaying only fragment ions as for small homologues so that the impression may result that the dimer 70 (n = 0) (Fujimoto et al., 1998) or the trimeric pinnatifolide (70, n = 1) from the red alga Laurencia pinnatifida (Viqar et al., 1991) is present; also CI delivers incomplete results, so that ESI or MALDI TOF are the methods of choice.

Most microbial butenolides are substituted at the double bond. So far, only 12 metabolites are 2,3-unsubstituted, 71 - 76 being recent examples (Mukku et al., 2000; Cho et al., 2001; Laatsch, unpublished results). Not much is known about their biological function.

The number of the reduced low-molecular butyrolactones is much higher, and more is known about their biological relevance: Some are autoregulators and initiate pigment or antibiotic production, and also antibiotic or antiviral activity was reported. The butyrolides 77 - 79 have been isolated recently from a marine streptomycete (Cho et al., 2001).
The related acylamino-butyrolides (acyl-homoserinlactones) have got much more attention due to their function as quorum sensing molecules. After $\gamma$-hydroxynorvaline lactone, N-acetyl-$\gamma$-hydroxyvaline lactone (80) provides now the third type of a 3-substituted homoserinelactone from bacteria (Hernandez et al., 2000a).

Simple seven-membered lactones are relatively rare in nature, in contrast to their frequently occurring six-membered homologues. The feigrisolides 60a, 60b and 62 have already been mentioned; (6R)-10-methyl-6-undecanolide (81a) and (6R,10S)-10-methyl-6-dodecanolide (81b) are recent examples from a marine Streptomyces albogriseolus isolate B6007 (Stritzke et al., 2004). Both lactones showed a weak antifungal and a pronounced antitumor activity.

\[
\text{81a: } R = \text{H} \\
\text{81b: } R = \text{Me}
\]

**Terpenes**

Terpenes are believed to be rare in bacteria and are more typical constituents of fungi. A number of sesquiterpenes has, however, been found among the odour components of S. citreus (Pollak et al., 1996) and other streptomycetes. Africantriol (82) from Streptomyces sp. GT 061115 is another bacterial example (Hu et al., 2003), although this compound has been isolated previously from the marine invertebrate Lemnalia africana.

The chemical investigation of the marine-derived Streptomyces sp. QD491 from Qingdao coast (China) delivered four sesquiterpenes, namely 10$\alpha$-hydroxyamorph-4-en-3-one (83), 10$\alpha$,11-dihydroxyamorph-4-ene (84), 10$\alpha$,14-dihydroxyamorph-4-en-3-one (85), 8$\alpha$,10$\alpha$,11-tri hydroxyamorph-3-one (86), and some trivial compounds (Wu et al., 2005); 84 had previously been obtained from Taiwania cryptomerioides Hayata. It exhibited moderate activity in the brine shrimp lethality test [He et al., 1997].
From the staurosporine producing streptomycete QD518 from the same shore in China, selina-4(14),7(11)-diene-8,9-diol (87) was obtained [Wu et al., 2005].

Recently, the cytotoxic indoles 88 - 90 having isoprenoid side chains were reported (Sanchez Lopez et al., 2003). They were produced by Streptomyces sp. BL-49-58-005 isolated from marine invertebrates, and are obviously products of a mixed biosynthesis. The nitril 88 can be derived by dehydration of the oxime 89, whose hydrolysis and reduction would deliver 90.

The irregular methylation pattern of the TPU-0037 components (91a - 91d) (Furumai et al., 2002) with an interesting activity against methicillin resistant Staphylococcus aureus strains from a marine Streptomyces platensis isolate TP-A0598 indicates that these compounds are certainly not terpenoids. Although the nitrogen containing terminal octahydronaphthalene unit occurs in at least 10 Streptomyces metabolites, this structural element is more typical for fungi like Alternaria, Fusarium, Phoma spp. and others. It is one of the rare cases where the bacterial and fungal metabolism results in related structures, and it should be of interest if also the gene sequences of their synthases reflect these similarities.

Peptides

Most of the about 500 Streptomyces peptides are cyclic and contain further rare structural elements such as chromophors or uncommon amino acids. In this respect, the puromycin re-
Resistance proteins 92 - 94 from *Streptomyces* sp. AP77 are quite normal (Woo et al., 2002). They show activity against *Pythium porphyrae*.

Heterocycles

While the bithiazolyl structural element is found often in a broad variety of microorganisms and even in cyanobacteria, the hexahydrobithiazolyl core of the antibiotics watasemycins A (94a) and B (94b) from *Streptomyces* sp. TP-A0597 is fairly rare in nature (Sasaki et al., 2002). It can be assumed that 94a and 94b are good ligands of iron(III) and bivalent metals, in
parallel to the closely related micacocidin C (96) from a terrestrial *Pseudomonas* sp. (Kobayashi *et al.*, 1998a,b).

Small heterocyclic compounds like the quinazolines 97 and 98 are found frequently (Speitling, 1998; Schroeder, 2002) and are not restricted to the marine habitat (Maskey *et al.*, 2004b).

**Others**

Two glycolipids (155) from marine streptomycetes are mentioned below. Also a number of small molecules (99 - 103) (Laatsch, unpublished results) belonging to different metabolic pathways was isolated from various sources, where no obvious biological activity is known (Maskey, 2001; Shaaban *et al.*, 2002). If these compounds are shunt products, side products of the primary metabolism or just metabolic waste, has still to be found out.

From the producer of the selinane 87, the simple 5,7-dihydroxy-5,6,7,8-tetrahydroazocin-2(1H)-one (104) was obtained [Wu *et al.*, 2005]. It is the first azocin-2-one from microorganisms and remarkably unstable: On standing in chloroform, a quantitative transformation into 1,2-dihydro-pyrrolizin-3-one (105) and the trimer 106 was observed, probably due to the influence of HCl, which induces a transannular reaction of 104.
Rare Actinomycetes

_Salinospora_ sp.

Morphologically indistinguishable from the _Micromonosporae_ are strains of the new sea water dependent genus _Salinospora_ (Fenical _et al._, 2002), which can be differentiated only by means of genetic markers. The first species was found by cytotoxicity screenings, which lead to the salinosporamides (Felting _et al._, 2003; Fenical _et al._, 2002) A (107), B (108) and E (111), novel ß-lactones with some relation only to omuralide (114) from _Streptomyces lactacystinaeus_ (Tomoda _et al._, 2000). Due to the high reactivity of the strained ß-lactone ring, the other salinosporamides (C, 109; D, 110; F, 112; G, 113) are formed as artifacts during workup (Fenical, 2003). Salinosporamide A (107) is active against colon cancer in the remarkable low concentration of 10 ng/ml.
Another marine filamentous bacterium (strain CNH-099) delivered several partially brominated and moderately cytotoxic naphthoquinones with a terpenoid ring extension (Hardt et al., 2000), namely isomarinone (115), hydroxydebromomarinone (116a), methoxydebromomarinone (116b), and neomarinone (117). Only the terrestrial naptherpines and naphthgeranines are similar (Wessels et al., 1991; Shinya et al., 1992).

\[ \text{115} \]
\[ 116a: R = H \]
\[ 116b: R = \text{Me} \]

117

The cytotoxic IB-96212 (118) from *Micromonospora* sp. L-25-ES25-008 is a common oligomycin derivative (Canedo et al., 2000; Fernandez-Chimeno et al., 2000),

\[ \text{118} \]

and also staurosporines are frequently found in terrestrial and marine streptomycetes. It can be speculated therefore that the staurosporines found in ascidians as *Eudistoma* sp. are synthesized by microbial symbionts and are just accumulated or modified by the host (Schupp et al., 1999). It is of interest that two new derivatives, 5'-hydroxystaurosporine (119a) and 4'-N-methyl-5'-hydroxystaurosporine (119b) have now been isolated from *Micromonospora* sp. L-31-CLCO-002 (Hernandez et al., 2000b).

\[ \text{119a: } R = H \]
\[ \text{119b: } R = \text{Me} \]

Compounds related with the recently described marine lorneamides A (120a) and B (120b) (Capon et al., 2000) are the terrestrial serpentemycins (Vertesy et al., 2004), serpentene, demetric acid, the marine ester 121 (Grzeganek, 2003), and the rubrenoic acids, the latter from the marine *Alteromonas rubra* (Holland et al., 1984). All these compounds displayed a weak antibacterial activity, the rubrenoic acids were also bronchodilatators.
Kahakamide A (122a) (Schumacher et al., 2001) from Nocardiopsis dassonvillei is the 4-methoxy derivative of neosidomycin from S. hygroscopicus and formally the partial hydrolysis product of the nitril SF-2140 from Actinomadura albolutea sf-2140 (FERM-p 5704), kahakamide B (122b) is the corresponding amide of 122a. All these compounds are fascinating, as two very rare structural elements are coming together, the 4-methoxy indole, and the indole-N-glycoside. The kahakamides are having a weak antibacterial activity.

The orange phenoxazin-3-one chromophor of the chandrananimycins A-C (123 - 125) (Maskey et al., 2003b) originates biosynthetically from an oxidative coupling of two molecules o-aminophenol and further derivatisation. Compounds of this type are rather common in bacteria, the actinomycins among them have certainly found the strongest interest (Mauger et al., 2005, in press). The chandrananimycins from Actinomadura sp. M045 have a significant cytotoxicity (IC$_{70} < 2$ µg/ml), 125 is in addition antibiotically active.

Ircinal (126a), ircinol (126b), several manzamines (127 - 130), and neokauluamine (131) have been obtained from the same marine Micromonospora sp. M42 isolated from a sponge (Hill et al., 2004). The co-existence of these compounds indicates that the β-carboline derivatives 127 - 130 are formed from ircinal (126a) and tryptamines via Pictet-Spengler reactions.
About 100 diazepines have been isolated from microbial sources, however, bacterial dibenzodiazepines were unknown so far. A first compound of this type, diazepinomicin (132) has been isolated now from a *Micromonospora* strain (Charan et al., 2004). Interestingly, the dibenzodiazepine core is linked to a farnesyl side chain.

Whereas the tetrapeptide 133 from a *Nocardiopsis* sp. is one of about 40 microbial tetrapeptides and a sequence isomer of fenestine A from the sponge *Leucophloeus fenestrata*,

126a
126b: OH instead of CHO
the cytotoxic and antibacterial quinone IB-00208 (134) from an *Actinomadura* sp. is unique so far (Malet-Cascon *et al.*., 2003). The next parallels are the terrestrial cervinomycins and the citreamicins.

Kadamycin A (137) with the novel hydroxylamino sugar kadamalose is a *nor*-N-methoxy-cinerubin R, and kadamycin B (138) is the corresponding spartanamycin A derivative (Maskey *et al.*., 2005). The only other hydroxylaminosugar-glycoside is dactylocycline A from a *Dactylosporangium* sp. (Tymiak *et al.*., 1992; Wells *et al*., 1992). Hydroxylamino sugars may be precursors of the various nitroso and nitro sugar derivatives as in dactylocycline B.

The 3-amino-2-butanone residue in barycine (139, 2-(1-methyl-2-oxo-propylamino)-benzoic acid) from *Streptomyces* sp. B6005 is extremely rare among the microbial metabolites and was found so far only in maniwamycin A (Nakayama *et al*., 1989) and 7-(1-methyl-2-oxopropyl)streptonigrin (Wang *et al*., 2002).
The new methoxy derivative methoxyneihumicin (140a) has been obtained from an uncharacterised marine bacterium NPS0002/0014 (Macherla et al., 2002), which might be related with *Micromonospora neihuensis*, the producer of the cytotoxic and antifungal neihumicin (140b) (Wu et al., 1988; Yang et al., 1988).

**Marine gliding Bacteria and Myxomycetes**

Especially due to the work of Hoefle and Reichenbach it is known that gliding bacteria (especially myxobacteria and myxomycetes) are extremely potent producers of highly active and structurally unique natural products. It was an obvious idea therefore, also to investigate such types isolated from marine samples. Only very few reports, however, were published so far.

The linear antifungal tetranes haliangicin A – D (141 - 145) from the NaCl requiring myxobacteria *Haliangium leuteum* AJ13395 seem not to have further parallels (Fudou et al., 2001; Kundim et al., 2003), and also the diterpenoid verrucosan derivatives 146a,b - 148 from *Saprospira grandis* (ATCC 23116) belonging to the CFB cluster are unique (Spyere et al., 2003).
Other Marine Bacteria

Among the metabolites from marine bacteria not belonging to actinomycetes or myxomycetes, two large groups can be distinguished, the peptides and the glycolipids. Wicke et al. (2000) isolated from a microbacterium isolated from a Mediterranean sponge a complex mixture 149a – 153 of diacylglycerolglycosides, which differ in the sugar part and the acyl pattern. The monosaccharides 149a/b were identified as glucofuranosides, whereas the disaccharides are gentiobiosyl-diglycerides (150) or glucopyranosyl-mannopyranosides (151 - 153) with α- or β-configuration. Similar (154, Lurtz et al., 2002) or identical compounds have also been isolated from other sources, e.g. from Bacillus pumilus associated with the ascidian Halocynthia aurantium (Kalinovskaya et al., 2000).

The lysophosphatidyl inositols 1 and 2 (155) were obtained from streptomycetes (Cho et al., 1999), however, the phosphatidyl glycerol 156 with an interesting cyclopropanated acid was found in a marine Pseudomonas sp. from the sponge Homophymia sp. (Bultel-Ponce 1999). The more complex 1,3-diphosphatidylglycerols (Wicke et al., 2000) 157 and 158 have been isolated from the a Microbacterium sp. (actinomycete); they exhibited a strong antitumor activity.
151a: \( R = H \)
151b: \( R = \text{Ac} \)
The acidic polysaccharide 159 was obtained from a marine *Pseudoalteromonas distincta* strain KMM 638 isolated from a sponge (Muldoon et al., 2001), the O-specific polysaccharide 160 comes from *Alteromonas marinoglutinosa* NCIMIB 1770 (Komandoña et al., 2001).

The recently published bacterial peptides can be summarised into three groups, the linear acylpeptides, the cyclopeptides and some very sulfur-rich thiazolylpeptides. The dehydropep-
tide bogorol A (161) from a *Bacillus* sp. is active against methicillin resistant *Staphylococcus aureus* and vancomycin resistant enterococci (Barsby *et al.*, 2001), the N-acylated aquachelins A-D (162a-d) from the marine bacterium *Halomonas aquamarina* strain DS40M3 are novel siderophores (Martinez *et al.*, 2000).

The marinobactins A-C, D1, D2, and E (163a-f) from the new genus *Marinobacter* resemble the aquachelins, however, are characterised by a unique α,γ-cyclodipeptide core of β-hydroxyaspartic acid and 2,4-diaminobutyric acid (Martinez *et al.*, 2000). They are siderophores as well.

The pseudoalterobactins A (164a) and B (164b) are siderophores from the marine *Pseudoalteromonas* sp. KP20-4 (Kanoh *et al.*, 2003). Their carboxybenzene sulfonic acid residue is unique in nature.
Petrobactin (165a) from *Marinobacter hydrocarbonoclasticus* is certainly not a peptide, however, has some similarity with 164a (Barbeau et al., 2002; Bergeron et al., 2003). The combination of the chelating properties of citric acid with a second chelator is rather common in nature and found e.g. in aerobactin, schizokinen, actinoferrin, etc. The combination with a catechol siderophor, however, is unique so far. Petrobactin sulfonate (165b) has been recently isolated from the oil-degrading marine bacterium *Marinobacter hydrocarbonoclasticus* (Hickford et al., 2004).

The cyclopeptide 166 (Mitova et al., 2003) from a *Pseudomonas* sp. from the sponge *Ircinia muscarum* and the weakly cytotoxic halolitoralins A (167), B (168) and C (169) from *Halobacillus litoralis* YS3106 resemble the previously discussed peptides (Yang et al., 2002). Their structures were elucidated by extended NMR, MS and chiral HPLC measurements.
Mitova et al. (2004) described recently a further series of cyclopeptides 170 - 175. Besides 170, all of them contain proline or hydroxyproline, which may give rise to the idea that nutrient constituents were incorporated. This may also be valid for the dipeptide 176 and the ornithine derivative 177 (Hernandez, 2004).
The mixirins A-C (179a – 179c) from a marine *Bacillus* sp. are related to the iturins, however, in the latter, Ser and Pro have changed places (Zhang *et al.*, 2004). The long-chain β-aminoacyl residue is a common constituent of many further cyclopeptides, e.g. the bacillomyccins and mycosubtilins.

From a further bacillus (*B. cereus* QN03323 from a marine sponge), the thiazolyl-dehydropeptides YM-266183 (178a) and YM-266184 (178b) were isolated. They are new members of a large group of related compounds, which are widespread over various bacterial taxa (Nagai *et al.*, 2003; Suzumura *et al.*, 2003) and are highly active against Gram-positive bacteria, however, not against Gram-negative microbes.

![Diagram of 180a and 180b](image)

180a: R = i-Bu
180b: R = 2-Bu

Two unusual chromopeptides alterochromide A and B (180a/b) were isolated as an inseparable mixture from a *Pseudoalteromonas piscicida* strain KMM 636 isolated from the sponge *Fascaplysinopsis reticulata* collected at the Greet Barier Reef (Speitling *et al.*, 2005). The only related chromopeptide is (the halogen-free) myxochromide A from a terrestrial myxomycete (Trowitzsch-Kienast *et al.*, 1993).

**Low-Molecular Weight Compounds**

At least 17 macrolactines are known from a deep-sea *Bacillus*. Some members of this series were already isolated in 1989 by Gustafson *et al.* (1989), further compounds were added in 2000 (181, 182) (Jaruchoktaweechai *et al.*, 2000) and 2001 (183 - 189) (Nagao *et al.*, 2001). All macrolactins exhibit a weak antibacterial activity.
Further korormicins 190 – 194 have been isolated from a marine *Pseudoalteromonas* sp. F-420 (Yoshikawa *et al.*, 2003). As 3, they are selective inhibitors of the primary sodium pump and are active against obligate marine Gram-negative bacteria. It is of interest that 193 contains bromine.
Acyl-thiazolylcarboxamides had been found before only in the few bacitracins from \textit{Bacillus subtilis} and \textit{B. licheniformis}. The recently described simple derivative bacillamide (195) from another \textit{Bacillus} sp. showed a reasonable activity against the dinoflagellate \textit{Cochlodinium polykrikoides} with an LC\textsubscript{50} value of 3.2 µg/ml (Jeong \textit{et al.}, 2003).

A number of \(\beta\)-phenylethylamides 196a - f had been isolated from a marine \textit{Bacterium} sp. GBF 102b (Maskey \textit{et al.}, 2002e), and later on, these and further amides 196g - k were obtained from many other marine and also terrestrial bacteria (Stritzke, 2003 and unpubl. results). It was surprising that these simple compounds showed a pronounced phycotoxicity (MIC 12.5 µg/ml) and even more, that closely related compounds had been patented as herbicides long ago (Zaweski, 1965; Kirino \textit{et al.}, 1983).

In contrast to most of the phenylethyl amides, the corresponding acyltyramines 197a-e (Stritzke, 2003 and unpubl. results) from the North Sea bacterium Hel 11 were already known also from plants, the isobutyramide 102 from a streptomycete had been already described above. The amide 197b is a moderate inhibitor of the porcine aldose reductase (Bahn \textit{et al.}, 1998).

A further simple amide 198 was obtained from \textit{Cytophaga} sp. strain AM13.1 (Shaaban \textit{et al.}, 2002) and the marine \textit{Vibrio parahaemolyticus} Bio249 (Veluri \textit{et al.}, 2003).
Small aliphatic compounds are certainly frequently occurring, however, difficult to isolate if other methods than GC are used. The amides 199 and 200 were obtained from *Cytophaga marinoflava* strain AM13,1 (Shaaban, 2004).

The isolation of the phenolic bisabolane sesquiterpenoid curcutetraol (201) is somewhat unexpected for a bacterium, as this type of compounds is typical for fungi. And indeed, Mülhaupt *et al.* (2005) isolated the same compound also from a marine fungus CNC-979. The corresponding acid, sydonic acid (202), is known from the terrestrial *Aspergillus sydowi*; also a cyclic form, sydowic acid, is known (Hamasaki *et al.*, 1978).

![Image of compound 201 and 202]

The combination of a diterpenoid basic structure with a pyridine ring is characteristic for fungi as well. Thallusin (203) with its labdane unit was isolated from an epiphytic marine *Cytophaga* so. YM2-23. With an activity of as low as 1 attogram/ml, it is probably the most active algal morphogenesisis inducer differentiation ever described (Matsuo *et al.*, 2005). The similarity with a series of fungal metabolites such as Pyripyropene-E (204) is obvious (Tomoda *et al.*, 1995). A related biological activity was, however, not reported for these compounds.

![Image of compound 203 and 204]

Three remarkable esters B-5354a-c (205 - 207) have been isolated from *Ruegeria* sp. SNAK 71896 and shown to be sphingosine kinase inhibitors (Kono *et al.*, 2000a,b). It should be mentioned that only one further occurrence of 4-amino-3-hydroxybenzoic acid (notoneso-mycin A) was reported before (Sasaki *et al.*, 1986a,b).
The *Roseobacter* clade seems to be specific marine and showed an interesting metabolic capacity in the past. Another impressive structure is that of the antibacterial tropodithietic acid (208), whose remarkable four-membered disulfide bridge was confirmed by X-ray analysis (Brinkhoff *et al.*, 2004; Liang, 2003). Isomers of 208 from a marine *Agrobacterium* and various *Pseudomonas* spp. have been described previously (Kawano *et al.*, 1997; 1998) as troposulfenin (210) and the tautomeric thietone thiotropocin (Tsubotani *et al.*, 1984; Kintaka *et al.*, 1984; Cane *et al.*, 1992), respectively. Their structures should be revised now into 208 (Liang *et al.*, unpublished). A minor component of strain T5 was identified as the hydroxytropodithietic acid 209 (Liang, 2003).

Thiazoloethanol (211a) has been isolated from an unidentified marine bacterium (M. Thorwest, A. Zeeck, unpublished). The corresponding methyl derivative sulfurol (211b) is a flavour component of *Boletales* (Rapior *et al.*, 1997) and part of vitamin B1.

Another simple sulphur compound is the thioester 212, an activated ester from the new species *Oceanibulbus indolifex* Hel 45 (Wagner-Döbler, 2004). This compound is new in nature, however, had been obtained previously by synthesis.

In the presence of selenomethionine (213) or selenoethionine (214), the selene-resistant marine *Bacillus* sp. No. 14 produced selenohomocystine (215), which is antibiotically active (Imada *et al.*, 2002).
ß-Carbolines, among them some alkaloids of the harman group, have been firstly isolated from plants, however, are known now also from many other sources. They are formed by condensation of tryptamines amines with carbonyl compounds and cyclisation of the intermediate imines, a sequence, which is called Pictet-Spengler reaction. Phenethylamines deliver correspondingly isoquinolines. Some complex examples were obtained from streptomycetes, but as arylethylamines are degradation products of the amino acids phenylalanine, tyrosine and tryptophane, similar structures were also formed in other bacteria.

Flazin (216) was isolated first from Japanese sake and soy sauce and is the Pictet-Spengler product (after subsequent dehydrogenation) of tryptophane and furfural (Gessner et al., 1988). The new ß-carbolin 217 can be derived in the same way from the naturally occurring 4-hydroxy-2-keto-butyraldehyde (Sparkes et al., 1969); both ß-carbolines were obtained from an unidentified North Sea bacterium Bio215 (Shaaban, 2004).

There are only a few examples that plant metabolites also occur in bacteria, the prenylated flavanone isoxanthohumol (218) from the North Sea isolate PIC009 being a new one (Shaaban, 2004). This compound was isolated first from the roots of Sophora flavescens (Kang et al., 2000), however, occurs also in hop and is an inhibitor of cyclooxygenases and lipoxygenase, has estrogenic, trypanocidal and antiviral properties and may have a potential as cancer chemopreventive agent.
The list of simple pyrazine derivatives has been extended by the new member 219 from Cytophaga sp. AM13.1, which was inactive against bacteria, fungi or algae (Shaaban et al., 2002).

Under oxidative conditions, indole derivatives may undergo a ring opening which delivers o-formylaminobenzenes and, after hydrolysis, the anilines. N-Acetylkynuramine (220), an oxidative degradation product of the bacterial metabolite N-acetyltryptamine, has now been isolated for the first time from a microorganism (Janibacter limosus Hel 1 from the German North Sea). From the same strain, the isoquinoline helquinolin (221) was isolated, which showed a selective activity against Gram-positive bacteria (Asolkar et al., 2004).

Methyl quinoline-2-one-4-carboxylate (222) was isolated together with nicotinamide (223) from a bacterium of the free water column Hel59b (Shaaban, 2004); other quinolones have been described previously (Bultel-Ponce et al., 1999). The yellow 2-methyl-pyrimidine-5-carboxamide 224 has been isolated from another unidentified marine bacterium (Thorwest et al., 2001). Related structures are part of the bleomycins and the boxazomycins; 224 is also a sub-structure of vitamin B₁.

A Halomonas sp. (strain RK 3771) from the North Sea turned out to be a talented producer of many amino acid-derived metabolites, e.g. isatin and some known indole derivatives, diketopiperazines, various new 2-aminophenoxazones (e.g. 225), and – for the first time from nature – 7-hydroxy-2H-benzo[1,4]thiazin-3-one (226) and the previously undescribed 3,4-di-(4'-hydroxyphenyl)pyrrole-2,5-dicarboxylic acid (227) (Liang, pers. communication). The unusual acetal 228 has been obtained from Vibrio angustum S14 (de Nys et al., 2001).
Feeding experiments of *Alteromonas violaceus* with [3,5-13C₂]p-hydroxybenzoic acid or [2-13C]proline resulted in a pentabromopseudilin (2) with a symmetrical isotope distribution on C-1',3' and C-2,5, respectively, which confirmed that also the pyrrole precursor must be symmetrical (Laatsch *et al.*, 1994; Peschke *et al.*, 2005). If both rings are connected via phenol oxidation, also the symmetrical dimers should be obtained, and this is the case, indeed. While hexabromobipyrrrole (229) is known for long (Burckholder *et al.*, 1966; Hanessian *et al.*, 1966; Lovel *et al.*, 1966), feeding experiments delivered now also the brominated biphenol 230 (Laatsch, unpublished results). This compound has recently been described from *Pseudoalteromonas phenolica* sp. nov. O-BC30T and was shown to have a remarkable activity against a methicillin-resistant *Staphylococcus aureus* strain (Isnansetyo *et al.*, 2003). Monomeric halogen phenols like 231 and 232 have been obtained only in feeding experiments with *Alteromonas luteo-violaceus* or from algae so far (Laatsch, unpublished results).

![Chemical Structures](image1)

While the characteristic earthy odour of actinomycetes is due to geosmin, the often very unpleasant smell of other bacteria has not found much attention. Dickschat has performed head space analyses of marine *Cytophaga* spp. and detected a number of mostly unsaturated methyl ketones 233 - 236 by GC/MS (Dickschat, 2003). Interestingly, most of these compounds were also found in the flavour of the gliding bacterium *Myxococcus xanthus*.

![Chemical Structures](image2)

It is obvious from the summary above, that in compounds from bacteria other than actinomycetales, especially those of the free water column or the German Wadden Sea, peptides and other aliphatic compounds dominate. Certain other structures like quinones or polyenes are extremely rare, not only in the recent past. Most compounds are of low molecular weight and cover a wide polarity range, as metabolic profiling by HPLC/MS showed (Fig. 4). In contrast, extracts of marine streptomycetes for comparison showed masses in the range of 400-1200, a narrow polarity profile and less background noise.
Bacteria of the Polar Sea Ice and Other Cold Habitats

It has been discussed above that the complexity of metabolite patterns of bacteria may decrease rapidly, if the environmental pressure is reduced, e.g. by repeated sub-cultivation in a monoculture. Oppositely, certain metabolites may be formed only in the presence of trigger substances, a situation which was interpreted as a chemical defence in the case of antibiotics, but can also be understood as a vivid chemical communication in life communities. Further examples were reported recently (Yakovleva et al., 1986). These mutualistic and commensal interactions can be expected in megadiversity places (hot spots like the Great Barrier Reef), however, dense bacterial populations were found surprisingly also in the sea ice of the polar regions (Staley et al., 1999).

A few antarctic isolates from rocky grounds have already been chemically investigated, and compounds, which resemble plastic additives have been obtained (see below). Bacteria from polar sea ice, however, have never been chemically investigated before. Other than tap water, freezing sea water is not forming a dense matter, but a sponge-like skeleton of ice crystals with brine channels in between, which are in connection with the sea water on the bottom. This material is translucent enough that in a depth of about 2 m below the surface a dense population of algae is found. The chlorophyll concentration may be higher than 2000 mg/m³, which is even more than in tropical oceans at algal blooms (Spindler et al., 1991).

These green or brown microalgae and diatoms are associated with a high variety of morphologically unique bacteria, which had not been inspected chemically before. The ratio of viable to total bacterial counts is extraordinarily high with sea ice samples. Percentages up to 80% could be determined with some Antarctic winter sea ice samples (Helmke et al., 1995). Furthermore most of the bacterial sea ice diversity turned out to be cultivatable (Brinkmeyer et al., 2003).

About 90% of the polar sea-ice bacteria are strictly cold adapted (psychrophilic) (Helmke et al., 1995) and must be handled and maintained permanently at temperatures below 10 °C. Their growth optima are at about 10 to 15 °C (Helmke et al., 2004); generation times can vary from about 5 days at -5 °C up to about 10 h at 10 °C. The mass fermentation which is needed if isolation and structure elucidation of metabolites are intended, required growth periods of

Figure 4. 3D-HPLC/MS profile of extracts from the North Sea bacterium Hel53 (left) and a marine streptomycete isolate (right)
4-6 weeks therefore. The temperature range of the psychrotolerant bacterial component is clearly broader and extends from about -5°C up to more than 30°C.

Most metabolites isolated so far from sea ice bacteria were obviously derived from the amino acid pathways: Simple indoles like tyramine, tyrosol, N-acetyl tryptamine, and again N-acetyl kynuramine (220), were isolated (Schroeder, 2002). Unique metabolites or metabolic capabilities, however, were rare. A very recent example of potent capabilities was an isolate of a flavobacterium T436 (gene bank accession no. AF 468417) from Arctic sea ice, which showed 98% homology with the 16S rRNA of Salegentibacter holothuriorum and formed an unprecedented variety of more than 20 nitro and dinitro compounds (237a-d to 246) (Schuhmann, 2005). Some of these metabolites had already been isolated from other sources or were synthesized, but were never found naturally in this complexity. Of special interest are the halogenated nitro compounds 242e and 242f which indicate that the producer possesses also halogenases or haloperoxidases. If the tryptamine derivative 246 is formed via an aminopyrrole precursor related to 247, or by cyclization of 245, has to be further explored.

Nothing is known about the biosynthesis of these compounds so far, and it cannot be excluded that they are formed by oxidation of amino precursors, similarly as in pyrrolnitrin (247) or chloramphenicol. The formation of 244a may indicate that also another pathway exists: the nitration of a nitrogen-free precursor 244b with inorganic nitrate as the nitrogen source as in 248 (Carter et al., 1987, 1989). The daidzein (244b) needed for this reaction is commonly present in the culture broth if soybean flour or malt extract are used for fermentation.
It has also to be further investigated if the nitro compounds could be formed by oxidation of the corresponding nitroso precursors. Nitrosophenols like 249 have been isolated from different bacterial sources and were shown to be iron chelators. It can be assumed that in the case of *Flavobacterium* sp. T436, precursors of the nitro compounds were synthesized for the same purpose, however, were oxidized by haloperoxidases.

Prenylated ubiquinones are very widespread among microorganisms and may serve as taxonomic markers. Side chains with a terminal acetone fragment as in 251 and 250 from *Pseudoalteromonas* sp. T268 (99% homology of 16S rRNA) are, however, very unusual (Schuhmann, 2005).

From the same strain, the benzoquinone 252 and the indolones 253a and 253b were isolated (Schuhmann, 2005). The latter had been obtained by Fenical (2001) before and is also formed easily in a solution of isatin (4) in acetone at room temperature.

Streptomycetes are rare in the polar habitats and seems to be isolated only from solid supports. Recent examples from Antarctica are the glaciapyrroles A-C (254-256) from a *Streptomyces* sp. NPS 008187 (Macherla *et al*., 2005). The conjugated pyrroloketone fragment is very rare amongst microbial products and was found so far only embedded in two few cytochalasans or as substructure of condensed ring systems.
Microbiaeratin (257) is the acetate an antibiotic TM-64 previously isolated from a *Thermoactinomyces* sp. The isolation from penguin excrements explains that the strain is thermophilic with an optimal growth temperature at 45 °C (Ivanova *et al.*, 2005).

**Bacterial Products of Uncertain Biogenetic Origin**

In most cases, for the mass fermentation of microorganisms complex media are used. It is obvious that also some of their constituents may be isolated and miss-interpreted as bacterial products, or will be modified in the bacterial metabolism as in a precursor-directed biosynthesis. Fats and peptides from fish meal, and the isoflavones daidzein (244b) and genistein from soybean flour are well-known examples; 7-O-methylgenisteine (258) from *Streptomyces* sp. B 4244 may result from such a precursor (Maskey *et al.*, 2003c).

Another group of frequently isolated compounds are the diketopiperazines. Most microbial examples contain proline or hydroxyproline, and as these amino acids occur in a high concentration in meat extract and as also these cyclodipeptides are already formed during sterilisation of the culture medium, some may be artefacts. On the other hand, even in recent articles (Trischmann *et al.*, 2004; De Rosa *et al.*, 2003) a pronounced and selective antibiotic activity is claimed which, however, could not be confirmed in all cases with material from other sources or synthesis.

It has been shown now that the D,D-diketopiperazines may have a high selective antibiotic activity, while the L,L-derivatives do not (Fdhila *et al.*, 2003). The D,D-piperazinediones 259 - 260 from marine bacteria were found to be highly active against *Vibrio anguillarum*. In most other cases, the configuration of these diketopiperazines has not been determined.
In the period of this report, several other compounds have been reported which may have an abiotic origin due to non-enzymatic reactions during work-up, modification of nutrient components or even pollution with xenobiotics as in the case of dioctyl phthalate. A biosynthetic origin cannot be excluded in all cases, however has to be proven carefully by further experiments, best by investigation of the biosynthesis.

The arctic *Streptomyces* sp. 1010 (Ivanova et al., 2001) contained the novel 2-amino-9,13-dimethyl heptadecanoic acid (261) and additionally the polyphenylether (262) and hexanedioic acid dioctyl ester (263). Compounds related with the latter are the many glycopeptides of the vancomycin type and especially the polyphenyl ethers isolated from brown algae by Glombitza *et al.* (1985).

Pharacine (Shaaban *et al.*, 2002) (264), a cyclic terephthalate, was isolated as a trace component from *Cytophaga* sp. AM13.1. It was previously known as a degradation product of certain polyesters, where it is found as mixture with higher homologues. Although the fermentation result was reproducible, only one further strain formed 264 so far, and higher oligomers as by the pyrolysis of terephthalates were not obtained, a confirmation as a natural product would need biosynthetic investigations.

A case of its own is the isolation of the indole derivatives 265 - 267 from *Vibrio para-haemolyticus* Bio249 (Veluri *et al.*, 2003). All these compounds are formal condensation products of simple aliphatic aldehydes or ketones with indole, and the ketone 266 e.g. is obtained indeed easily by reaction of indole with diacetyl. Indole is a main product of the isolate Bio249, however, some of the condensation products (but not all) are already present in fresh extracts from agar plates.

\[
R = i-\text{Bu} \\
R = 2-\text{Bu} \\
R = \text{Bzl}
\]
Unexpected is the unequivocal identification of N-phenyl-1-naphthylamine (271) as a natural product from a streptomycete B8335 (Shaaban, 2004). This compound has been used as an antioxidant and fluorescent dye in tissue cultures, however, occurs only a second time in nature as a substructure in 10-hydroxy-18-N-1-naphthyl-N-phenylaminobetaenone (272) (Ebel, 2002); also the isomeric 2-naphthyl-N-phenylaminobetaenon has been described (Brauers et al., 2000).

During the past four years, nearly 250 new metabolites have been isolated from marine bacteria, and many of them have been patented due to their relevant biological activities. Interestingly, in the same period much less compounds have been published from bacteria of terrestrial origin. This relation confirms again the importance of the marine habitat for natural product chemistry and is a strong incentive for further investigations.

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