may play a crucial role in controlling Listeria in T cell incompetent mice.

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Brominated diphenyl ethers from a marine bacterium associated with the sponge *Dysidea* sp.

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Summary. Bacteria *Vibrio* sp. isolated from the sponge *Dysidea* sp. were shown to biosynthesize brominated diphenyl ethers. We identified one of the bacterial brominated metabolites, using gas liquid chromatography and mass spectrometry to compare this product with standard 3,5-dibromo-2-(3',5'-dibromo-2'-methoxyphenoxy)phenol. The latter has been isolated from ethanol extracts of the sponge *Dysidea* sp.
Key words. Marine bacteria; sponges; *Dysidea sp.*

In recent years, several reports have appeared in the literature about the microbial biosynthesis of some metabolites which had earlier been isolated from marine macroorganisms[1-3]. In the present work we show that symbiotic microorganisms of the sponge *Dysidea* sp. can synthesize physiologically active compounds which belong to the group of brominated diphenyl ethers. To identify brominated bacterial metabolites we employed tetrabrominated diphenyl ether **1**, isolated from the ethanol extract of the sponge *Dysidea* sp. as a standard. The H^1 and C^{13} NMR spectra of **1** were identical to those of 3,5-dibromo-2-(3',5'-dibromo-2'-methoxyphenoxy)phenol from *Dysidea fragilis*[4].

Eight pure bacterial cultures were isolated from two specimens of *Dysidea* sp. collected near the islands Tutuila and Ofu (Eastern Samoa) in June 1989, during the 9th cruise of the R/V "Akademik Oparin". All the bacteria isolated were grown during 96–100 h at 30 °C in a thermostat in 1-l flasks with 500 ml of medium (peptone 5 g, yeast extracts 2.5 g, $MgSO_4 \cdot 7H_2O$ 0.1 g, seawater 1 l). Evaporated butanol extracts from culture broths were tested for the presence of brominated metabolites, using negative ion mass spectrometry (NIMS) with direct inlet for detection of characteristic ions of Br at m/z 79 and 81. Thus the presence of brominated compounds was demonstrated in the culture broths of two bacterial strains of the genus *Vibrio*. One of these strains, KMM 9-81-1, was cultivated under the above conditions.

The filtered culture broth was extracted by n-butanol and the butanolic extract evaporated in vacuo. The dry concentrate was fractionated in the system: water:ethanol:chloroform (1:1:1). Substances that gave a color reaction with diazotized benzidine, characteristic for phenol compounds, were present in the chloroform

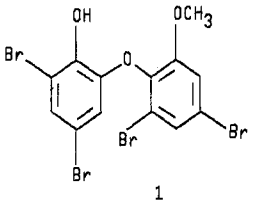

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Figure 1. 3,5-dibromo-2-(3',5'-dibromo-2'-methoxyphenoxy)phenol **1** from the sponge *Dysidea* sp. and from bacterium *Vibrio* sp.

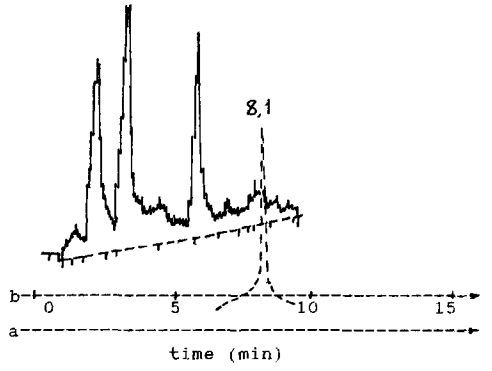

Figure 2. Total ion current for brominated diphenyl ether **1**, (a), and the fraction containing brominated derivatives (b), chromatographed on SE-54 (25 × 0.32 mm) programmed at 8 °C/min from 200 °C to 280 °C.

layer. After evaporation of the chloroform layer, the residue was subjected to chromatography on silica gel with chloroform: ethylacetate (20:1). The fractions were tested for the presence of brominated compounds with the help of negative ion mass spectrometry (Electron Impact 70 eV). The chosen fractions containing brominated derivatives were pooled and purified on Sephadex LH-20 with chloroform-ethanol (7:1). The purified fraction was analyzed by GLS-MS in positive ion mode (EI-70 eV), using a solventless injector for the chromatogram registration.

The chromatogram showed a lot of peaks, including peaks in the expected region, and the exact definition of the peaks conforming to brominated compounds is very difficult (fig. 2). Therefore the same fraction was analyzed by GLC-MS in resonance electron capture mode (REC GLC-MS) at an electron energy related to the maximum yield of ion current of standard **1** (about

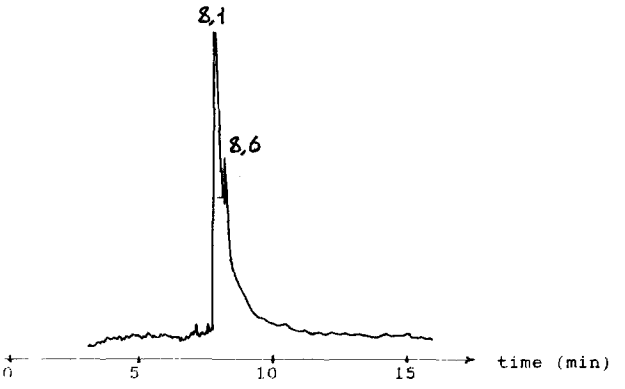

Figure 3. Total ion current (REC) for the fraction containing brominated derivatives and chromatographed on SE-54 (25 m × 0.32 mm) programmed at 8 °C/min from 200 °C to 280 °C.

4 ev; energy is stated before experiment by inserting **1** using the direct inlet system). Using this method, only two peaks were detected on the chromatogram. The retention time of the first of them coincides with that of **1** (fig. 3).

In the second GLC-MS experiment the mass spectra (EI) of two compounds which had retention times of 8.1 and 8.6 min, were recorded. The mass spectrum of the first compound was identical to the spectrum of the standard tetrabrominated diphenyl ether **1** (m/z 536(16), 534(65), 532(100), 530(70), 528(18), 440(14), 438(42), 436(43), 434(15), 374(9), 372(17), 370(8). The second compound has the molecular mass of 604. The multiplet nature of its spectrum in the molecular ion region (m/z 608(16), 606(65), 604(100), 602(70), 600(18)), indicates that the second substance contains four bromine atoms.

Thus, it was shown for the first time that bacteria living in association with the sponge *Dysidea* sp. are responsible for the production of brominated diphenyl ethers.

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