thick, and oriented parallel to (010) was prepared from the olivine crystal (composition determined by microprobe analysis). Examination of the sample by optical microscopy before the experiment revealed no inclusions or heterogeneities other than subboundaries, in agreement with the observations of S. H. Kirby and M. W. Wegner [*Trans. Am. Geophys. Union* **54**, 452 (1973)] and M. W. Wegner and J. M. Christie [*Contrib. Mineral. Petrol.* **43**, 195 (1971)] on olivine from the same locality. The disk was encased in a stainless steel container which included "momentum trap" plates [D. G. Doran and R. K. Linde, *Solid State Phys.* **19**, 262 (1966)], and the container was impacted with a 16-mm, tungsten flyer-plate launched from a high-performance propellant gun.
9. We calculated the peak pressure, using standard impedance match techniques for multiple shock reverberations [J. M. Walsh and R. H. Christian, *Phys. Rev.* **97**, 1544 (1955); L. V. Al'tshuler, *Sov. Phys. Usp.* **8**, 59 (1965); R. V. Gibbons and T. J. Ahrens, *J. Geophys. Res.* **76**, 5489 (1971)] based on the Hugoniot equation-of-state data (*11*) for tungsten and stainless steel, and it is accurate to within about 5 percent. The time at peak pressure is based on a calculation of the type given by G. R. Fowles [*J. Appl. Phys.* **31**, 655 (1960)] and by Al'tshuler (see above).
10. Carried out with the 1-Mev electron microscope at the U.S. Steel Research Laboratory, Monroeville, Pa., and at 200 kv at the U.S. Geological Survey, Reston, Va.
11. R. G. McQueen, S. P. Marsh, J. W. Taylor, J. N. Fritz, W. J. Carter, in *High Velocity Impact Phenomena*, R. Kinslow, Ed. (Academic Press, New York, 1970), p. 294.
12. We made the simplifying assumption that the temperature in the sample which has attained peak pressure by multiple reverberations is approximately the same as the temperature of the container achieved by a single shock to peak pressure.
13. T. J. Ahrens, F. D. Tsay, D. H. Live, *Geochim. Cosmochim. Acta* (Suppl. 7) (1976), p. 1143. This is a maximum estimate since it is for the attainment of peak pressure by a single shock [see L. V. Al'tshuler (*9*)].
14. T. J. Ahrens and J. D. O'Keefe, *Moon* **4**, 214 (1973).
15. D. R. Waldbaum, *Nature (London)* **232**, 545 (1971).
16. N. L. Bowen and J. F. Schairer, *Am. J. Sci.* **29**, 161 (1935).
17. E. C. T. Chao, in *Researches in Geochemistry*, P. Abelson, Ed. (Wiley, New York, 1967), vol. 2, p. 204. Note that thetomorphic and diaplectic are equivalent terms.
18. T. J. Ahrens, J. H. Lower, P. L. Lagus, *J. Geophys. Res.* **76**, 518 (1971); T. J. Ahrens and C. F. Petersen, in *The Application of Modern Physics to the Earth and Planetary Interiors*, S. K. Runcorn, Ed. (Wiley-Interscience, New York, 1969), p. 449. The Hugoniot data are consistent with results from static, high-pressure experiments [L. Liu, *Nature (London)* **262**, 770 (1976); *Geophys. Res. Lett.* **2**, 417 (1975); *Nature (London)* **258**, 510 (1975)] which indicate major, reconstructive transformations at these pressures in magnesian olivine, for example, to $(Mg,Fe)SiO_3$ perovskite + (Mg,Fe)O rock salt phases [see R. Jeanloz and T. J. Ahrens, in *High Pressure Research: Applications to Geophysics*, M. Manghnani and S. Akimoto, Eds. (Academic Press, New York, 1977), p. 439]. Direct evidence for similar major, reconstructive transformations and reactions in nesosilicates under shock can be found in L. Liu [*Earth Planet. Sci. Lett.* **26**, 425 (1975)], T. J. Ahrens and E. K. Graham [*ibid.* **14**, 87 (1972)], and H. Schneider and U. Hornemann [*Naturwissenschaften* **62**, 296 (1975)].
19. See B. J. Skinner and J. J. Fahey, *J. Geophys. Res.* **68**, 5595 (1963), and references therein.
20. L. Liu and A. E. Ringwood, *Earth Planet. Sci. Lett.* **28**, 209 (1975); A. E. Ringwood and A. Major, *ibid.* **12**, 411 (1971). In these experiments, $CaSiO_3$ perovskite formed (and identified) at high pressure could only be quenched to a glass.
21. The free energy of a crystal is increased by an amount of the order of $1/2\ \mu b^3$ per atomic length of dislocation (where μ is the shear modulus and b is the average length of the Burgers vectors of the dislocations). Taking the dislocation density to be about 10^{12} cm^{-2} or more in the tangles, $b \sim 5$ Å, $\mu = 80 \times 10^9$ pascals, and assuming that a typical difference in free energy between silicate crystals and their glasses is about 5 kilojoules per mole, it is possible for the glass to have a lower free energy than the dislocated crystal. See also R. M. J. Cotterill, E. J. Jensen, W. Damgaard Kristensen, and R. Paetsch [*J. Phys. (Paris)* **36**, C2 (1975)].
22. D. E. Grady, in *High-Pressure Research: Applications to Geophysics*, M. Manghnani and S. Akimoto, Eds. (Academic Press, New York, 1977), p. 389; ———, W. J. Murri, P. S. DeCarli, *J. Geophys. Res.* **80**, 4857 (1975); D. E. Grady and W. J. Murri, *Geophys. Res. Lett.* **3**, 472 (1976).
23. R. M. J. Cotterill, *Phys. Lett.* **60A**, 61 (1977).
24. We are grateful for discussions with I. Jackson and L. Liu, and for reviews from A. Albee and D. Grady, all of which were very helpful. This research was supported by NASA grant NGL 05-002-105 and contract NAS9-14749 (U.S. Steel Research Laboratory). Contribution No. 2889, Division of Geological and Planetary Sciences, California Institute of Technology.

24 March 1977; revised 25 April 1977

Polychlorinated Biphenyls: Penetration into the Deep Ocean by Zooplankton Fecal Pellet Transport

Abstract. *High concentrations of polychlorinated biphenyls (PCB's) were found in fecal pellets from natural populations of euphausiids collected in the Ligurian Sea. Since biogenic particulate products, especially fecal pellets, are known to sink rapidly and intact to the ocean bottom, the transport of PCB's by such sinking particles could be an important mechanism which contributes to the penetration of PCB's into the deep sea.*

The rate of vertical mixing in the ocean (*1*) is too slow to account for the quantities of polychlorinated biphenyls (PCB's) found in Atlantic and Mediterranean abyssal sediments (*2*) if one assumes that they penetrate into the deep ocean only in the dissolved state. An alternative explanation is that PCB's are carried to the sediments by rapidly sinking particles. Several investigators have hypothesized that sinking biogenic material such as zooplankton fecal pellets and molts may accelerate the downward vertical transport of certain metals and radionuclides (*3*). Analytical work has shown that both euphausiid fecal pellets and molts contain significant quantities of trace elements (*4, 5*) and radionuclides (*6*); therefore, the sinking of these particulate products, especially fecal pellets, is strongly implicated in the removal of many such elements from surface waters. Experimentally determined sinking rates of several hundred meters per day (*7–9*) for zooplankton fecal pellets and molts are compatible with the hypothesis that these products could act as effective conveyors of surface-introduced pollutants into the deep sea. Recent field studies (*9, 10*) have shown conclusively that zooplankton fecal pellets not only penetrate to great depth (2000 to 4000 m) intact but also in large numbers—650 pellets per square meter per day. We have found that freshly released euphausiid fecal pellets collected from natural populations contain relatively high concentrations of PCB's, and we propose that such biogenic particles make a significant contribution to the vertical transport of PCB's in the ocean.

The euphausiid *Meganyctiphanes norvegica* was examined since it is an abundant member of the zooplankton community in the western Mediterranean and North Atlantic and sufficient biological data on the production rate of particulate products have been compiled for this species (*4, 11*). During 1974–1975 *M. norvegica* and the microplankton which serve as its food were collected at a station 5 km off Villefranche-sur-Mer, France. We fished for euphausiids and microplankton at night with an Isaacs-Kidd mid-water trawl and a 1-m plankton net (76-μm mesh aperture), respectively, by making several short oblique tows between 100 m and the surface. All microplankton samples were carefully examined and found to be free from adhering tar, oil, and paint chips.

Glass and metal implements cleaned in advance were used to sort euphausiids from other species in order to avoid contaminating the samples. Euphausiid fecal pellets and molts were collected on board and later in the laboratory by methods described elsewhere (*11, 12*) with the exception that all containers were either glass or metal. Although extreme care was taken to avoid unnecessary contact between the samples and plastic materials, some contact inevitably occurred between the organisms and the nylon plankton nets. Cross-contamination of PCB's between plankton and nylon nets can occur (*13*); however, extraction of the nets used in our study indicated that PCB contamination from this material was negligible.

Samples were analyzed by standard procedures (*14*) with modifications to adapt to small sample quantities. All samples were frozen, lyophilized, and pulverized in preparation for extraction. The relatively abundant samples, such as microplankton (0.1 to 1.5 g dry) and euphausiids (1 to 8 g dry), were subjected

to extraction with hexane in a Soxhlet extractor (8 hours at four cycles per hour). We batch-extracted the less abundant samples, fecal pellets (15 to 90 mg dry) and molts (50 to 150 mg dry), by shaking with hexane in sealed, conical-bottomed, centrifuge tubes. We removed the extracts by first centrifuging to settle the sample residue and then transferring the supernatant solvent with the aid of a Pasteur pipette. Each sample was extracted ten times. Interfering substances were eliminated from the hexane extracts by chromatography on Florisil and by treatment with concentrated H_2SO_4. It was not necessary to remove fatty substances by acetonitrile-hexane partitioning. Hexane extracts were then further concentrated to a convenient volume (usually 0.2 to 0.5 ml) in a Kuderna-Danish concentrator and analyzed by gas chromatography. Precautions were employed to ensure that contamination from chemicals, apparatus, and glassware was negligible (*15*). During the November 1974 collection, a surface water sample was also collected and analyzed for PCB's according to the method of Harvey and Steinhauer (*16*). The PCB concentration (DP-5) was 2.5 ng/liter, a value that is considered representative for these waters (*17*).

Concentrations of PCB's in euphausiid bodies, molts (*18*), and fecal pellets as well as in the microplankton upon which they feed are given in Table 1. Fecal pellets contained the highest concentrations of PCB's in all samples examined. The PCB concentration in feces (wet weight) from the November 1974 collection was 1.5×10^6 greater than that present in the surrounding water. Furthermore, the PCB concentrations in feces (dry weight) ranged from 3.5 to 21 times higher than those in the food organisms which formed the feces. A similar concentrating process has been found to occur for several trace elements and radionuclides in the same species (*4–6*).

Recently, estimates have been made of PCB deposition rates into sediments in the northwestern Mediterranean region (*19*). These estimates are based on sediment concentrations in a series of cores taken at a depth of 400 m off the coast of Nice, France. Computed values range from approximately 80 to 125 μg of PCB (DP-5) per square meter per year over a period of 15 to 20 years.

It is conceivable that PCB's could be transported to sediments by way of fecal pellets, molts, eggs, and carcasses released from an overlying euphausiid population. Molting, egg laying, and death (carcass production) in euphausiids are discontinuous processes compared to defecation. Hence, the production rates for these products are much lower than that for fecal pellets (*4, 8*). This lower production rate and the fact that PCB concentrations in molts and whole euphausiids appear to be much lower than corresponding concentrations in feces indicate that defecation should be far more effective in removing PCB's from surface waters than the discontinuous processes. Accordingly, we have attempted to calculate roughly how much of the total vertical flux of PCB's into sediments in this area could be attributed to sinking fecal pellets from an overlying population of *M. norvegica*. Production rates of fecal pellets for this euphausiid have been found to range between approximately 1.8 and 5.1 percent of the organism's dry body weight per day, depending on the available food supply (*4*). From the data of Franqueville (*20*), we have computed that the annual average biomass of *M. norvegica* in the Ligurian Sea is approximately 0.17 mg (dry weight) per cubic meter. Coupling these data with the mean fecal pellet PCB concentration [$\simeq$ 17,000 μg/kg (dry weight)] for the three sampling periods shown in Table 1 leads to values for fecal-released PCB's ranging from 5.2×10^{-5} to 15×10^{-5} μg m^{-3} day^{-1}. If these data are integrated over the photic zone (top 75 m) where the majority of the fecal pellets are released during the time the euphausiids are actively feeding, we obtain delivery rates of PCB's to sediments of 1.4 to 4.1 μg m^{-2} year^{-1}, which are between one and two orders of magnitude lower than estimates based on deposition by all routes. However, measurements of zooplankton biomass (*20, 21*) made in the same region indicate that *M. norvegica* comprises only 1 to 5 percent of the total zooplankton biomass. If PCB concentrations in feces and fecal pellet production rates measured in *M. norvegica* are typical of those for other zooplankton species (notably copepods, which probably form the bulk of the zooplankton biomass), the delivery rates arising from zooplankton defecation will approach those based on PCB concentrations in the sediments. Our calculations are admittedly crude because of the limited data available and are subject to many assumptions that may not be entirely valid; nevertheless, the close similarity between PCB flux rates derived from fecal pellet data and those based on sediment values suggests that sinking zooplankton fecal pellets contribute significantly to the downward vertical transport of surface-introduced PCB compounds.

Table 1. Polychlorinated biphenyls (*15*) in euphausiids (*Meganyctiphanes norvegica*), their particulate products, and microplankton which serve as the euphausiids' food. Water sampled during the November 1974 collection contained 2.5 ng of PCB per liter.

Sample	Ratio of wet weight to dry weight	DP-5 [μg/kg (dry weight)]
November 1974		
Whole animal	4.7	620
Molts	4.6	1,400
Fecal pellets	4.4	16,000
Microplankton*	10.7	4,500
January 1975		
Whole animal†		260, 290
Molts		170
Fecal pellets		4,800
March 1975		
Whole animal		38
Molts		Not detectable
Fecal pellets†		11,000; 38,000
Microplankton*		1,800

*Principally copepods, phytoplankton, and detritus. †Two separate samples.

D. L. ELDER
SCOTT W. FOWLER
International Laboratory of Marine Radioactivity, Musée Océanographique, Principauté de Monaco

References and Notes

1. W. S. Broecker, *Chemical Oceanography* (Harcourt Brace Jovanovich, New York, 1974), p. 59.
2. G. R. Harvey and W. G. Steinhauer, in *Environmental Biogeochemistry*, J. O. Nriagu, Ed. (Ann Arbor Science Publishers, Ann Arbor, Mich., 1976), vol. 1, p. 203; D. L. Elder, J. P. Villeneuve, P. Parsi, G. R. Harvey, in *Activities of the International Laboratory of Marine Radioactivity, 1976 Report* (International Atomic Energy Agency, Vienna, 1976), p. 136.
3. C. Osterberg, A. G. Carey, H. Curl, *Nature (London)* **200**, 1276 (1963); F. G. Lowman, T. R. Rice, F. A. Richards, in *Radioactivity in the Marine Environment* (National Academy of Sciences, Washington, D.C., 1971), p. → J. H. Martin, *Limnol. Oceanogr.* **15**, 756 (1970); S. Krishnaswami and M. M. Sarin, *Earth Planet. Sci. Lett.* **32**, 430 (1976); S. Krishnaswami, D. Lal, B. L. Somayajulu, R. F. Weiss, H. Craig, *ibid.*, p. 420; M. P. Bacon, D. W. Spencer, P. G. Brewer, *ibid.*, p. 277; Y. Nozaki, J. Thompson, K. K. Turekian, *ibid.*, p. 304.
4. L. F. Small, S. W. Fowler, S. Keckes, in *Radioactive Contamination of the Marine Environment* (International Atomic Energy Agency, Vienna, 1973), p. 437.
5. G. Benayoun, S. W. Fowler, B. Oregioni, *Mar. Biol.* **27**, 205 (1974); S. W. Fowler and B. Oregioni, in *Activities of the International Laboratory of Marine Radioactivity, 1974 Report* (International Atomic Energy Agency, Vienna, 1974), p. 55; S. W. Fowler and G. Benayoun, *Mar. Sci. Commun.* **2**, 43 (1976).
6. R. D. Cherry, S. W. Fowler, T. M. Beasley, M. Heyraud, *Mar. Chem.* **3**, 105 (1975); M. Heyraud, S. W. Fowler, T. M. Beasley, R. D. Cherry, *Mar. Biol.* **34**, 127 (1976); J. J. W. Higgo, R. D. Cherry, M. Heyraud, S. W. Fowler, *Nature (London)* **266**, 623 (1977).
→ T. J. Smayda, *Mar. Geol.* **11**, 105 (19 → *Limnol. Oceanogr.* **14**, 621 (19 → S. W. Fowler and L. F. Small, *ibid.* **17**, 293 (1972).
8. L. F. Small and S. W. Fowler, *Mar. Biol.* **18**, 284 (1973).
9. P. H. Wiebe, S. H. Boyd, C. Winget, *J. Mar. Res.* **34**, 341 (1976).
10. H.-J. Schrader, *Science* **174**, 55 (1971).
11. S. W. Fowler, G. Benayoun, L. F. Small, *Thalassia Jugosl.* **7**, 35 (1971); S. W. Fowler, L. F. Small, S. Keckes, *Mar. Biol.* **11**, 45 (1971).
12. J. La Rosa, *Deep-Sea Res.* **23**, 995 (1976).

13. G. R. Harvey and J. M. Teal, *Bull. Environ. Contam. Toxicol.* **9**, 287 (1973).
14. *Pesticide Analytical Manual* (U.S. Department of Health, Education, and Welfare, Food and Drug Administration, Rockville, Md., 1968), vol. 1
15. The PCB content of routine procedural blanks was measured concurrently with that of samples; the results were always less than 10 percent of the sample values. We carried out the quantification by relating strip chart peak heights produced by samples to those of a standard solution of Phenoclor DP-5. In the calculations we used the sum of the heights of the six major peaks.
16. G. R. Harvey and W. G. Steinhauer, *J. Mar. Res.* **34**, 562 (1976).
17. D. L. Elder, *Mar. Pollut. Bull.* **7**, 63 (1976); _____ and J. P. Villeneuve, *ibid.* **8**, 19 (1977).
18. The relationship between the PCB concentration in whole bodies and molts was not consistent for the three collections. In November 1974 PCB concentrations in molts were relatively high. Molts represent about 8 percent of the euphausiid's dry weight; therefore, it can be calculated that approximately 18 percent of the euphausiid's PCB body burden was associated with the outer surface of the animals collected at that time. On the other hand, the fraction associated with molts collected in January 1975 was only about 5 percent. The fluctuation in the relationship between the concentrations in whole animals and molts could be explained if the predominant route of accumulation of PCB's in the exoskeleton is by surface adsorption from the surrounding water. This mode of uptake is thought to play an important role in the accumulation of organochlorine compounds by euphausiids and other small aquatic invertebrates [see J. L. Cox, *Fish. Bull.* **69**, 627 (1971); R. F. Addison, in *Effects of Pollutants on Aquatic Organisms*, A. P. M. Lockwood, Ed. (Cambridge Univ. Press, Cambridge, England, 1976), p. 127]. If this is the case, then significant short-term changes in PCB concentrations in the water would be more closely reflected by PCB concentrations in the molts than those in whole bodies which include tissues not in direct contact with the water. On the other hand, whole animal PCB concentrations should be more representative of long-term integrated PCB concentrations in the water mass or in the food, or both.
19. D. L. Elder and G. R. Harvey, in preparation.
20. C. Franqueville, *Tethys* **6**, 741 (1975).
21. P. Nival, S. Nival, A. Thiriot, *Mar. Biol.* **31**, 249 (1975).
22. The International Laboratory of Marine Radioactivity operates under a tripartite agreement between the International Atomic Energy Agency, the government of the Principality of Monaco, and the Oceanographique Institute at Monaco. This research was funded in part by United Nations Environment Program contracts FP/053-76-09(978) and 0800-74-007. We thank Station Zoologique de Villefranche, France, for ship time and assistance in sampling. We thank Drs. C. Osterberg, R. Fukai, and R. D. Cherry for critical comments on the manuscript.

9 February 1977; revised 30 March 1977

Primary Bioassay of Human Tumor Stem Cells

Abstract. *A simple method has been developed to support human tumor stem cell colony growth in soft agar. The technique appears suitable for culture of a variety of neoplasms of differing histopathology. Tumor stem cell colonies arising from different types of cancer have differing growth characteristics and colony morphology. This bioassay should be suitable for clinical studies of effects of anticancer drugs or irradiation on human tumor stem cells.*

Tumor stem cells are the cell renewal source of a neoplasm and also serve as the seeds of metastatic spread of cancer. Studies of transplantable tumors in animals indicate that tumor stem cell colony-forming assays (in vivo or in vitro) can be used to study the biological properties of these cells and to delineate differences in individual sensitivity to a variety of chemotherapeutic agents (*1*, *2*). For example, the development of an in vitro colony-forming assay for stem cells from transplantable mouse myeloma (*3*) (a plasma cell neoplasm) permitted detailed analysis of the effects of anticancer drugs in vitro, and the assays are predictive of therapeutic responses even in animals with advanced mouse myeloma (*1*).

The ability to grow colonies from primary tumor cell explants in semisolid culture media has even greater potential application. Unfortunately, primary explantation of human tumors for colony formation in vitro has met with little success. One major problem has been the creation of an environment that gives tumor cells a selective advantage over normal cells. Several investigators have had occasional success in obtaining colony growth in soft agar with pediatric solid tumors (*4*). Most recently, the effect of drugs on human stem cell colonies has been studied with the use of xenografts established in nude mice and then culturing cells from these grafts in agar in diffusion chambers intraperitoneally implanted in mice that had been irradiated (*5*). However, such multiple-step systems have not been clinically practical. A standard colony-forming assay for human tumor stem cells could be used to determine the sensitivity of tumor cells from individual patients to drugs, irradiation, and other therapeutic modalities and would permit the demonstration of resistant clones of cells in previously treated patients. The development of such a colony-forming assay seems especially important in view of evidence (*6*) indicating that the only valid measure of drug efficacy in killing an established culture of human lymphoma cells was the inhibition of their colony-forming capability.

Using the studies of mouse myeloma stem cells as a model, we developed an in vitro assay that permitted colony formation by human myeloma stem cells in soft agar and appeared to preclude growth of normal hematopoietic precursors. Using bone marrows or malignant effusions as the source of metastatic tumor cells, we subsequently proceeded to other tumor types.

A feeder layer that was analogous to those used for murine systems was required for support of human tumor colony growth (*7*, *8*). While several feeder layers proved useful for our studies on humans, the preferred one consisted of a "conditioned medium" prepared from the adherent spleen cells of BALB/c mice that had been primed with 0.2 ml of mineral oil injected intraperitoneally 4 weeks previously. The adherent cells were obtained as follows. The spleens were teased with needles to form a single-cell suspension, and 5×10^6 cells were placed in a 60-mm Falcon petri dish for 2 hours to permit the cells to adhere. The dishes were then rinsed three times in cold phosphate-buffered saline. (All tissue culture media, buffers, and serums were obtained from Grand Island Biological Co.) Cells were incubated for 3 days at 37°C in RPMI 1640 medium with 15 percent heat-inactivated fetal calf serum (*7*). The conditioned medium was decanted and centrifuged at 400*g* for 15 minutes, and the supernatant was then passed through a 0.45-μm Nalgene filter and stored at −20°C.

Bone marrow cells were collected aseptically (in preservative-free heparin) from consenting normal volunteers and patients with various neoplasms with known bone marrow involvement. Malignant ascites was collected (in heparin) by paracentesis. Red blood cells were removed from the bone marrow samples by sedimentation in 3.0 percent dextran-saline, and the supernatant bone marrow cells were collected and then washed three times in Hanks balanced salt solution with 10 percent fetal calf serum that had been inactivated by heat. The number of viable cells was determined by hemocytometer counts with trypan blue.

Cells to be tested were suspended in 0.3 percent Bacto agar (Difco) in CMRL 1066 supplemented with 20 percent horse serum, penicillin (100 unit/ml), streptomycin (2 mg/ml), glutamine (2 m*M*), $CaCl_2$ (4 m*M*), insulin (3 unit/ml), asparagine (0.1 mg/ml), and DEAE dextran (0.5 mg/ml) to yield a final concentration of 5×10^5 cell/ml. 2-Mercaptoethanol was added at a concentration of $5 \times 10^{-5} M$ immediately before the cells were plated (*9*). A portion (1 ml) of the resultant mixture was pipetted onto a 1.0-ml feeder layer (which combined 0.2 ml of conditioned medium in 0.5 percent Bacto agar) in 35-mm plastic petri dishes. Cultures were incubated at 37°C in 5 percent