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19. Subsampling RCB Cores from the Western Woodlark Basin (ODP Leg 180) for Microbiology[1]

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ABSTRACT

The existence of significant bacterial populations in deep subseafloor sediments has been established over the last decade through the analysis of samples obtained from the Ocean Drilling Program (ODP). To date, core material analyzed for a range of microbiological studies has been obtained either using advanced piston coring (APC) or extended core barrel (XCB) technology and thus has been restricted to softer sediments.

Bacteria are thought to exist much deeper than samples currently analyzed (750 meters below seafloor [mbsf]), and further investigation will require sampling and analysis of more indurated sediments obtained using rotary core barrel (RCB) technology. Unlike APC and XCB cores, RCB core samples have variable outer diameters and do not fit tightly in the plastic core liners used by ODP. Thus, the outer layers of RCB cores are open to contamination and exposure to oxygen during coring, recovery, storage, and subsequent handling.

A hydraulic cutting system was developed to obtain a sample of the "pristine" inner core of the sample under sterile, anoxic conditions. In addition, as RCB samples are too hard to use intact core techniques, samples were powdered and slurried prior to further analysis. The cutting rig worked well on indurated sediments from Leg 180, the first ODP cruise in which RCB core samples have been used for a suite of microbiological analysis (activity measurements, enrichments, and enumeration) in indurated sediments. Data from these samples demonstrate culturable anaerobic bacteria and realistic rates of anaerobic bacterial activity (sulfate reduction, methanogenesis, and thymidine in-

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corporation) in the deepest samples from the deep subseafloor biosphere analyzed to date for microbial populations (842 mbsf) and activities (800 mbsf). The new sample handling technique preserves the anoxia of the core with no evidence of contamination, and application of this new technique will permit microbiological investigation of deeper indurated deep-sea sediments.

INTRODUCTION

In just over a decade, the existence of significant bacterial populations in deep subseafloor sediments has been established (Parkes et al., 2000). This has been largely discovered through the analysis of samples obtained from the Ocean Drilling Program (ODP) (Table T1). In most cases, microbiological analysis was restricted to enumeration of the total bacterial population in small (≤5 mL) subsamples of sediment. To date, more than 1200 such samples have been analyzed. These data show that bacterial distributions in deep marine sediments generally decrease rapidly with increasing depth and conform to the model of Parkes et al. (1994), although they vary with the oceanographic setting (Parkes et al., 2000).

T1. Previous ODP/DSDP microbiological investigations, p. 10.

On four previous occasions, a far more detailed program of microbiological analysis has been conducted on intact whole-round core (WRC) samples. Subsampling was conducted with modified (luer end removed) sterile plastic 5-cm³ syringes under anaerobic and sterile conditions, inserted vertically into the WRC. Potentially contaminated sediment near the original core liner was not sampled, and insertion of sterile plugs to replace the removed sediment prevented the core from collapsing and the possible introduction of sediment from next to the liner (Parkes et al., 1995). The syringe subcores were sealed with a suba seal and then processed further. This included injection and incubation with radiotracer, which enabled activity measurements to be conducted on intact and minimally disturbed cores (Parkes et al., 1995). These techniques were employed in four detailed investigations, which studied microbiological populations and processes at the Peru Margin (Leg 112 [Parkes et al., 1990]), Japan Sea (Leg 128 [Cragg et al., 1992b]), Cascadia Margin (Leg 146 [Cragg et al., 1997]), and Blake Ridge (Leg 164, [Wellsbury et al., 2000]). Data included total bacterial populations; specific types of culturable bacteria; potential rates of sulfate reduction, methanogenesis from hydrogen/carbon dioxide or acetate; thymidine incorporation; methane oxidation; and the enrichment and isolation of unique new organisms (Bale et al., 1997; Barnes et al., 1998). The core material analyzed from all these previous legs was obtained either using advanced piston coring (APC) or extended core barrel (XCB) technology.

A range of data (see review [Parkes et al., 2000]) suggests that bacteria will be present much deeper than samples currently analyzed (750 meters below seafloor [mbsf] [Wellsbury et al., 2000]), and further investigation will require sampling and analysis of more indurated sediments. These indurated sediments are typically obtained using rotary core barrel (RCB) technology. Unlike APC and XCB cores, RCB core samples have variable outer diameters and do not fit tightly in the plastic core liners used by ODP. Thus, there is considerable potential for contamination of the outer layers of the sediment core and exposure to oxygen during coring, recovery, storage, and subsequent handling. Several problems prevent syringe subsampling of indurated RCB core sam-

ples. The sediment is too hard, and the force required to insert the syringes can split the syringes. The sediment tends to collapse during subcoring, as it is not supported by the core liner, with the probability of outer contaminated sediment being mixed into the sample. The hardness of the sediment in the syringe subcores makes it difficult to accurately inject the radioisotope because of difficulty in inserting the microsyringe needle. In addition, low porosity causes pressure to build up during injection, causing the isotope to leak out after injection and/or the microsyringe to leak.

A completely new sampling procedure had to be devised to overcome these difficulties. Homogenization of the sediment and the use of sediment slurries seemed the only realistic way forward, and this approach has been used for most other deep microbiological investigations (Chapelle and Bradley, 1996; Fredrickson and Phelps, 1997; Jones et al., 1989; Kotelnikova and Pedersen, 1998; Krumholz et al., 1997). Homogenization of the sediment means that it was even more important to remove the potentially contaminated and partially oxidized outer surface layer of the RCB core. It is also desirable to remove the outer layer of the core prior to subsampling for microbiological analysis. We decided to devise a system to do this using a hydraulic cutter, which would avoid the serious risk of cross-contamination of the inner core when manual scraping/paring is used to remove the outer layer (Fredrickson and Phelps, 1997). In addition, hydraulic subcoring should be much quicker than manual paring and reduce exposure of these anaerobic cores to trace oxygen or hydrogen when in glove bags. Leg 180 was the first ODP cruise in which RCB indurated sediment samples were analyzed for a suite of microbiology studies (bacterial numbers, activity, and enrichments).

METHODS

Shipboard Sampling and Handling

Twenty-six WRCs were taken for microbiological analysis at two sites (1109 and 1115) on the northern margin of the spreading tip in the Woodlark Basin extension system (Taylor, Huchon, Klaus, et al., 1999). Ten of these cores were obtained using rotary drilling.

Whole-Round Cores

All samples were taken from 25-cm WRCs for measurement of potential bacterial activity and estimation of viable bacterial numbers. The WRC samples were taken using a sterile cutting rig (Parkes et al., 1995), flushed with sterile oxygen-free nitrogen (OFN) to maintain anaerobic conditions, and sealed with sterile core end caps. Capped WRCs were stored in gas-tight anaerobic bags (Cragg et al., 1992a) in the ship's cold room at 4°C and transported back to the laboratory in insulated trunks containing wet ice and ice packs. The samples remained cold throughout transportation.

Laboratory Sample Handling

APC and XCB Cores

All sample handling was performed under aseptic anaerobic conditions (Parkes et al., 1995). WRCs were cut into 5-cm sections, from each

of which ten 5-cm³ syringe subcores were removed for radiotracer activity measurements and one 5-cm³ syringe subcore for most probable number viable counts. Syringe subcores were taken from the center of the WRC, avoiding sediment near the core liner to avoid the possibility of contamination.

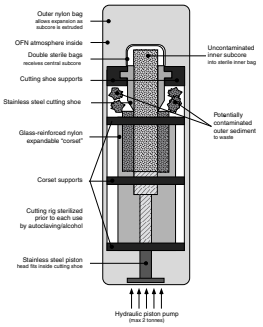
RCB Cores

A special cutting rig was constructed to obtain a sterile subcore from the center of the RCB core under anoxic conditions (Fig. F1). The RCB core was removed from the core liner in a laminar flow cabinet and the potentially contaminated end of the core removed (~2 cm) with a sterile stainless steel cleaver. The core was supported in an expandable glass-reinforced nylon "corset" and transferred to the sterile cutting rig. To accommodate the varying core diameters produced by rotary coring, a range of expandable corsets of glass-reinforced nylon were produced with varying inner diameters but constant outer diameters to fit in the supports. Each corset was designed to expand up to 4 mm (e.g., 55–59, 60–64, and 65–69 mm).

The whole cutting rig system was enclosed within a presterilized nylon bag filled with OFN to maintain sterile anoxic conditions. A hydraulic pump (maximum = 2 tonnes) was used to force the core through a sterile stainless steel cutting shoe (diameter = 40 mm) with the central subcore extruded into a (double lined) sterile nylon bag. At the end of the extrusion process, the last ~2 cm of potentially contaminated core remained in the cutting shoe. The double-layer nylon bag containing the sterile subcore was removed from the rig and sealed with a heat sealer. The sterile subcore was then transferred to a laminar flow cabinet in its sealed sterile nylon bag.

Unlike APC/XCB core samples, RCB samples were too hard to use intact core techniques (e.g., those described by Parkes et al., 1995). Instead, the material was powdered and slurried. The subcored material was carefully crushed with stainless steel hammers while remaining in its bag. The double thickness of the bag prevented holes from developing in the outer bag as a result of abrasion during the crushing process. The resulting fine powder was slurried (25% v/v) with a sterile nutrient-free mineral salts solution (Wellsbury et al., submitted [N1]). Microbiological data from these sites are published in detail elsewhere (Wellsbury et al., submitted [N1]).

F1. Diagram of subcoring rig for RCB cores, p. 9.



RESULTS AND DISCUSSION

In general, the cutting rig worked well on the indurated sediments from Sites 1109 and 1115, although there were some minor problems. For example, extreme care must be taken to avoid possibly splitting the double nylon collector bags during the core-crushing process. In addition, the process was relatively slow, which could lead to problems in changes in sample temperature, particularly in samples from hot sediments. Autoclaving the stainless steel assemblies took a relatively long time, so the turnaround time (>~2 hr) before further samples could be handled was limited. However, the RCB-cored material from Leg 180 was sufficiently friable to effectively disperse within the mineral salts solution after stirring for ~1 hr. Work is under way to refine this prototype system to overcome the minor problems detailed above.

Other authors have noted the importance of not only removing potential contamination in outer layers but also handling samples quickly

to limit exposure to oxygen. Rochelle et al. (1994) used a molecular genetic approach to investigate the effects of sample handling on bacterial communities in an APC sediment core from the Japan Sea (Leg 128). They demonstrated that the composition of the bacterial community in the outer layer, which had been exposed to air during handling, was markedly different to that contained in the pristine core center and was dominated by aerobic bacteria. However, contamination checks carried out during Leg 185 (Plank, Ludden, Escutia, et al., 2000; Smith et al., 2000) demonstrated that APC cores and the interior of RCB cores were not contaminated by fluorescent microbeads during the drilling and core recovery process.

The samples from the Woodlark Basin are the deepest samples ever analyzed for microbial populations (842 mbsf) and activities (800 mbsf). Bacteria were present in both Sites 1109 and 1115 to depths of 746 and 801 mbsf, respectively (Taylor, Huchon, Klaus, et al., 1999). Total bacterial populations, culturable bacteria, and bacterial activities (sulfate reduction, methanogenesis, and thymidine incorporation) generally decrease with increasing depth (Table T2) (Wellsbury et al., submitted [N1]). The presence of culturable bacteria and realistic potential anaerobic bacterial activity rates in samples from RCB cores obtained from these sites (Table T2) shows that the sample handling technique preserved the anoxia of the core with no evidence of contamination.

T2. Bacterial populations and activities, Sites 1109 and 1115, p. 11.

Implications of the New Technique

Although total bacterial populations decrease with increasing depth, the rate of decline does not steepen in deeper layers. This indicates that bacteria should be present much deeper than the depths currently analyzed (Parkes et al., 2000). The effective analysis of RCB core material will be essential for investigating the microbiology of deeper, and more consolidated, sediments than previously possible. In addition, experimental evidence has shown that the reactivity, and hence bioavailability, of buried organic matter increases with temperature (Wellsbury et al., 1997). As temperature increases with depth, this increasing bioavailability provides a mechanism for sustaining bacteria in deep formations. Furthermore, further additional bacterial substrates (e.g., acetate and H_2) are produced by thermal alteration of buried organic matter at higher temperatures (>80°C) (Cooles et al., 1987). As bacteria can live at temperatures up to 113°C (Stetter, 1996), in sediments with a typical thermal gradient of 30°C/km they can potentially be present to ~4 km depth. Thus, the investigation of deeper, more indurated samples is essential for further deep biosphere research. The new core handling approach described here is an important development for analyzing these deeper sediments.

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REFERENCES

Bale, S.J., Goodman, K., Rochelle, P.A., Marchesi, J.R., Fry, J.C., Weightman, A.J., and Parkes, R.J., 1997. *Desulfovibrio profundus* sp. nov., a novel barophilic sulfate-reducing bacterium from deep sediment layers in the Japan Sea. *Int. J. Syst. Bacteriol.,* 47:515–521.

Barnes, S.P., Bradbrook, S.D., Cragg, B.A., Marchesi, J.R., Weightman, A.J., Fry, J.C., and Parkes, R.J., 1998. Isolation of sulfate-reducing bacteria from deep sediment layers of the Pacific Ocean. *Geomicrobiol. J.,* 15:67–83.

Chapelle, F.H., and Bradley, P.M., 1996. Microbial acetogenesis as a source of organic acids in ancient Atlantic Coastal Plain sediments. *Geology,* 24:925–928.

Cooles, G.P., Mackenzie, A.S., and Parkes, R.J., 1987. Non-hydrocarbons of significance in petroleum exploration: volatile fatty acids and non-hydrocarbon gases. *Mineralog. Mag.,* 51:483–493.

Cragg, B.A., 1994. Bacterial profiles in deep sediment layers from the Lau Basin (Site 834). *In* Hawkins, J., Parson, L., Allan, J., et al., *Proc. ODP, Sci. Results,* 135: College Station, TX (Ocean Drilling Program), 147–150.

Cragg, B.A., Bale, S.J., and Parkes, R.J., 1992a. A novel method for the transport and long-term storage of cultures and samples in an anaerobic atmosphere. *Lett. Appl. Microbiol.,* 15:125–128.

Cragg, B.A., Harvey, S.M., Fry, J.C., Herbert, R.A., and Parkes, R.J., 1992b. Bacterial biomass and activity in the deep sediment layers of the Japan Sea, Hole 798B. *In* Pisciotto, K.A., Ingle, J.C., Jr., von Breymann, M.T., Barron, J., et al., *Proc. ODP, Sci. Results.,* 127/128 (Pt. 1): College Station, TX (Ocean Drilling Program), 761–776.

Cragg, B.A., and Kemp, A.E.S., 1995. Bacterial profiles in deep sediment layers from the eastern equatorial Pacific Ocean, Site 851. *In* Pisias, N.G., Mayer, L.A., Janecek, T.R., Palmer-Julson, A., and van Andel, T.H. (Eds.), *Proc. ODP, Sci. Results,* 138: College Station, TX (Ocean Drilling Program), 599–604.

Cragg, B.A., Law, K.M., Cramp, A., and Parkes, R.J., 1997. Bacterial profiles in Amazon Fan sediments (Sites 934, 940). *In* Flood, R.D., Piper, D.J.W., Klaus, A., and Peterson, L.C. (Eds.), *Proc. ODP, Sci. Results,* 155: College Station, TX (Ocean Drilling Program), 565–571.

————, 1998. The response of bacterial populations to sapropels in deep sediments of the Eastern Mediterranean (Site 969). *In* Robertson, A.H.F., Emeis, K.-C., Richter, C., and Camerlenghi, A. (Eds.), *Proc. ODP, Sci. Results,* 160: College Station, TX (Ocean Drilling Program), 303–307.

Cragg, B.A., Law, K.M., O'Sullivan, G.M., and Parkes, R.J., 1999. Bacterial profiles in deep sediments of the Alboran Sea, western Mediterranean, Sites 976–978. *In* Zahn, R., Comas, M.C., and Klaus, A. (Eds.), *Proc. ODP, Sci. Results,* 161: College Station, TX (Ocean Drilling Program), 433–438.

Cragg, B.A., and Parkes, R.J., 1994. Bacterial profiles in hydrothermally active deep sediment layers from Middle Valley (NE Pacific), Sites 857 and 858. *In* Mottl, M.J., Davis, E.E., Fisher, A.T., and Slack, J.F. (Eds.), *Proc. ODP, Sci. Results,* 139: College Station, TX (Ocean Drilling Program), 509–516.

Cragg, B.A., Parkes, R.J., Fry, J.C., Weightman, A.J., Maxwell, J.R., Kastner, M., Hovland, M., Whiticar, M.J., Sample, J.C., and Stein, R., 1995. Bacterial profiles in deep sediments of the Santa Barbara Basin, Site 893. *In* Kennett, J.P., Baldauf, J.G., and Lyle, M. (Eds.), *Proc. ODP, Sci. Results,* 146 (Pt. 2): College Station, TX (Ocean Drilling Program), 139–144.

Cragg, B.A., Parkes, R.J., Fry, J.C., Weightman, A.J., Rochelle, P.A., and Maxwell, J.R., 1996. Bacterial populations and processes in sediments containing gas hydrates (ODP Leg 146: Cascadia Margin). *Earth Planet. Sci. Lett.,* 139:497–507.

Cragg, B.A., Summit, M., and Parkes, R.J., 2000. Bacterial profiles in a sulfide mound (Site 1035) and an area of active fluid venting (Site 1036) in hot hydrothermal sediments from Middle Valley (northwest Pacific). *In* Zierenberg, R.A., Fouquet, Y.,

Miller, D.J., and Normark, W.R. (Eds.), *Proc. ODP, Sci. Results,* 169 [Online]. Available from World Wide Web: <http://www-odp.tamu.edu/publications/169_SR/chap_02/chap_02.htm>. [Cited 1999-12-15]

Fisk, M.R., Giovannoni, S.J., and Thorseth, I.H., 1998. Alteration of oceanic volcanic glass: textural evidence of microbial activity. *Science,* 281:978–980.

Fredrickson, J.K., and Phelps, T.J., 1997. Subsurface drilling and sampling, *In* Hurst, C.J., Knudsen, G.R., McInerney, M.J., Stetzenbach, L.D., and Walter, M.V. (Eds.), *Manual of Environmental Microbiology:* Washington, D.C. (ASM Press), 526–540.

Furnes, H., Thorseth, I.H., Tumyr, O., Torsvik, T., and Fisk, M.R., 1996. Microbial activity in the alteration of glass from pillow lavas from Hole 896A. *In* Alt, J.C., Kinoshita, H., Stokking, L.B., and Michael, P.J. (Eds.), *Proc. ODP, Sci. Results,* 148: College Station, TX (Ocean Drilling Program), 191–206.

Giovannoni, S.J., Fisk, M.R., Mullins, T.D., and Furnes, H., 1996. Genetic evidence for endolithic microbial life colonizing basaltic glass/seawater interfaces. *In* Alt, J.C., Kinoshita, H., Stokking, L.B., and Michael, P.J. (Eds.), *Proc. ODP, Sci. Results,* 148: College Station, TX (Ocean Drilling Program), 207–214.

Jones, R.E., Beeman, R.E., and Suflita, J.M., 1989. Anaerobic metabolic processes in the deep terrestrial subsurface. *Geomicrobiol. J.,* 7:117–130.

Kotelnikova, S., and Pedersen, K., 1998. Distribution and activity of methanogens and acetogens in deep granitic aquifers at Åspö Hard Rock Laboratory, Sweden. *FEMS Microbiol. Ecol.,* 26:121–134.

Krumholz, L.R., McKinley, J.P., Ulrich, G.A., and Suflita, J.M., 1997. Confined subsurface microbial communities in Cretaceous rock. *Nature,* 386:64–66.

Mather, I.D., and Parkes, R.J., 2000. Bacterial profiles in sediments of the eastern flank of the Juan de Fuca Ridge, Sites 1026 and 1027. *In* Fisher, A., Davis, E.E., and Escutia, C. (Eds.), *Proc. ODP, Sci. Results,* 168: College Station, TX (Ocean Drilling Program), 161–165.

Oremland, R.S., Culbertson, C., and Simoneit, B.R.T., 1982. Methanogenic activity in sediment from Leg 64, Gulf of California. *In* Curray, J.R., Moore, D.G., et al., *Init. Repts. DSDP,* 64 (Pt. 2): Washington (U.S. Govt. Printing Office), 759–762.

Parkes, R.J., Cragg, B.A., Bale, S.J., Getliff, J.M., Goodman, K., Rochelle, P.A., Fry, J.C., Weightman, A.J., and Harvey, S.M., 1994. Deep bacterial biosphere in Pacific Ocean sediments. *Nature,* 371:410–413.

Parkes, R.J., Cragg, B.A., Bale, S.J., Goodman, K., and Fry, J.C., 1995. A combined ecological and physiological approach to studying sulphate reduction within deep marine sediment layers. *J. Microbiol. Methods,* 23:235–249.

Parkes, R.J., Cragg, B.A., Fry, J.C., Herbert, R.A., and Wimpenny, J.W.T., 1990. Bacterial biomass and activity in deep sediment layers from the Peru margin. *Philos. Trans. R. Soc. London A,* 331:139–153.

Parkes, R.J., Cragg, B.A., and Wellsbury, P., 2000. Recent studies on bacterial populations and processes in marine sediments: a review. *Hydrogeol. Rev.,* 8:11–28.

Plank, T., Ludden, J.N., Escutia, C., et al., 2000. *Proc. ODP, Init. Repts.,* 185 [CD-ROM]. Available from: Ocean Drilling Program, Texas A&M University, College Station TX 77845-9547, USA.

Rochelle, P.A., Cragg, B.A., Fry, J.C., Parkes, R.J., and Weightman, A.J., 1994. Effect of sample handling on estimation of bacterial diversity in marine sediments by 16S rRNA gene sequence analysis. *FEMS Microbiol. Ecol.,* 15:25–226.

Smith, D.C., Spivack, A.J., Fisk, M.R., Haveman, S.A., Staudigel, H., and ODP Leg 185 Shipboard Scientific Party, 2000. Tracer-based estimates of drilling-induced microbial contamination of deep sea crust. *Geomicrobiol. J.,* 17:207–219.

Stetter, K.O., 1996. Hyperthermophilc procaryotes. *FEMS Microbiol. Rev.,* 18:149–158.

Taylor, B., Huchon, P., Klaus, A., et al., 1999. *Proc. ODP, Init. Repts.,* 180 [CD-ROM]. Available from: Ocean Drilling Program, Texas A&M University, College Station, TX 77845-9547, U.S.A.

Thorseth, I.H., Torsvik, T., Furnes, H., and Muehlenbachs, K., 1995. Microbes play an important role in the alteration of oceanic crust. *Chem. Geol.,* 126:137–146.

Torsvik, T., Furnes, H., Muehlenbachs, K., Thorseth, I.H., and Tumyr, O., 1998. Evidence for microbial activity at the glass-alteration interface in oceanic basalts. *Earth Planet. Sci. Lett.,* 162:165–176.

Wellsbury, P., Goodman, K., Barth, T., Cragg, B.A., Barnes, S.P., and Parkes, R.J., 1997. Deep marine biosphere fueled by increasing organic matter availability during burial and heating. *Nature,* 388:573–576.

Wellsbury, P., Goodman, K., Cragg, B.A., and Parkes, R.J., 2000. The geomicrobiology of deep marine sediments from Blake Ridge containing methane hydrate (Sites 994, 995, and 997). *In* Paull, C.K., Matsumoto, R., Wallace, P.J., and Dillon, W.P. (Eds.), *Proc. ODP, Sci. Results,* 164: College Station, TX (Ocean Drilling Program), 379–391.

Wellsbury, P., Mather, I.D., and Parkes, R.J., 2001. Bacterial abundances and porewater acetate concentrations in sediments of the Southern Ocean (Sites 1088 and 1093). *In* Gersonde, R., Hodell, D.A., and Blum, P. (Eds.), *Proc. ODP, Sci. Results,* 177 [Online]. Available from World Wide Web: <http:www-odp.tamu.edu/publications/177_SR/chap_03/chap_03.htm>.

Whelan, J.K., Oremland, R., Tarafa, M., Smith, R., Howarth, R., and Lee, C., 1986. Evidence for sulfate-reducing and methane producing microorganisms in sediments from Sites 618, 619, and 622. *In* Bouma, A.H., Coleman, J.M., Meyer, A.W., et al., *Init. Repts. DSDP,* 96: Washington (U.S. Govt. Printing Office), 767–775.

Figure F1. Schematic diagram (plan view) of subcoring rig to obtain uncontaminated control subcores from RCB cores under sterile anoxic conditions. OFN = oxygen-free nitrogen.

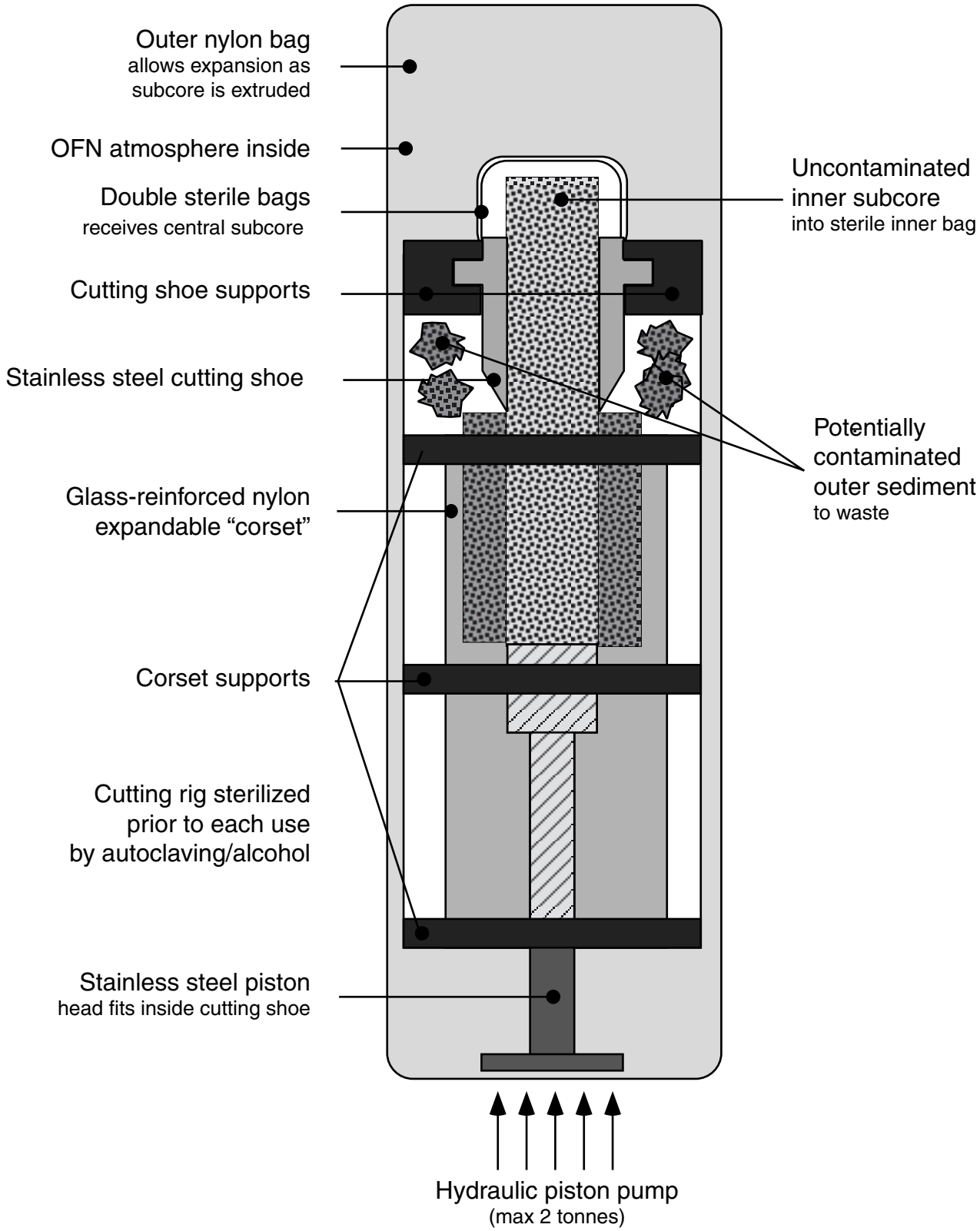

Table T1. Ocean Drilling Program (ODP) and Deep-Sea Drilling Project (DSDP) Legs during which microbiological investigations have been made.

Leg	Location	Related publication
64	Gulf of California	Oremland et al. (1982)
96	Gulf of Mexico	Whelan et al. (1986)
112*	Peru Margin	Parkes et al. (1990)
128*	Japan Sea	Cragg et al. (1992b)
135	Lau Basin	Cragg (1994)
138	Eastern Equatorial Pacific	Cragg and Kemp (1995)
139	Juan de Fuca Ridge	Cragg and Parkes (1994)
146*	Cascadia Margin	Cragg et al. (1995); Cragg et al. (1996)
148	Costa Rica Rift	Fisk et al. (1998); Furnes et al. (1996); Giovannoni et al. (1996); Thorseth et al. (1995); Torsvik et al. (1998)
155	Amazon Fan	Cragg et al. (1997)
160	Mediterranean I	Cragg et al. (1998)
161	Mediterranean II	Cragg et al. (1999)
164*	Blake Ridge	Wellsbury et al. (2000)
168	Juan de Fuca Ridge	Mather and Parkes (2000)
169	Juan de Fuca Ridge	Cragg et al. (2000)
177	Southern Ocean	Wellsbury et al. (2001)
180*	Woodlark Basin	This study; Wellsbury et al. (submitted [N1])
185	Izu-Mariana Margin	Plank, Ludden, Escutia, et al. (2000); Smith et al. (2000)

Note: * = legs from which whole-round core samples were studied.

Table T2. Comparison of bacterial populations and activities in samples, Sites 1109 and 1115.

			Culturable bacteria (MPN)			Potential bacterial activity			
Depth (mbsf)	Drilling method	Total bacteria (cells/cm^3)	Fermenters (cells/cm^3)	Autotrophic acetogens (cells/cm^3)	Heterotrophic acetogens (cells/cm^3)	Sulfate reduction (nmol/mL/day)	Methanogenesis from H_2/CO_2 (nmol/mL/day)	Methanogenesis from acetate (nmol/mL/day)	Thymidine incorporation (pmol/mL/day)
Site 1109									
0.05	APC	331.0×10^6	5050	94400	150	2.663	0.000	24.0	2.32
89.1	APC	4.3×10^6	21	39	8	0.160	0.003	0.06	0.92
582.72	RCB	0.8×10^6	0	0	21	0.002	0.001	0.14	0.02
Site 1115									
1.20	APC	42.7×10^6	1.76×10^7	26	16	1.257	0.020	2.90	0.21
309.7	RCB	1.8×10^6	92	3	0	0.002	0.000	8.90	0.11
797.89	RCB	0.4×10^6	10	0	7	0.002	0.000	0.08	0.06

Notes: MPN = most probable number. APC = advanced piston corer, RCB = rotary core barrel.

CHAPTER NOTE*

N1. Wellsbury, P., Mather, I.D., and Parkes, R.J., submitted. Geomicrobiology of low organic carbon sediments in the Woodlark Basin (ODP Leg 180). *FEMS Microbiol. Ecol.*

*Dates reflect file corrections or revisions.