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Kieler Meeresforsch., Sonderh. 8, 178-181. Kiel 1991

Isolation and identification of anaerobic sulfur dependent thermophilic bacteria from two new hydrothermal sites in SW Pacific (Lau Basin and North Fiji Basin)

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Introduction

Extremely thermophilic bacteria were first discovered in freshwater environments, but recently many species were isolated from shallow marine environments (STETTER 1986). Since 1977, several deep-sea hydrothermal vents have been discovered in the Eastern Pacific (East Pacific Rise, Juan de Fuca Rise, Guaymas Basin) and the mid Atlantic. Deep-sea hydrothermal ecosystems are characterized by a wide variation of temperature and chemical properties. Because of the hydrostatic pressure existing at these depths, water is still liquid at temperature above 100 °C. Such environment was an unique opportunity for studying life in extreme conditions of pressure and temperature. Only few species of ultrathermophilic bacteria have been isolated from deep-sea hydrothermal sites and characterized: *Methanococcus jannaschi* (JONES et al. 1983) and other *Methanococcus* sp. from East Pacific Rise at 21°N; *Staphylothermus marinus* (FIALA et al. 1986) from East Pacific Rise at 11°N; *Desulfurococcus* sp. (JANNASCH et al. 1988) from East Pacific Rise at 11°N; *Methanopyrus* (HUBER et al. 1989) from Guaymas Basin; isolate ES1 (PLEDGER and BAROSS 1989) from Juan de Fuca Ridge and *Archaeglobus profundus* (BUGGRAF et al. 1990) from Guaymas Basin.

During May and July 1989 two oceanological cruises "Biolau" and "Starmer" were organized to explore new hydrothermal sites in back arc basins of the SW Pacific with the deep-sea man-operated submersible "Nautile". During the "Biolau" cruise in the Lau Basin, active hydrothermal chimneys venting fluids with temperature up to 400 °C were discovered. In the North Fiji Basin, explored during the French-Japanese cruise "Starmer", active chimneys were also found, with maximum temperature around 300 °C. Associated fauna was mainly composed of gastropods and bivalves (JOLLIVET et al. 1989).

Results and discussion

Hydrothermal fluids were sampled using titanium bottles. Samples of chimneys, rock debris, sediment and invertebrates were collected with the arm of the sub-

mersible, and transferred to the surface and mother ship in an insulated box. Before inoculation, invertebrates were dissected and selected tissues were ground in sterile seawater. Rock and chimney fragments were transferred into sterile vials containing sterile seawater, immediately reduced by addition of Na_2S or titanium citrate, flushed with N_2 and then vigourously shaken. On board, enrichment cultures were performed on two different sulfur containing media: ZoBell sulfur medium, pH 7.5 (BELKIN and JANNASCH 1985), SME medium, pH 6.5 (STETTER et al. 1983) at 55 and 95 °C. Back in the laboratory subcultures were done with different complex media including ZoBell sulfur medium and SME medium, at temperatures between 60 and 95 °C. Purification was performed by streaking on "Gelrite" sulfur medium or by serial dilutions. Twenty-four isolates were obtained by streaking on solid medium at 80 °C, 3 at 95 °C and 13 isolates at 60 - 65 °C. Nineteen isolates were obtained by serial dilutions at 85 - 90 °C.

Isolates were mainly obtained from sediment or chimney samples (Fig. 1). All the isolates are heterotrophic anaerobes, and no growth was detected in absence

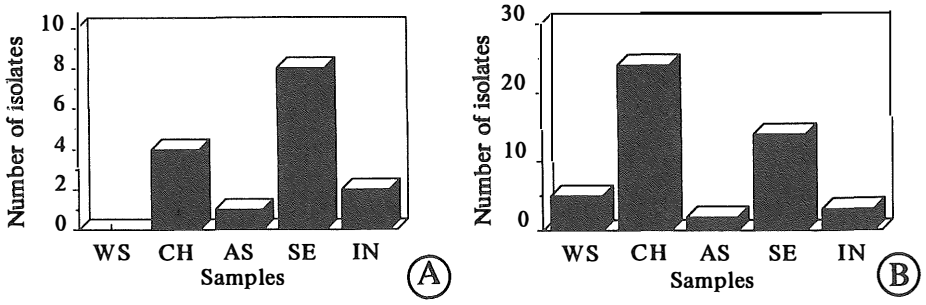

Fig. 1. Origin of isolates from Lau Basin and North Fiji Basin at 60 - 65 °C (A) and at 80 - 95 °C (B). WS: water samples; CH: chimney and rock debris samples; AS: artificial substratum; SE: sediment samples; IN: invertebrate samples.

of sulfur. Most of the isolates tested are able to use yeast extract, peptone, brain heart infusion and sucrose as substrate. Some of them can grow on starch, glucose or pyruvate (Table 1). Optimal growth temperature was determined for 25 strains isolated at 80 °C. They ranged from 65 °C to 90 °C, the doubling time varying from 34 min to 3 h at optimal temperature.

By phase contrast microscopy and scanning electron microscopy isolates growing between 80 and 95 °C appeared as regular or irregular coccoid cells occuring singly, by pairs or in aggregates of 10 to 20 cells. Cells were from 1 to 3 µm in diameter. Isolates at 60 - 65 °C showed three different morphological types: coccoid cells and rod-shaped rigid cells, one with a diameter of 0.4 µm (8 isolates) the other with a diameter of 0.8 µm; both showed various length up to 70 µm.

From preliminary lipid analysis and antibiotic resistances, at least 27 isolates could be assigned to Archaebacteria. Comparison of isolates was first conducted by total protein profiles in SDS-PAGE. The 44 isolates tested, grown under the same culture conditions, showed quite different patterns.

Table 1. Carbon substrates supporting growth. Isolates were grown in sulfur containing seawater, pH 7.5 supplemented with various carbon sources.

Substrates	Brain heart infusion	Yeast extract	Peptone	Glucose	Pyruvate	Starch	Sucrose
Number of isolates tested	29	33	33	33	33	33	29
Number of positive cultures	28	28	30	2	4	16	28

A comparison of the genotypic characteristics of the isolates, using ribosomal RNA gene restriction patterns (GRIMONT and GRIMONT 1986), is now in progress. This approach should indicate the level (strain, species or genus) of this apparent and rather unexpected diversity.

References

BELKIN, S. and H.W. JANNASCH, 1985. A new extremely thermophilic sulfur-reducing heterotrophic marine bacterium. Arch. Microbiol. 141, 181-186.

BUGGRAF, S., H.W. JANNASCH, B. NICAULOS and K.O. STETTER, 1990. *Archaeglobus profundus* sp. nov. represents a new species within the sulfate reducing archaebacteria. System. Appl. Microbiol. 13, 24-28.

FIALA, G., K.O. STETTER, H.W. JANNASCH, T.A. LANGWORTHY and J. MADON, 1986. *Staphylothermus marinus* sp. nov., represents a novel genus of extremely thermophilic submarine heterotrophic archaebacteria growing up to 98 °C. System. Appl. Microbiol. 8, 106-113.

GRIMONT, F. and P.A.D. GRIMONT, 1986. Ribosomal, ribonucleic acid restriction patterns as potential taxonomic tools. Ann. Inst. Pasteur/Microbiol. 137 B, 165-175.

HUBER, R., M. KURR, H.W. JANNASCH and K.O. STETTER, 1989. A novel group of abyssal methanogenic bacteria *(Methanopyrus)* growing at 110 °C. Nature 342, 833-834.

JANNASCH, H.W., C.O. WIRSEN, S.J. MOLYNEAUX and T.A. LANGWORTHY, 1988. Extremely thermophilic fermentative archaebacteria of the genus *Desulfurococcus* from deep-sea hydrothermal vents. Appl. Environ. Microbiol. 54, 1203-1209.

JOLLIVET, D., J. HASHIMOTO, J.M. AUZENDE, 1989. Premières observations de communautés animales associées à l'hydrothermalisme arrière arc du bassin Nord-Fidjien. Cr. Acad. Sci. Paris. 309, 301-308.

JONES, W.J., J.A. LEIGH, F. MAYER, C.R. WOESE and R.S. WOLFE, 1983. *Methanococcus jannaschi* sp. nov. an extremely thermophilic methanogen from submarine hydrothermal vents. Arch. Microbiol. 136, 254-261.

PLEDGER, R.J. and J.A. BAROSS, 1989. Characterization of an extremely thermophilic archaebacterium isolated from a black smoker polychaete (*Paralvinella* sp.) at the Juan de Fuca Ridge. System. Appl. Microbiol. 12, 249-256.

STETTER, K.O., 1983. *Pyrodictium* gen. nov., a new genus of submarine disc-shaped sulfur-reducing archaebacteria growing optimally at 105 °C. System. Appl. Microbiol. 4, 535-551.

STETTER, K.O., 1986. Diversity of extremely thermophilic archaebacteria. In: T.D. BROCK (ed.), Thermophiles: General, molecular and applied microbiology, John Wiley and son.