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27. We thank R. Wallace for expert assistance in the transgenic experiments. Supported in part by grants from the American Business for Cancer Research Foundation and E. I. duPont de Nemours and Co., Inc.

8 January 1987; accepted 3 March 1987

# Dual Infection of the Central Nervous System by AIDS Viruses with Distinct Cellular Tropisms

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**Human immunodeficiency virus (HIV) is the causative agent of the acquired immune deficiency syndrome (AIDS). A large number of AIDS patients show evidence of neurologic involvement, known as AIDS-related subacute encephalopathy, which has been correlated with the presence of HIV in the brain. In this study, two genetically distinct but related viruses were isolated from one patient from two different sources in the central nervous system: brain tissue and cerebrospinal fluid. Both viruses were found to replicate in peripheral blood lymphocytes, but only virus from brain tissue will efficiently infect macrophage/monocytes. The viruses also differ in their ability to infect a brain glioma explant culture. This infection of the brain-derived cells in vitro is generally nonproductive, and appears to be some form of persistent or latent infection. These results indicate that genetic variation of HIV in vivo may result in altered cell tropisms and possibly implicate strains of HIV with glial cell tropism in the pathogenesis of some neurologic disorders of AIDS.**

ACQUIRED IMMUNE DEFICIENCY syndrome (AIDS) is associated with a broad spectrum of clinical disorders (*1*). An increasing fraction of AIDS cases are recognized with disorders of the central nervous system (CNS) known as AIDS-related subacute encephalopathy (*2*). These disorders are correlated with the presence of HIV, the human immunodeficiency virus, in the CNS, which can be detected by virus isolation as well as by direct analysis by hybridization (*3, 4*). At least one of the cell types infected by HIV in brain tissue appears to be a macrophage/monocyte-like cell, presumably derived from infiltration of monocytes from the peripheral blood (*5*). However, some studies suggest that HIV can also infect cells of CNS origin (*6*).

We have studied virus from cerebrospinal fluid and brain tissue of patients with AIDS encephalopathy. In one patient, the virus in the cerebrospinal fluid was genotypically different from the virus isolated from brain tissue of the same individual. Furthermore, the cell-free virus isolated from the cerebrospinal fluid infected a primary brain glioma explant in culture. The virus from brain tissue efficiently infected macrophage/monocytes. These results suggest that genotypic variation in HIV within one individual may influence the pathogenesis of disease by conferring distinct tropisms for different systemic compartments.

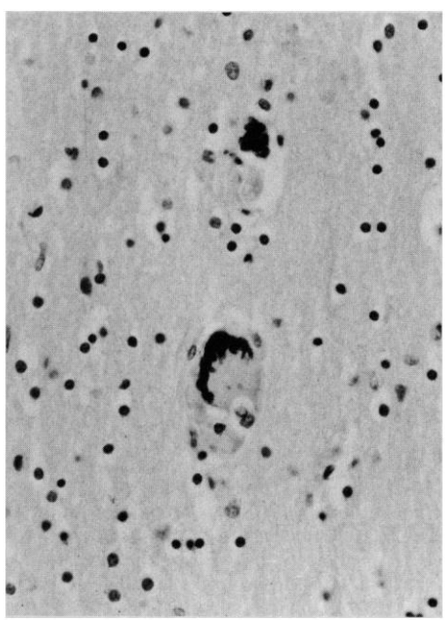

**Fig. 1.** Multinucleate giant cells in brain of patient J.R. Tissue samples from frontal lobe specimens were fixed with Formalin and stained with hematoxylin and eosin. The perivascular multinucleated giant cell syncytia are often observed in cases of AIDS encephalopathy (*5*).

Patient J.R. died with Kaposi's sarcoma and severe AIDS encephalopathy. The brain showed extensive leukoencephalopathy, and characteristic multinucleated giant cell syncytia were observed in pathologic specimens of frontal lobe brain tissue taken at autopsy (*7*). As shown in Fig. 1, these syncytia are morphologically similar to those previously described as being of macrophage/monocyte origin and shown to express HIV RNA detected by in situ hybridization (*5, 6*). Virus was isolated from various tissue sources by infection of lectin-activated normal human peripheral blood lymphocytes (PBL). The presence of infectious virus in the cultures was assayed by measuring reverse transcriptase (RT) activity in the supernatant fluid, and detecting viral antigens in the supernatant fluid by an enzyme-linked immunosorbent assay (ELISA; Cellular Products and Abbott) and viral DNA in the infected cells by Southern hybridization (*8*) (Fig. 2).

Viruses isolated from different sources were analyzed by restriction enzyme digestion. Viral DNA was isolated from infected PBL 11 to 14 days after cocultivation. This short period of growth in culture was chosen to minimize the possibility that selection for genetic changes would occur in vitro. Thus the viruses isolated would be most closely representative of virus resident in infected tissues.

Two genotypically distinct viruses were obtained from the CNS of patient J.R. The genome of virus isolated from the cell-free cerebrospinal fluid was distinct by restriction enzyme analysis from that of virus isolated from cells derived from the same sample (Fig. 2). Cerebrospinal fluid is generally free of cells in normal healthy individuals (*9*). The large number of cells in the cerebrospinal fluid in this case probably resulted either from cells of the CNS or from peripheral blood mononuclear cells infiltrating the cerebrospinal fluid, as is often observed in CNS disorders (*9*). We tested the possibility that the cell-associated

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virus from cerebrospinal fluid was derived from brain tissue by analyzing viruses isolated directly from both frontal lobe and cerebellum brain tissue. Comparison of their genomes demonstrated that virus isolated from the brain tissue samples was identical to virus isolated from cells of cerebrospinal fluid (Fig. 2). Brain tissue also contains numerous blood capillaries; thus, this virus may have been derived from either infiltrating macrophage/monocyte cells or cells in the blood capillaries, both originally derived from the peripheral circulation. Peripheral blood was not available from this patient; therefore, it is not possible conclusively to assign the cellular source of this virus isolate. The source of the distinct extracellular virus isolated from cell-free cerebrospinal fluid is unknown, but was probably from cells of the CNS.

We further characterized the genomes of the two J.R. isolates in an attempt to determine whether they were derived by mutation directly from each other, or whether they represented independent viruses. Comparison of the restriction enzyme maps of the two isolates indicated that the differences between the two strains (4 differences of 26 restriction enzyme sites) were fewer than differences observed between distinct isolates from different individuals (for example, the isolate from J.R.). Cerebrospinal fluid (CSF) differs from HTLV-III$_B$ in 15 of 33 sites and from associated retroviruses in 13 of 33 sites. Thus, it is likely that the two viruses had a common ancestor and diverged from each other during the course of infection in vivo; however, molecular cloning and nucleic acid sequence analysis will be necessary to clearly establish the extent of relatedness between these strains. We will term these viruses HIV(JR-CSF) and HIV(JR-FL) for isolates derived from the cerebrospinal fluid and frontal lobe, respectively.

We investigated the possible neurotropic nature of both viruses by testing their ability to replicate in cultured cells derived from a brain glioma explant (Fig. 3). Brain cells derived from a glioma grown for 4 months in culture were inoculated with both HIV(JR-CSF) and HIV(JR-FL), and with a prototype HIV strain, HTLV-III$_B$. The cells were infected with an equivalent amount of virus, as measured by RT activity [$1.5 \times 10^5$ cpm; for HTLV-III$_B$ this is equivalent to $3 \times 10^4$ plaque-forming units on MT-4 cells (*10*)]. Whereas all three viruses would efficiently infect PBL, only HIV(JR-CSF) persisted in the infected glial cells (Fig. 3). Production of virus was low, and appeared in a transient fashion; therefore, infection of the glioma cultures was confirmed by cocultivating the cells at 19 days after infection with normal PBL. Virus production was apparent within 7 days after cocultivation (Fig. 3). By restriction enzyme analysis, the recovered virus appeared to be identical to HIV(JR-CSF). Neither HIV(JR-FL) nor the prototype HTLV-III$_B$ strain replicated in these brain cultures, and virus was not detected after cocultivation of these glial cell cultures with normal human PBL. The specific cell type in the explant cultures infected by HIV(JR-CSF) is unknown. The cultures stained for markers consistent with mixtures of oligodendrocytes (30 to 40%), astrocytes (70 to 80%), and some fibroblasts (see Fig. 3). We tested the glial cultures for macrophage/monocytes, since other investigators had previously shown that these cells could be infected by some strains of HIV (*11, 12*). No macrophage/monocytes were detected in these cultures by three different monoclonal antibodies (legend to Fig. 3). Furthermore, macrophage/monocytes are generally trypsin-resistant, and the glioma cultures were passaged by trypsinization for 4 months prior to infection, and five times after infection, prior to rescue with PBL. Attempts to infect a cloned oligodendrocyte cell line (MG138 clone 5C5, brain endothelial cell

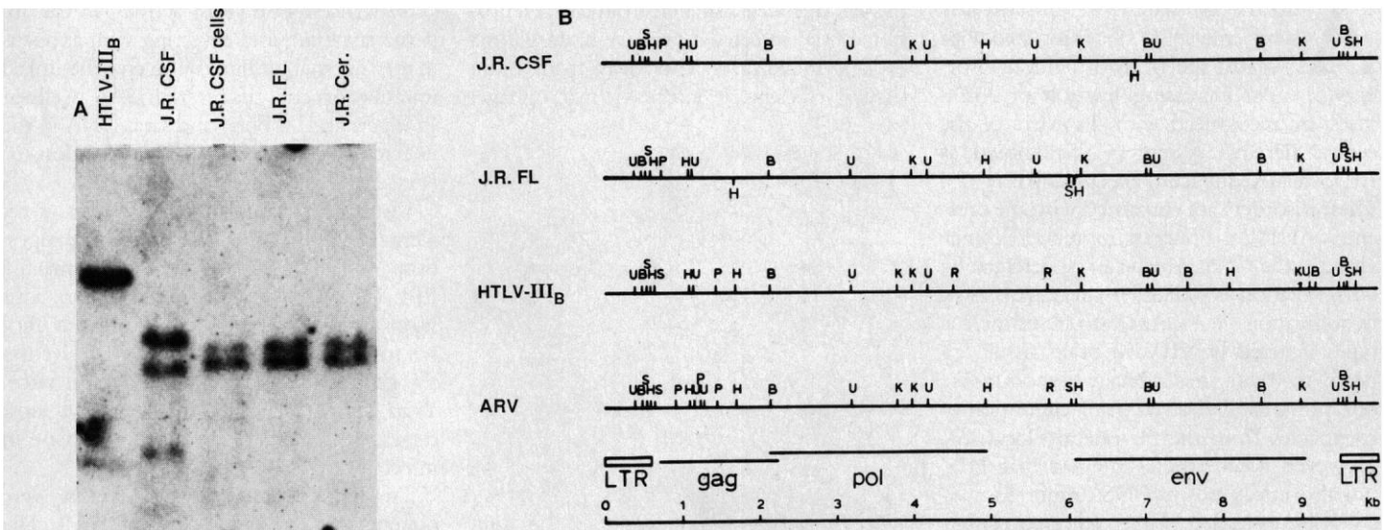



**Fig. 2.** (**A**) Detection of HIV DNA sequences after infection of normal human PBL. From patient J.R., cerebrospinal fluid (CSF) and tissue specimens were isolated postmortem. The CSF was filtered free of cells. For virus isolation from CSF, 1.0 ml of the filtered fluid was used to directly infect $5 \times 10^6$ phytohemagglutinin (PHA)-activated normal PBL. Isolation of the other viruses was performed by cocultivation of tissue samples [$1 \times 10^6$ CSF cells, fragments of brain tissue (total, about 1 cm$^3$)] with $5 \times 10^6$ PHA-activated normal lymphocytes. Cultures were propagated in Iscove's medium, 20% fetal calf serum, and 10 ng/ml of recombinant IL-2. Virus was assayed 11 days after infection for RT activity as described (*10*), for antigens by ELISA (Cellular Products), and for viral DNA by Southern hybridization (*8*) performed on the Hirt supernatant fraction of cells (*14*). DNA was extracted from the cells at day 11 for J.R. Southern hybridization (*7*) was performed on 15 μg of Hind III–digested Hirt supernatant fraction DNA for J.R. Total DNA from the H9 cells infected with HTLV-III$_B$ is shown for comparison. The hybridization probe consisted of a molecular clone of circular ARV DNA, ARV-7B (*15*). The abbreviations JR-CSF, JR-CSF cells, JR-FL, and JR-Cer. indicate isolates of virus from cerebrospinal fluid and from cells of the cerebrospinal fluid, frontal lobe brain tissue, and cerebellum brain tissue, respectively. (**B**) Restriction endonuclease cleavage map for HIV (JR-CSF) and HIV(JR-FL). Restriction maps were constructed by Southern hybridization with specific fragments of the ARV-7B clone. Sites distinct between the two J.R. viruses are indicated below the viral genomes. Restriction endonuclease cleavage map of the same enzymes for HTLV-III$_B$ (*16*) and ARV (*15*) are shown for comparison. Abbreviations: B, Bgl II; H, Hind III; K, Kpn I; P, Pst I, R, Eco RI; S, Sst I; U, Pvu II; LTR, long terminal repeat. The positions of *gag*, *pol*, and *env* genes for HTLV-III$_B$ are indicated.

cultures (HBE2), neuroblastoma cell lines (LAN-1, LAN-5), and astrocyte cell lines (U251MG, A172MG, and D54MG) were unsuccessful.

The glioma cultures were also infected with various doses of the three viruses (Table 1). Under these conditions it was clear that isolate HIV(JR-CSF) can infect this glioma culture, whereas the other J.R. virus and HTLV-III$_B$ cannot. Thus, these results demonstrate that isolate JR-CSF has a greater tropism for some cell types derived from the CNS than do other isolates of HIV, including one isolated at the same time from the same individual. It will be necessary to examine more glial cell types to test the generality of this observation.

The mechanism of virus persistence in these brain explant cultures may be due to a very low level of viral replication in one of the multiple cell types present. However, other means of viral persistence, such as some form of latent infection or stable internalization of input virions followed by release, cannot be excluded.

Other investigators detected HIV in macrophage/monocyte cells by in situ hybridization of brain tissue (*5, 6*), and others demonstrated that some isolates of HIV from brain tissue will efficiently infect primary macrophage/monocyte cultures (*11, 12*). We tested the ability of these viral isolates to replicate in primary peripheral blood macrophage/monocyte cells. Only the J.R. virus isolated from brain tissue efficiently and productively infected the macrophage cultures. The cerebrospinal fluid virus resulted in only a low level of production by 14 days after infection. Attempts to infect a macrophage/monoctye cell line (U937) were unsuccessful with both J.R. viruses, though HTLV-III$_B$ did productively infect these cells. These results, together with those from infection of the glioma explant cultures, confirm that the two J.R. viruses are biologically distinct.

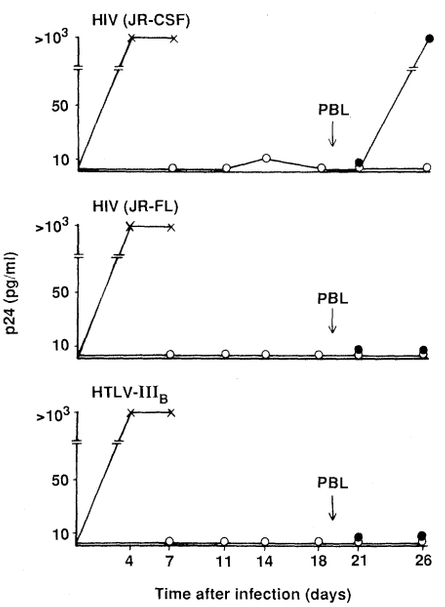
Our data provide direct evidence that different strains of HIV can simultaneously coexist in the same individual in different tissues. We suggest that at least one mechanism for the different tissue distribution is genetic variation in HIV, which results in distinct cellular tropisms for the virus. In other individuals whom we have studied, viruses identical by gross restriction enzyme mapping were detected in different body compartments. For example, in a second patient (J.P.) with AIDS encephalopathy, virus isolates from brain tissue, cerebrospinal fluid, and peripheral blood were identical by restriction enzyme analysis. However, it is possible that genetic variation in these J.P. virus isolates would be revealed by biological tests. The J.P. viruses were distinct from both strains isolated from patient J.R., consistent with previous studies of AIDS virus genetic variation, where comparison of viral genomes from different individuals reveals wide heterogeneity (*3, 13*).

Given the pattern of cellular tropism, it is likely that HIV(JR-FL) is derived from macrophage/monocyte cells evident at least in part as giant cell syncytia in J.R. brain tissue (Fig. 1). HIV(JR-CSF) is not derived from cells present in the cerebrospinal fluid, nor is this virus the predominant strain present in brain autopsy samples. However, current virus isolation and culture systems for HIV would not selectively detect a minor subpopulation of one virus type present in brain tissue. Therefore, the virus in the cerebrospinal fluid may have been derived from a minor infected cell population of the brain tissue that was selectively shed into the cerebrospinal fluid. Alternatively, the virus may have replicated in cells of the brain

**Table 1.** Infection of glioma and macrophage/monocyte cells by different HIV strains. Glioma cultures were infected with the indicated amounts of HIV (as assayed by RT activity), and assayed at the indicated times for HIV p24 antigens as described (see Fig. 3), with the exception that PBL were cocultivated on day 14 after infection. Macrophage cultures were prepared by adherence to culture dishes for 4 days, and adherent cells were infected with the indicated amounts of HIV in the presence of 10 μg/ml of Polybrene for 2 hours. Medium was changed each 3 to 4 days, and virus was assayed on days 7 or 11 and day 14 after infection. HIV levels are expressed as picograms of p24 per milliliters, as calculated relative to p24 standards (Abbott Laboratories).

| Virus | | Glioma | | | | Macrophage | |
|---|---|---|---|---|---|---|---|
| Strain | Amount (cpm) | Days after glioma infection | | Days after PBL coculture | | Days after macrophage infection | |
| | | Day 11 | Day 14 | Day 7 | Day 14 | Day 11 | Day 14 |
| HIV(JR-CSF) | $1.5 \times 10^5$ | <5 | <5 | 151 | $5.4 \times 10^4$ | 16 | 77 |
| HIV(JR-CSF) | $3.0 \times 10^4$ | <5 | <5 | 20 | $2.8 \times 10^3$ | <5 | <5 |
| HIV(JR-CSF) | $6.0 \times 10^3$ | <5 | <5 | <5 | <5 | <5 | <5 |
| HIV(JR-FL) | $1.5 \times 10^5$ | <5 | <5 | <5 | <5 | $9.2 \times 10^3$ | $1.5 \times 10^4$ |
| HIV(JR-FL) | $3.0 \times 10^4$ | <5 | <5 | <5 | <5 | $5.9 \times 10^3$ | $5.7 \times 10^3$ |
| HIV(JR-FL) | $6.0 \times 10^3$ | <5 | <5 | <5 | <5 | $1.7 \times 10^3$ | $6.4 \times 10^3$ |
| HTLV-III$_B$ | $1.5 \times 10^5$ | <5 | <5 | <5 | <5 | <5 | 11 |
| HTLV-III$_B$ | $3.0 \times 10^4$ | <5 | <5 | <5 | <5 | <5 | <5 |
| HTLV-III$_B$ | $6.0 \times 10^3$ | <5 | <5 | <5 | <5 | <5 | <5 |

**Fig. 3.** Kinetics of HIV replication in cultured glial cells. Cultured cells ($2 \times 10^5$ per 60-mm dish) of a brain glioma explant were infected with HTLV-III$_B$, HIV(JR-CSF), and HIV(JR-FL) for 2 hours in the presence of 10 μg/ml of Polybrene. Equivalent amounts of RT activity were used for infection; in this case, $1.5 \times 10^5$ cpm. The infected glioma cells were passaged each 3 to 4 days by trypsinization (total, five times), and the supernatant assayed for virus antigens by ELISA (Cellular Products and Abbott Laboratories) after 100-fold concentration (open circles). Other portions of virus ($1.5 \times 10^5$ cpm) were used for direct infection of PBL (x). Nineteen days after infection, $1 \times 10^6$ infected glial cells were cocultivated with $1 \times 10^6$ PHA-activated normal human PBL. Virus production from these cultures was assayed as above (closed circles). Essentially, equivalent results were obtained with the Cellular Products and Abbott assay methods. The optical density reading from the Abbott method was converted to picograms of p24 per milliliter of culture supernatant, based on p24 standards. The RT activity ($1.5 \times 10^5$ cpm) for HTLV-III$_B$ corresponded to $3 \times 10^4$ plaque-forming units of virus in MT-4 T-cells (*9*). Quantitative titration of these primary isolates of HIV(JR-CSF) and HIV(JR-FL) by the plaque-forming assay was not possible. The glioma culture was prepared by explant, and passaged in culture for 4 months prior to infection. The phenotype of the cells appeared to be approximately 5 to 10% fibroblasts, 70 to 80% astrocytes, and 30 to 40% oligodendrocytes. No macrophage/monocyte cells were detected. The antibodies used were rabbit antibody to human fibronectin (1/20, Accurate); rabbit antibody to bovine glial fibrillary acidic protein (1/20, Accurate); rabbit antibody to bovine galactocerebrosides (1/25, J. Merrill); mouse monoclonal antibody to human Thy 1 (1/100, ab 390, B. Seeger); and macrophage/monocyte-specific mouse monoclonals, OKMI (1/50, Ortho), Leu M5 (1/50, Becton Dickinson), and MY4 (1/50, Coulter).



distinct from those obtained in the autopsy tissue sample. The specific cell type in the CNS infected by this virus requires further investigation.

It is noteworthy that the patient J.R. had a particularly rapid encephalopathic disease course. His sexual partner also died of AIDS with similarly rapid CNS deterioration, suggesting a similar etiology. One possibility worth considering is that the infection of the CNS by two distinct viruses with different cellular tropisms may have contributed to the pathogenesis of this AIDS subacute encephalopathy. It will be important to fully characterize HIV isolates from the CNS of other individuals with regard to genetic variation and cellular tropism. Such variations may be important in determining clinical phenotypes, in transmission of HIV by different body fluids, and may ultimately be of clinical relevance in altering the disease manifestations of AIDS.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17. We thank S. Clark for the vector used to synthesize recombinant IL-2; P. Luciw for the ARV-7B molecular clone; R. Gallo for HTLV-III$_B$; B. Seeger for Thy-1 monoclonal antibody and astroglial and neuroblastoma cell lines; A. Diagne and Y. Koyanagi for technical assistance; A. Cann, J. Gasson, D. Golde, D. Ho, J. Rosenblatt, H. Temin, and W. Wachsman for comments on the manuscript; and W. Aft for editorial assistance. This work was supported by ACS JFRA-99 and the California Universitywide Task Force on AIDS.

20 January 1987; accepted 7 April 1987


# Isolation of Extremely Thermophilic Sulfate Reducers: Evidence for a Novel Branch of Archaebacteria


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**Extremely thermophilic archaebacteria are known to be metabolizers of elemental sulfur and the methanogens. A novel group of extremely thermophilic archaebacteria is described, which consists of sulfate-respiring organisms that contain pure factor 420 and that have been isolated from marine hydrothermal systems in Italy. They possess a third type of archaebacterial RNA polymerase structure previously unknown, indicating an exceptional phylogenetic position. Most likely, this group represents a third major branch within the archaebacteria. The existence of sulfate reducers at extremely high temperatures could explain hydrogen sulfide formation in hot sulfate-containing environments, such as submarine hydrothermal systems and deep oil wells.**


TWO MAIN SUBDIVISIONS OF ARchaebacteria are distinguished: one comprising the methanogenic bacteria and the extreme halophiles, and a second consisting of the sulfur-metabolizing extreme thermophiles (*1*). These branches can be recognized on the basis of a comparison of RNA polymerase structures (*2*), 16*S* ribosomal RNA (rRNA) sequences (*1*), and metabolic properties. Members of the group of archaebacterial sulfur metabolizers are able to grow by either oxidizing or reducing elemental sulfur, depending on the genera (*3, 4*). Energy conservation by dissimilatory sulfate reduction has been observed up to now exclusively within some eubacteria (*5*). Eubacterial sulfate reducers are mesophiles or moderate thermophiles and play an important role in the global sulfur cycle (*5*). We describe here a novel group of extremely thermophilic sulfate reducers that belong to the archaebacterial kingdom.

To study microbial life at extremely high temperatures, we collected 45 anaerobic samples (*6*) of hot sediments from marine hydrothermal systems in the vicinities of Vulcano and Stufe di Nerone, Italy. The original temperatures of the sediments ranged between 70° and 100°C. In the laboratory, oxygen-free marine mineral medium supplemented with 0.1% (w/v) yeast extract (Difco) was inoculated with portions of each sample (*7, 8*) and then incubated at 85°C (gas phase: $N_2/CO_2$ = 80/20). After 1 week we observed coccoid bacterial cells in 28 of the 45 cultures that we attempted to form. They showed a strong blue-green fluorescence under the ultraviolet (UV) microscope at 420 nm characteristic of methanogenic bacteria (*8*).

Surprisingly, however, only traces of methane (about $10^{-4}$ times that produced by a methanogen culture of comparable cell density) were detected. The novel organisms were cloned by plating on culture medium solidified by 1.5% agar (Oxoid). The plates were incubated anaerobically at 70°C (*8*). Isolate VC-16 from Vulcano was the first

**Table 1.** Substrates for growth of isolate VC-16. Portions of 20-ml anaerobic sulfate- or thiosulfate-containing mineral medium (MGG) (*7*) were supplemented with possible substrates (2 g/liter) and then inoculated (1% inoculation) with strain VC-16. Incubation was usually at 85° and 65°C in the case of glucose and pyruvate, respectively. Substrate utilization was determined after four transfers in sequence into the same culture medium (1% inoculation), each followed by an incubation period. Acids were added as sodium salts.

| Class of nutrients | | |
|---|---|---|
| Intermediates in anaerobic degradation | Sugar | Complex substrates |
| Molecular hydrogen,* formate, formamide, L(+)- and D(−)-lactate, pyruvate | Glucose | Yeast extract, casamino acids,* beef extract, peptone, cell homogenates of *E. coli, Lactobacillus bavaricus* and *Methanothermus fervidus* |

*Growth only in thiosulfate-containing medium.


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