

添付資料3-6

OriV(vegetative replication origin)遺伝子領域の解析

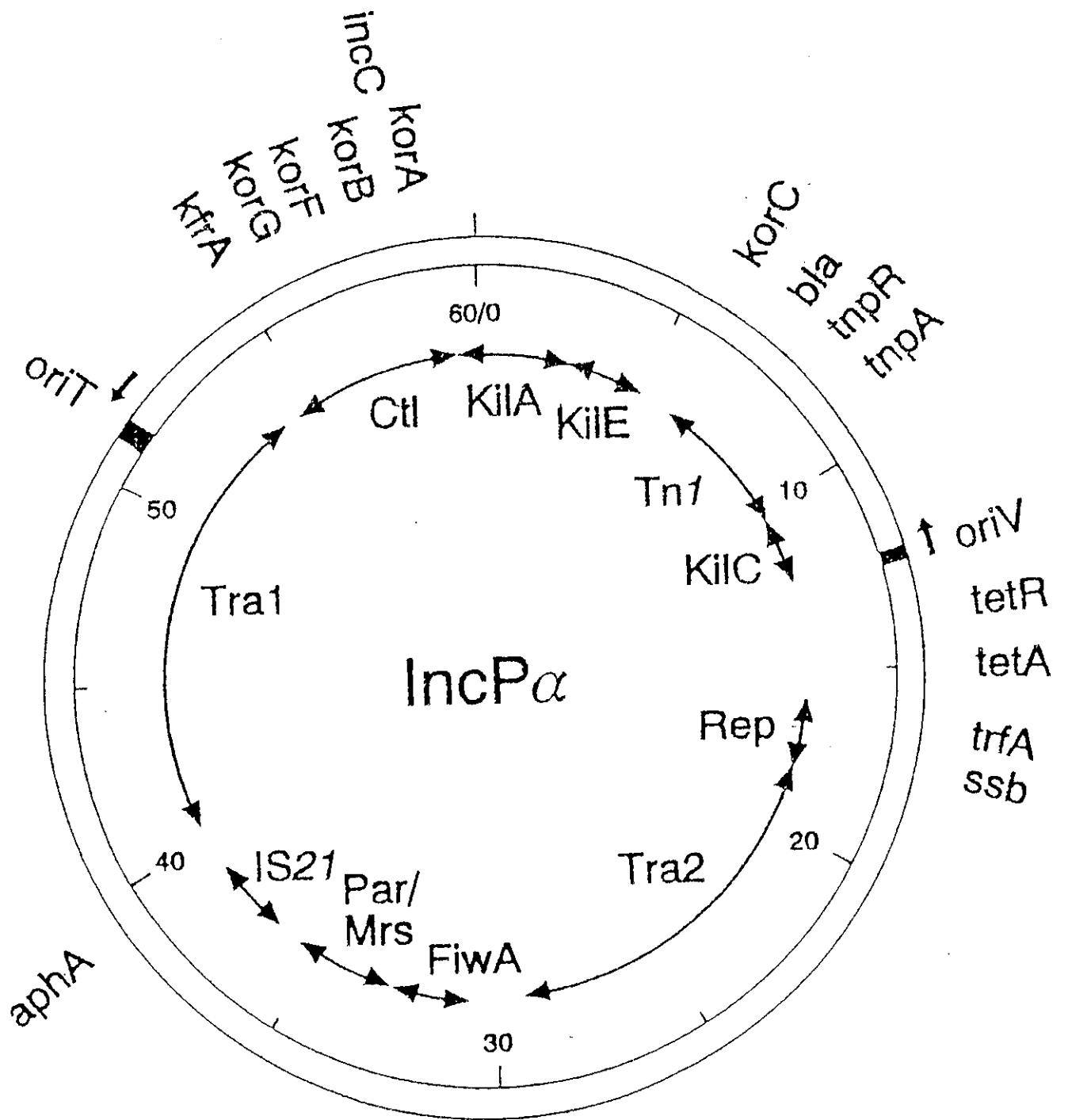
図は OriV(vegetative replication origin)領域のプラスミドが休止期の状態から DNA 複製を始める開始点を示したものである。TrfA は oriV の iteron に結合すると oriV は活性化され一方向の DNA 複製を開始する。またその間に蛋白質 DnaA が結合するサイト dnaA Box が3カ所にみられる。遺伝子 trfA は oriV を含む上流域に存在し、その蛋白質 TrfA は2種類 TrfA1, TrfA2 である。iteron1 と iteron10 の距離は 1090bp である。

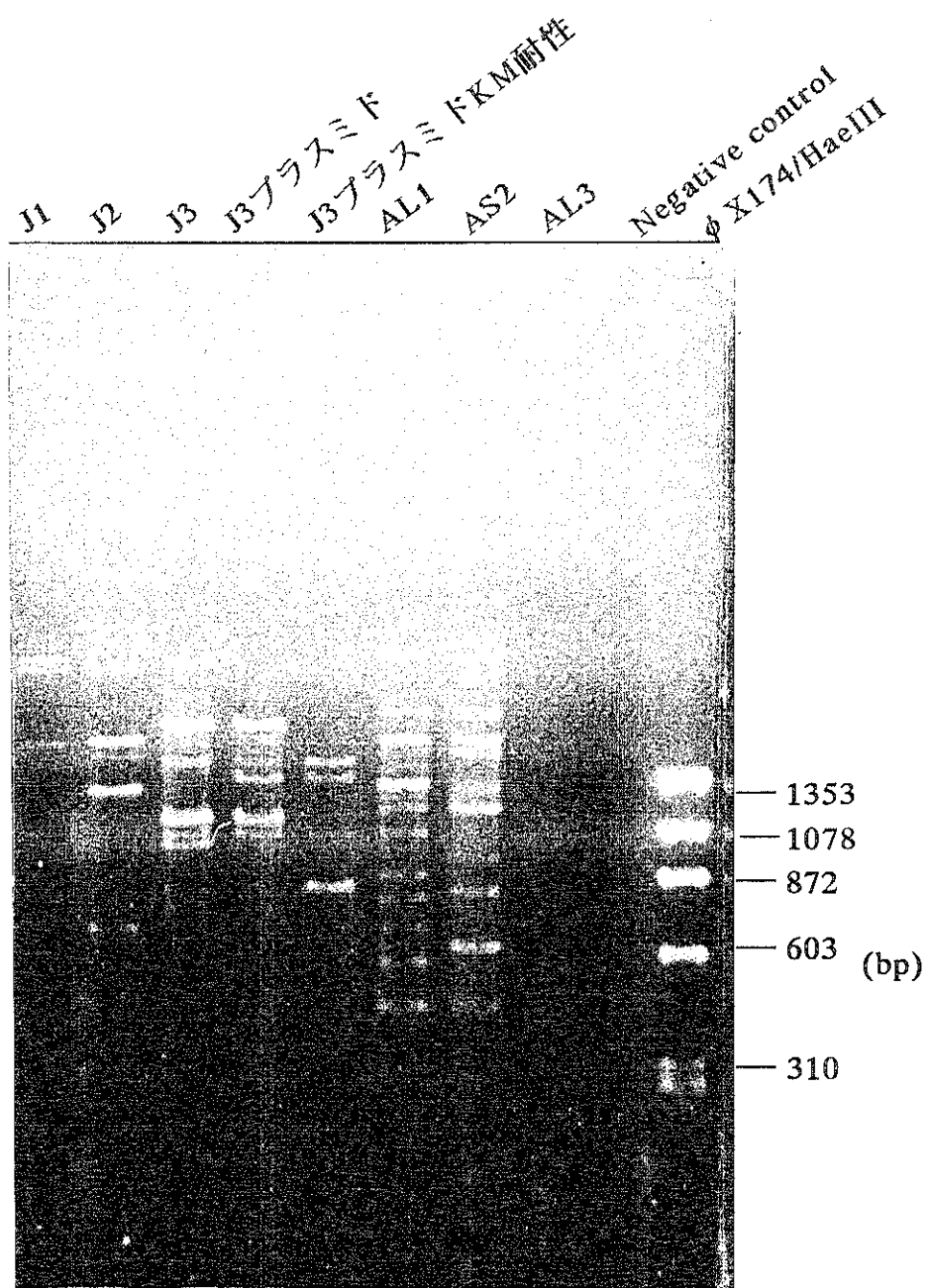
iteron1 と iteron10 は逆向きに塩基配列が並び、iteron1 から iteron9 までは同方向に塩基配列が並んでいる。また iteron4 と iteron5 の間の距離が他と比べて大きいことから(間に dnaA が2つ入っている)、ループには役割を分担する2つの iteron ブロックがあると考えられる。バンドのパターン変化を示すものと考えられる。

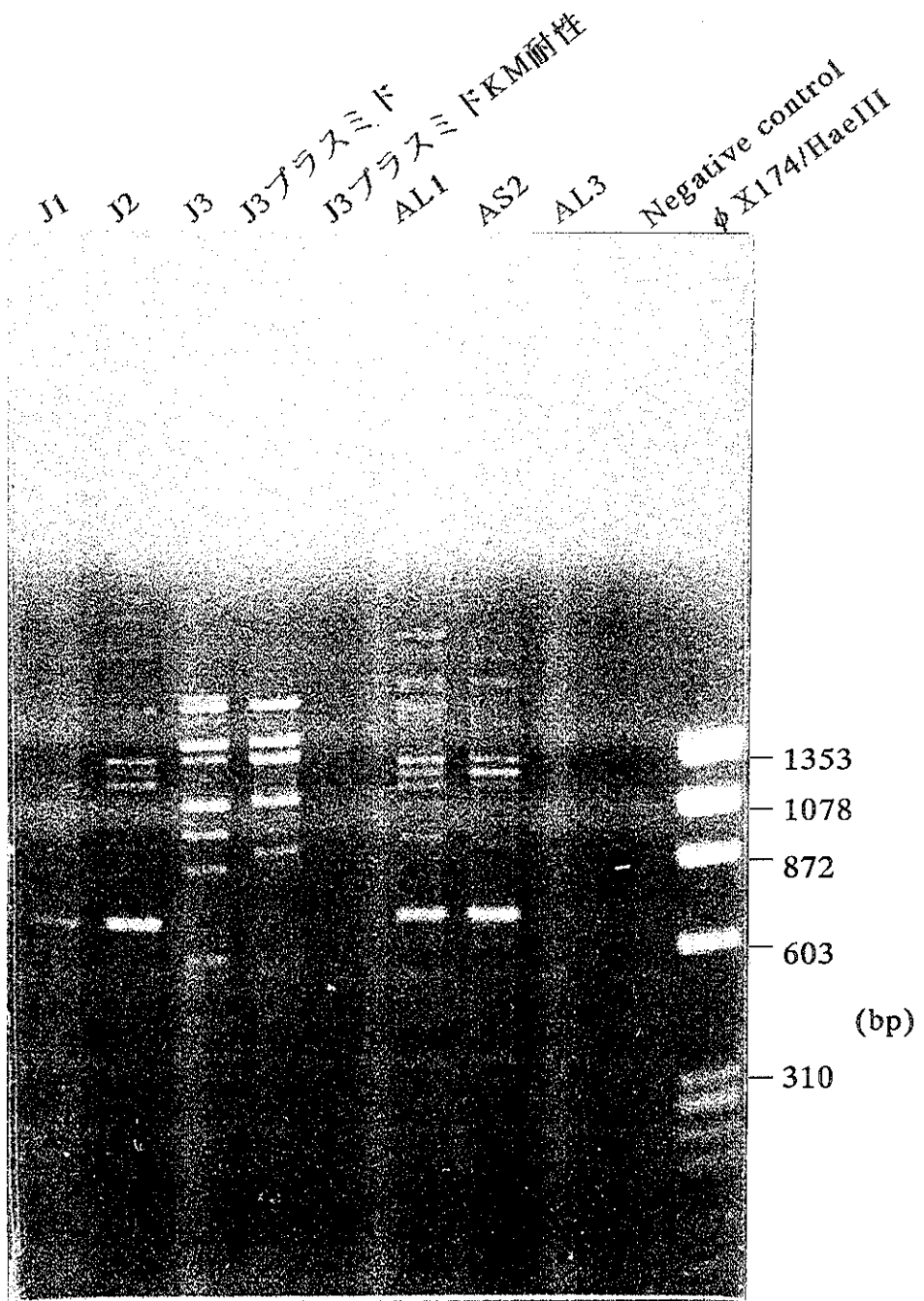
写真は iteron1 と iteron10 を用いて遺伝子増幅プライマーとし、PCR 増幅を行ったものである。マグネシウム濃度を 1.5mM と一定にし、アニーリング温度を45°C、50°C、55°C、60°Cに上昇させていった。徐々にバンド数が減少していった。

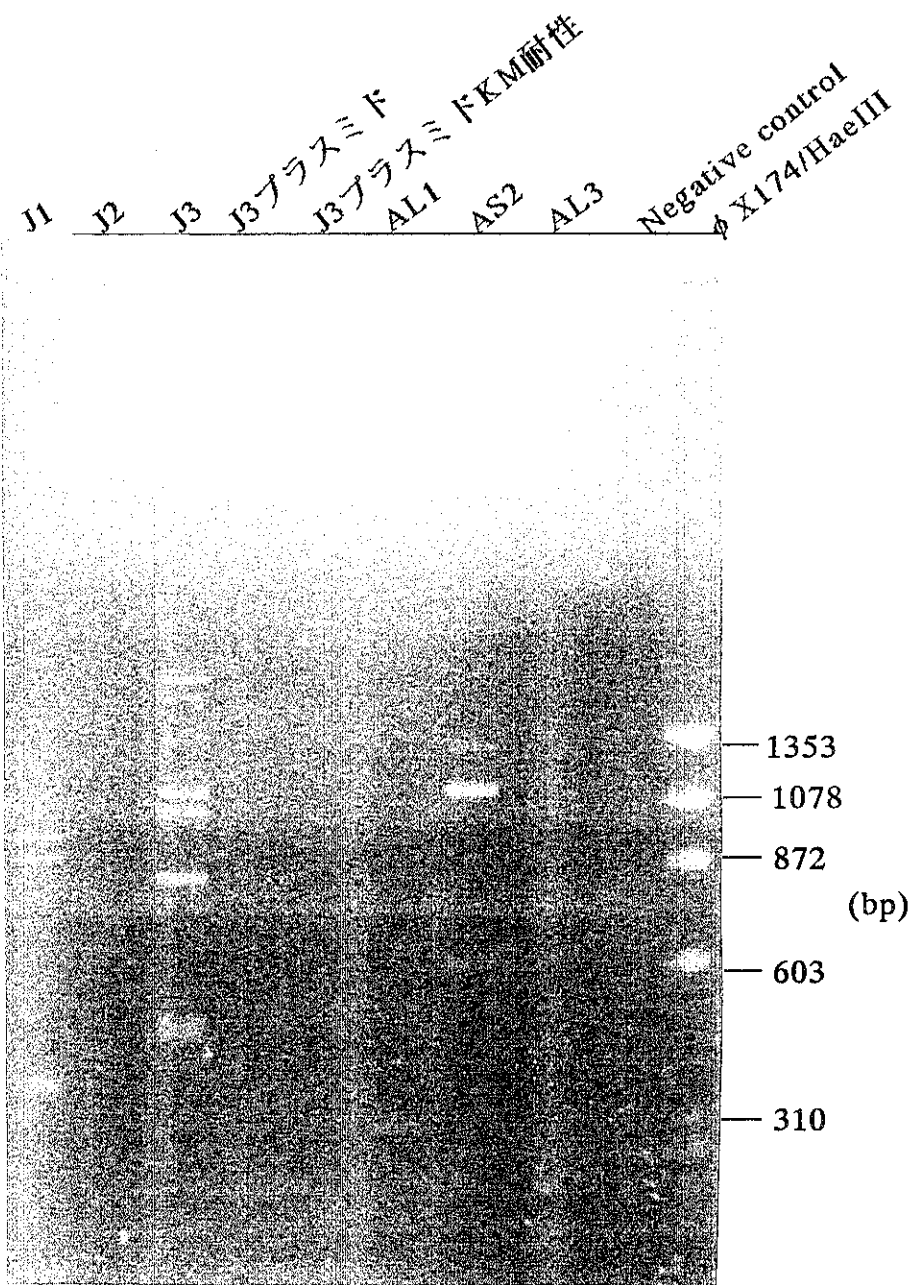
B. midousuji は発育温度が62°C以上であることから、このような温度シークエンスを行った。

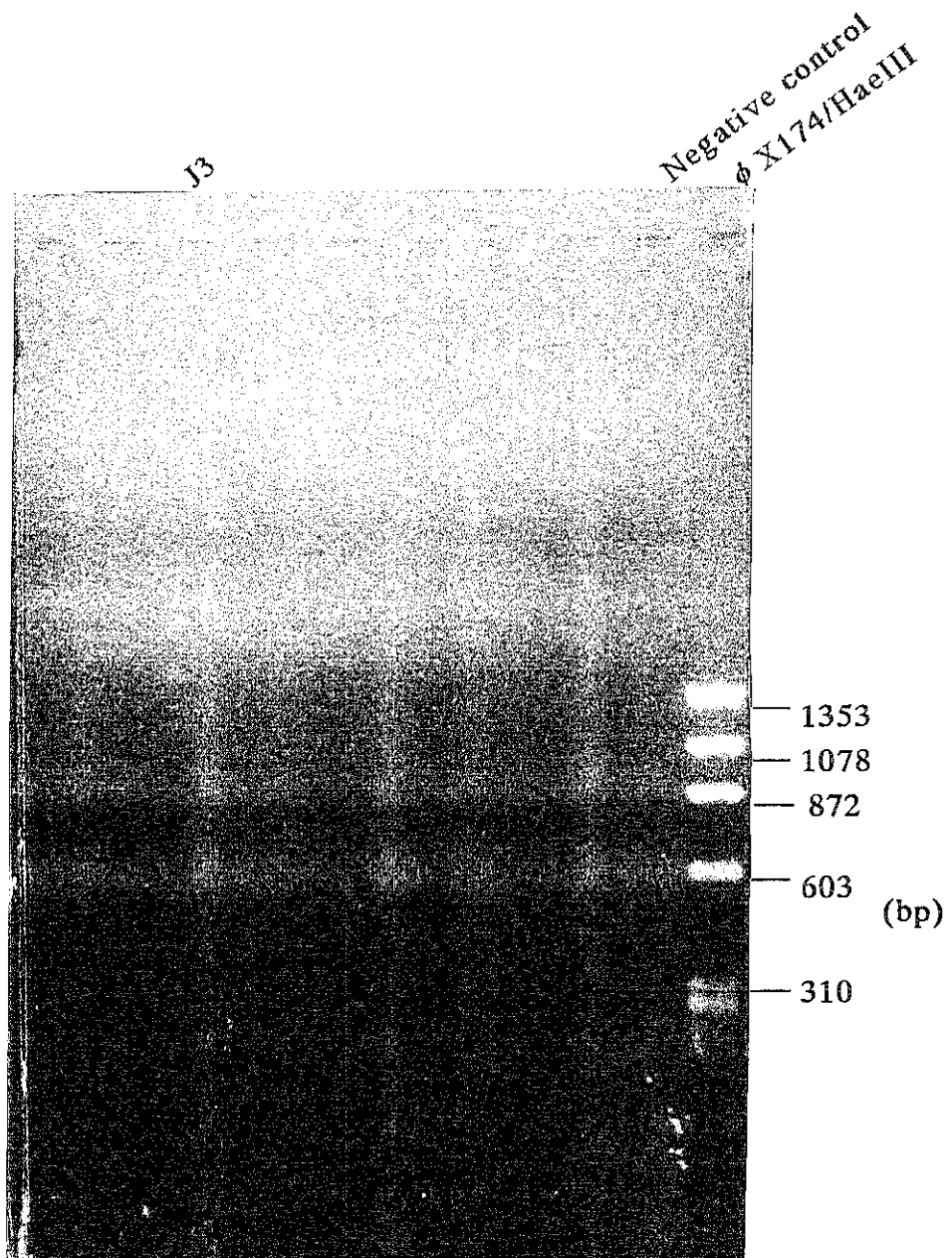
Stringencyが高い状況でバンドが出現していることから、*B. midousuji* に iteron 遺伝子塩基配列が存在するものと考えられる。











添付資料3—7

平成10年度の生活安全総合研究推進事業(外国への日本人研究者派遣事業)による米国コロロンビア大学地球環境研究所(Lamont Earth Observatory)でのニューヨーク、ジャマイカ湿地帯環境改善計画シンポジウム(The Jamaica Bay Meeting)での口頭発表抄録(1999年2月1日)

Human Health, Biodiversity and Industrial Pollution in Jamaica Bay, NY

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Anthropogenic pollution of the marine environment has significant adverse effects on endangered species, and on suitability for human recreational use. Industrial pollution is also very likely to impact adversely on human health as regards both environmental exposures to teratogens and carcinogens, as well as exposures to toxic algal blooms and human infectious agents. The restoration of the Jamaica Bay National Seashore presents itself as a possible paradigm for highly-urbanized salt march ecosystems throughout the industrialized world. Since this ecosystem has been, and continues to be, heavily impacted by exogenous contaminants it provides an excellent opportunity to study the nature of the relationship between biodiversity and pollution.

One aspect of the proposed study would require assessment of biodiversity of macrofauna and macroflora, and its often inverse correlation with increasing levels of pollution. This study would require extensive sampling of soil and water, and testing for industrial contaminants including metals, solvents, polychlorinated biphenyls (PCB), dioxin, polyaromatic hydrocarbons (PAH), and other xenobiotics. Localized sampling of macroflora and macrofauna would permit assessment of bioaccumulation of specific target chemicals, including mercury, lead, dioxin, PCB and PAH. These studies would require significant expenditures for mass spectrometric analyses of soils, water, and biological samples.

In this way connections could possibly be made linking specific areas of pollution, including their geophysical characters, and oceanographic properties to bioaccumulation in certain elements of the food chain. Only by understanding these relationships could specific steps could be proposed to address problematic components of the ecosystem.

From the anthropocentric perspective, the obvious area of investigation would be

determination of the impacts of sewage seepage, storm runoff, and sludge disposal on levels of pathogenic microflora (e.g. coliform bacteria and hepatitis viruses). Traditional bacteriological laboratory methods, and also molecular biology techniques such as the polymerase chain reaction (PCR), would be required for this aspect of the study. In addition, the various sources of pollution may impact on nutrient loading, decline in predators, loss of natural filters, and introduced species, to contribute to red tides and other kinds of harmful algal blooms. Thus, in addition to concerns about infectious agents and unanswered questions of long-term toxicities due to increased cancer and birth defects in local communities, there is a very real concern for acute toxicity in humans including gastrointestinal and neurological symptoms.

A quantitative assessment of these complex biophysical processes on human health would require mathematical modeling including the potential impacts of chaos theory, and application of traditional risk assessment methods.

Finally, an area that is perhaps least well understood is the ability of marine ecosystems, and in particular salt marshes where biodegradative processes are highly active to naturally buffer exogenous impacts. Our specific interest centers on microfauna including species of *Bacillus* to mediate biodegradation of environmental contaminants. Our recent studies indicate that thermophilic bacteria isolated from compost are capable of digesting organic (fish carcasses) and as well as inorganic (plastics) pollutants. Specific plasmid-encoded genes were found to be responsible for these various biodegradative properties, and these were linked to antibiotic resistance genes. Our studies also suggest that bacteriological toxicity toward algae could potentially be a natural method for buffering against anaerobic conditions. The possibility exists that similar bacteriological systems may exist in industrially impacted salt march ecosystems. Studies of bacterial evolution indicate that under certain environmental conditions processes in addition to random mutation may drive genotypic and phenotypic changes.

Taken together these findings suggest the need for investigation of the biodiversity of microflora at various sites within the marshlands with various human impacts. This study would also require bacteriological and molecular biological techniques such as PCR.

口頭発表に対する Raymond N. Sambrotto, Senior Research Scientist, Lamont-Doherty, EarthObservatory (sambrott@ldeo.columbia.edu 61 Rt. 9W/ P.O. Box 1000Palisades, NY 10964 U.S.A. phone: 914-365-8402 fax: 914-365-8150)から Environmental Genetics Laboratory at Lamont, Columbia University 開設の通知

Title: Development of a Genetic Analysis Facility at Lamont and its Application to Environmental Problems

Introduction and rationale:

There has been an explosive growth in the capabilities and applications of biotechnology over the last two decades. Many of what were once cutting edge laboratory techniques in the analysis of DNA have quickly been transformed into almost routine procedures. Most of these advances have been driven by medical applications, as the entire human genome will soon be mapped ahead of schedule.

We propose to establish the capabilities to extract, amplify and make preliminary analyses of DNA here at Lamont. These capabilities already exist on the Columbia campus at the Biology Dept. and Medical School.

However, unlike these other labs., the Lamont Genetic Analysis Facility will be devoted to environmental samples. The scientific return on this investment can be enormous, and the costs for establishing such a facility are not great. Space exists in Sambrotto's lab. (with minor modification) for this facility.

Applications:

- Analysis of microbial populations associated with polluted soils and sediments (O'Driscoll and Hoshina).
- Cladistic studies of marine and estuarine plankton (Sambrotto)
- Cladistic studies of endangered or other critical populations (CERC).

Funding sources:

- EPA
- NSF, particularly their BioComplexity Program. The estimated budget for research projects in this area alone is \$45M, and the President's request to Congress for next year suggests significantly

greater funding.

- NIH (in collaboration with health researchers).

添付資料3-8

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2. Jean Armengaud, Birgitta Happe, Kenneth N. Timmis Genetic Analysis of Dioxin Dioxygenase of *Sphingomonas* sp. Strain RW1:Catabolic GenesDispersed on the Genome Journal of Bacteriology 180: (15) 3954-3966, 1998
3. Jean Armengaud, Kenneth N. Timmis, Rolf-Michael Wittich A Functional 4-Hydroxysalicylate/Hydroxyquinol Degradative Pathway Gene Cluster Is Linked to the Initial Dibenzo-*p*-Dioxin Pathway Genes in *Sphingomonas* sp. Strain RW1 Journal of Bacteriology 181: (11) 3452-3461, 1999
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embIAJ233425.1|PVU233425 Proteus vulgaris 16S rRNA gene (strain DSM 30118)
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発表関連

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19990625

以降は雑誌/図書等に掲載された論文となりますので、
「研究成果の刊行に関する一覧表」をご参照ください。

「研究成果の刊行に関する一覧表」

Bacterium capable of biodegradation of dioxin-like substances, Sadayori Hoshina, David H. Figurski, I. Bernard Weinstein, Hiroshi Gohda and Tohru Furuichi, Organohalogen Compounds, vol.40, pp503-505, 1999

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