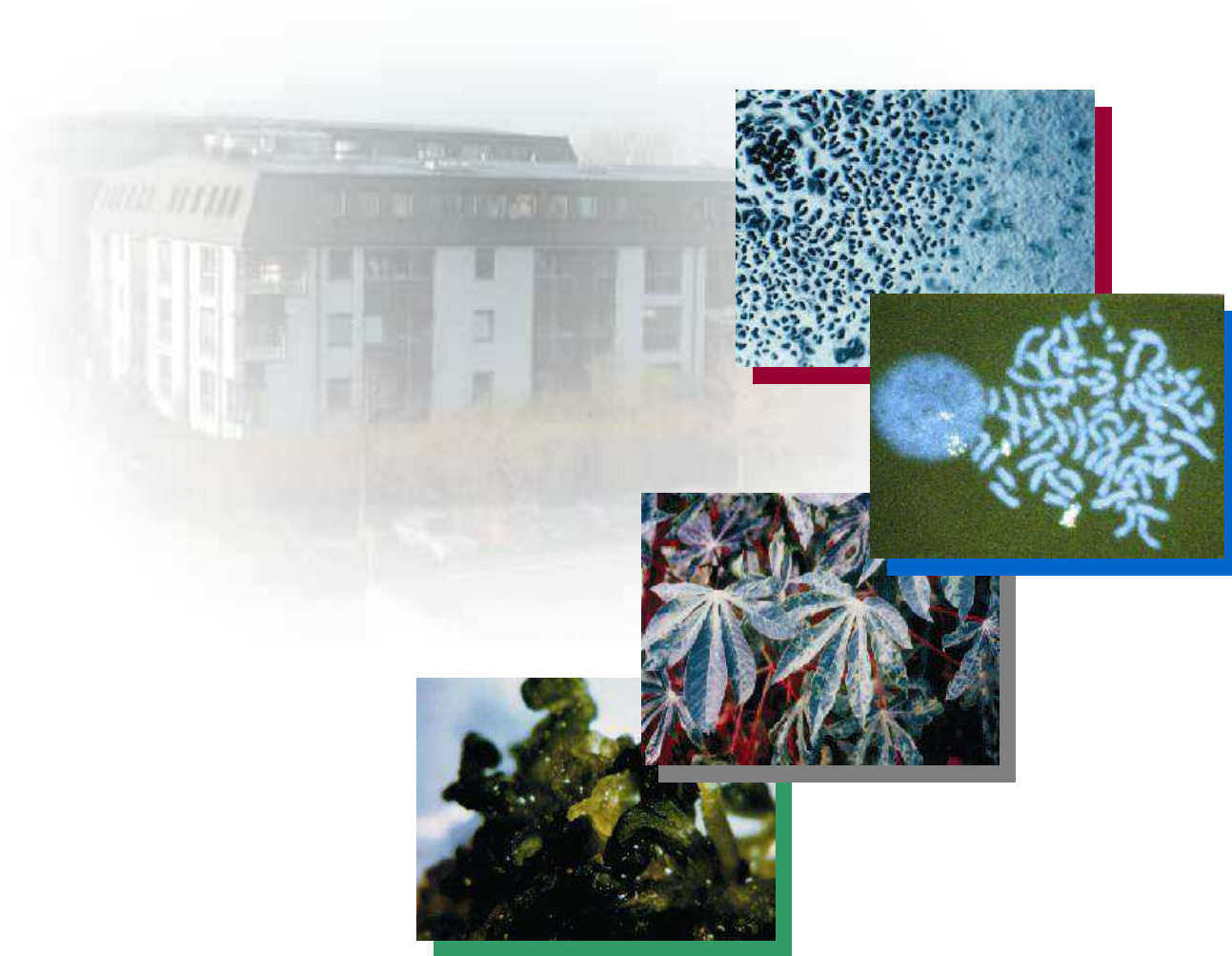


New *Actinobacteria* showing novel Properties isolated from uncommon Habitats

Microorganisms

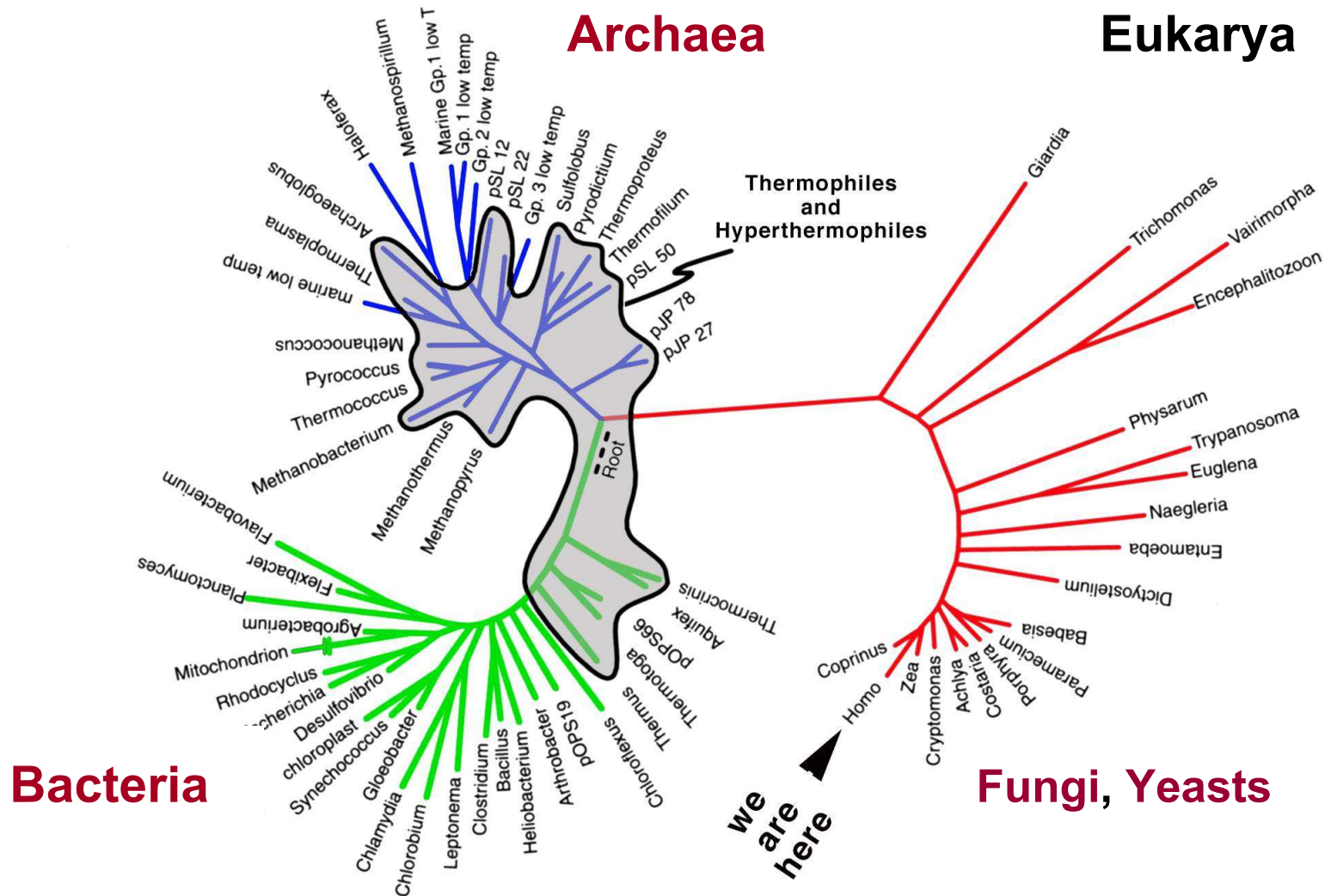


DSMZ - The German Collection of Microorganisms and Cell Cultures



Unrooted phylogenetic Tree based on the Results of comparative 16S and 18S rRNA Gene Sequencing

Microorganisms



Class *Actinobacteria*

Subclass *Acidimicrobidae* Order *Acidimicrobiales* Family *Acidimicrobiaceae*
 Subclass *Rubroacteridae* Order *Rubroacterales* Family *Rubroacteraceae*
 Subclass *Coriobacteridae* Order *Coriobacteriales* Family *Coriobacteriaceae*
 Subclass *Sphaerobacteridae* Order *Sphaerobacterales* Family *Sphaerobacteraceae*
 Subclass *Actinobacteridae* Order *Actinomycetales*

Suborder *Actinomycineae*

Family
Actinomycetaceae

Suborder *Micrococcineae*

Families
Micrococcaceae
Brevibacteriaceae
Cellulomonadaceae
Dermabacteraceae
Dermatophilaceae
Intrasporangiaceae
Jonesiaceae
Microbacteriaceae
Promicromonosporaceae

Suborder *Corynebacterineae*

Families
Corynebacteriaceae
Dietziaceae
Gordoniaceae
Mycobacteriaceae
Nocardiaceae
Tsukamurellaceae

Suborder *Micromonosporineae*

Family
Micromonosporaceae

Suborder *Propionibacterineae*

Families
Propionibacteriaceae
Nocardiodaceae

Suborder *Pseudonocardineae*

Family
Pseudonocardaceae

Suborder *Streptomycineae*

Family
Streptomycetaceae

Suborder *Streptosporangineae*

Families
Streptosporangiaceae
Nocardiopsaceae
Thermomonosporaceae

Suborder *Frankineae*

Families
Frankiaceae
Acidothermaceae
Geodermatophilaceae
Microsphaeraceae
Sporichthyaceae

Suborder *Glycomycineae*

Family
Glycomycetaceae

Order *Bifidobacteriales* Family *Bifidobacteriaceae*

FIG. 2. Proposed hierarchic classification system of the class *Actinobacteria* based on the phylogenetic analyses of the 16S rDNA/rRNA sequence data.

Discovery of useful secondary Metabolites

ex Hopwood, D. A. (2007), Streptomyces in Nature and Medicine: the Antibiotic Makers

Microorganisms



Antibiotics produced by **Fungi**, by non actinomycete **bacteria**, by **Actinomycetes**

The Actinomycetes: the good guys and the bad guys

Their good side

1. Production of Antibiotics (8000) mainly by Streptomycetes (about 100 are in use)
2. Recycling plant and animal debris into humus
difficulty degradable natural Polymers e. g. natural rubber (Polyisoprenes):
Gordonia polyisoprenivorans, *G. westfalica*
Keratin: *Pilimelia columellifera*, *Amycolatopsis keratiniphila*
Degradation of Xenobiotics e.g. polycyclic aromatics:
Mycobacterium hodleri, *M. chlorophenicum*,
3. Bio-leaching of ore: strains from alkaline slag dump *Nocardiopsis metallica*
4. Atmospheric nitrogen fixing:
Frankia alni, *Micromonospora coriariae*, *Kribella lupini*
5. Producer of Amino Acids: *Corynebacterium glutamicum*

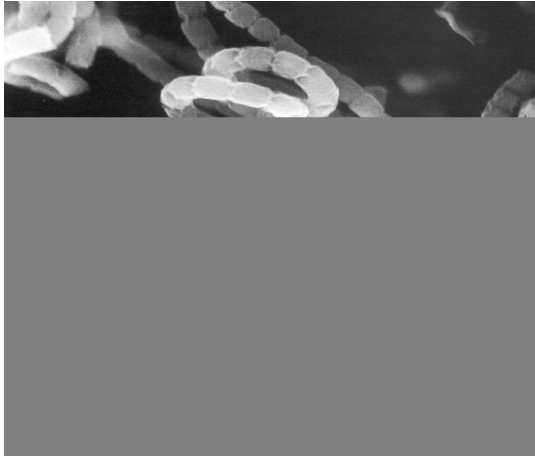
Their dark side

1. The most dangerous Infection Disease in the world Tuberculosis caused by an Actinomycete, *Mycobacterium tuberculosis*.
Nocardiosis another lung disease: *Nocardia exalbida*, *N. arthritidis*
2. Producer of toxic secondary metabolite: *Nocardiopsis umidicoholae*

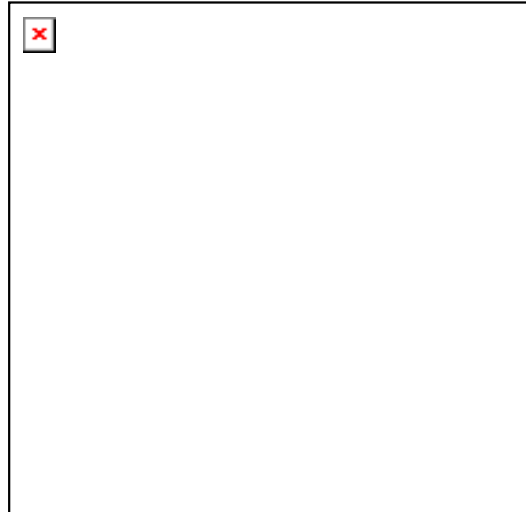
- 1. Phylogeny of Actinomycetes**
- 2. Specific Methods for Isolation of Actinomycetes**
- 3. Polyphasic Classification of Actinomycetes**
- 4. Isolation and Identification of Actinomycetes from different Habitats**

Morphology of Actinomycetes

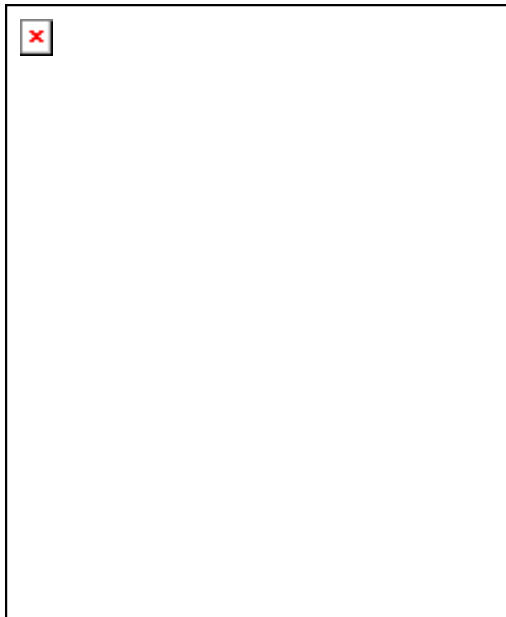
(ex. Our invisible Partners, Meiji 1992)



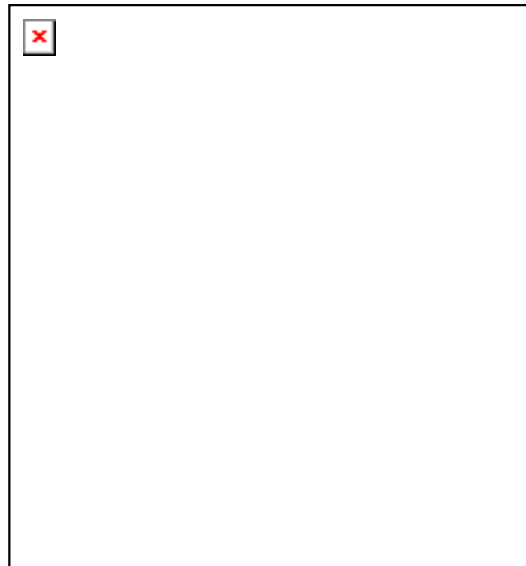
Streptomyces nojiriensisの胞子連鎖 (×13,000)
Streptomyces属の菌群では気菌糸上に胞子の長い連鎖を作るのが特徴である。胞子表面は平滑状、粗面状、イボ状、トゲ状、毛状など多彩である。この写真の菌株は野尻湖畔で採取され、抗生物質ノリマイシンを生産する。



Nocardia brasiliensisの菌糸 (×17,000)
抗酸性であり、菌糸は分断して胞子となる。この種は最初ブラジルの風土病の起因菌として発見された。



Streptosporangium viridogriseum SF2381株の胞子のう (×2,100)
胞子のうの膜が厚く別属として独立すべきとする説が有力になってきた。



Streptosporangium roseum SF2294株の胞子のう (×7,000)
気菌糸の先端に球状の胞子のうを形成する菌群。胞子のうにはらせん状に胞子が並んで入っている。胞子のう胞子には運動性がないのが特徴である。

Specific Methods for the Isolation of Actinomycetes

1. Membrane Filter Technique
2. Anderson Sampler
3. Bait Technique

**Isolation of Actinomycetes of an Indoor Plaster Sample
using the Membrane Filter Technique
(Sample Id 4893)**



Isolation of Micro-organisms from an Indoor Plaster Sample (Id 4893)



Isolation of Actinomycetes from Indoor Plaster Sample using the Membrane Filter Technique (Sample Id 4757)



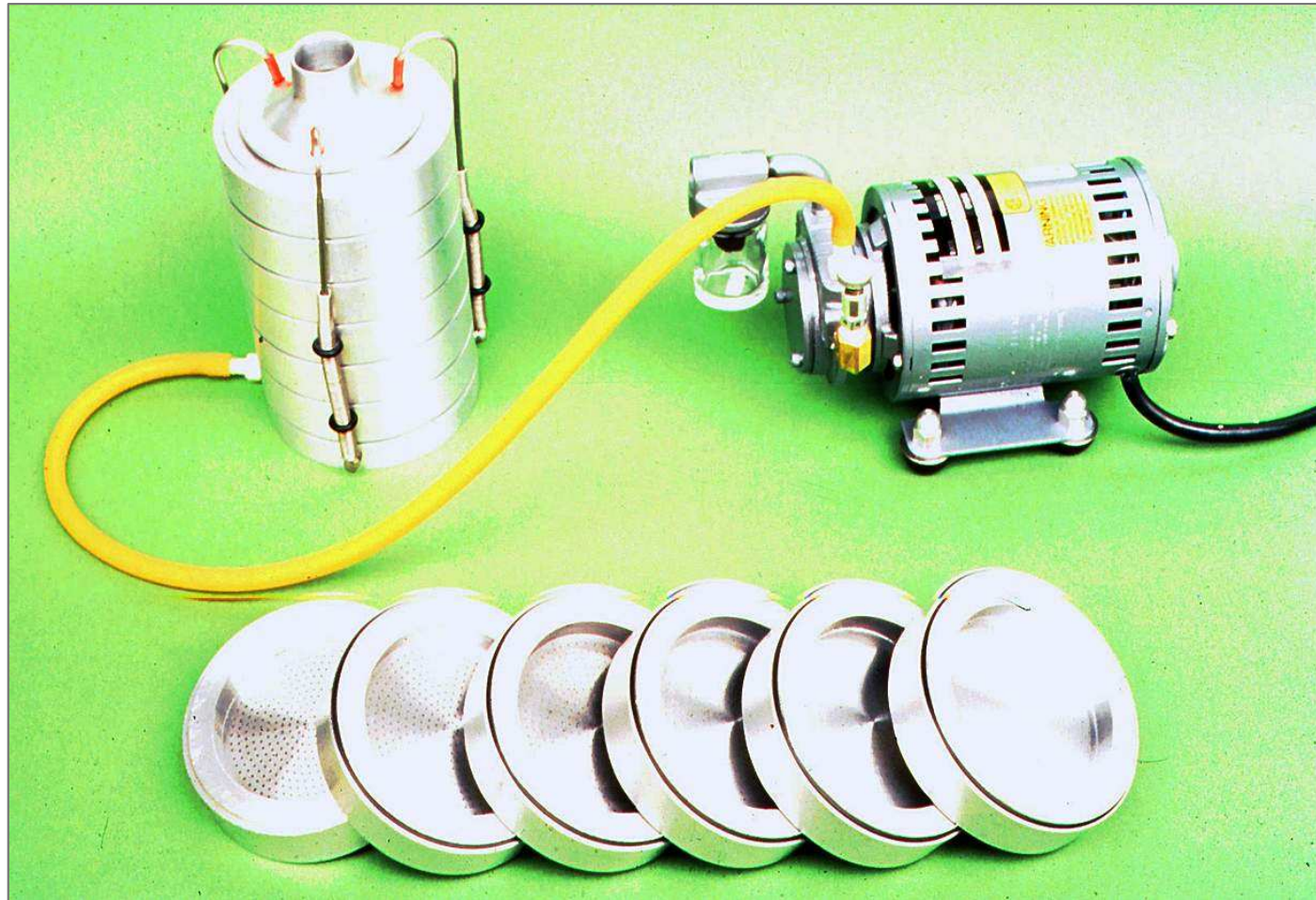
Isolation of Actinomycetes from Indoor Plaster by Membrane Filter Technique (Sample Id 4757 Section)



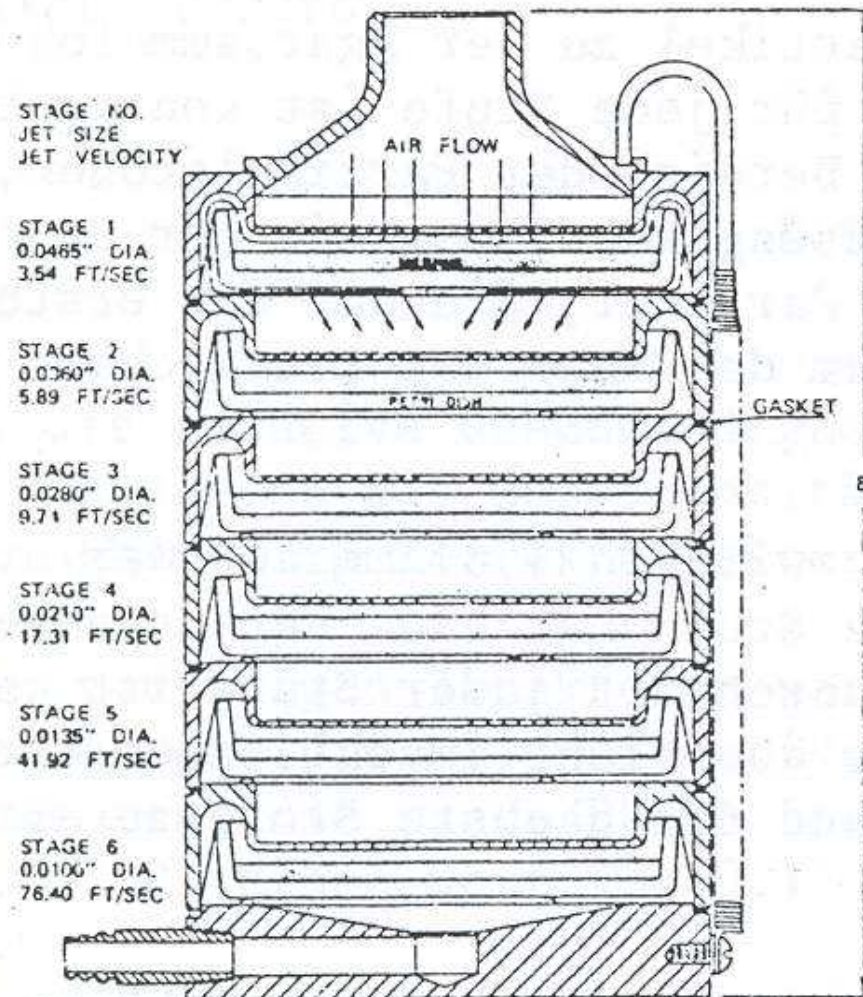
Viable (Microbial) Particle Sizing Sampler

6 Stage Andersen Sampler

Microorganisms

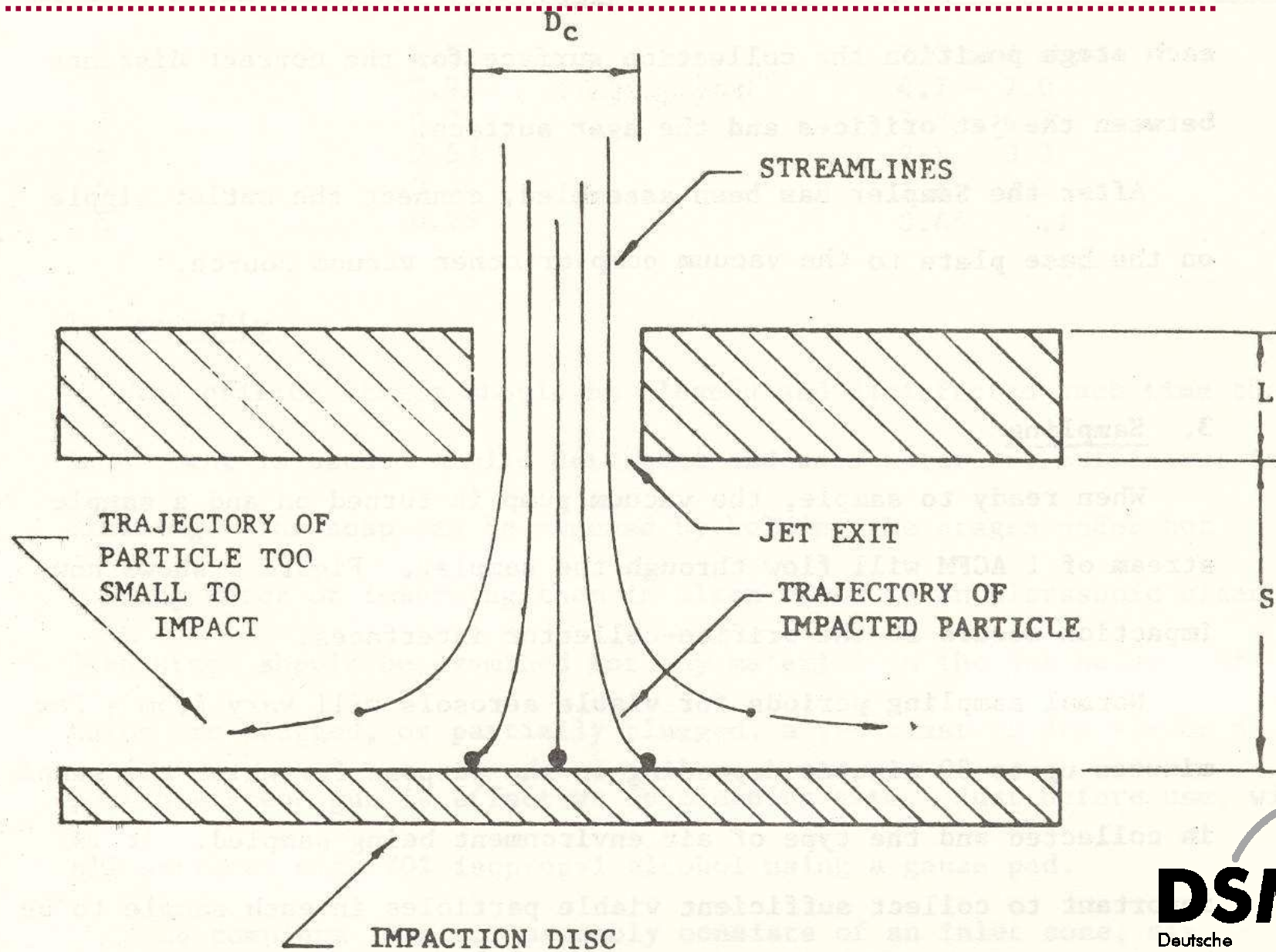


Schematic Drawing of an Anderson 6 Stage Bacteria Sampler

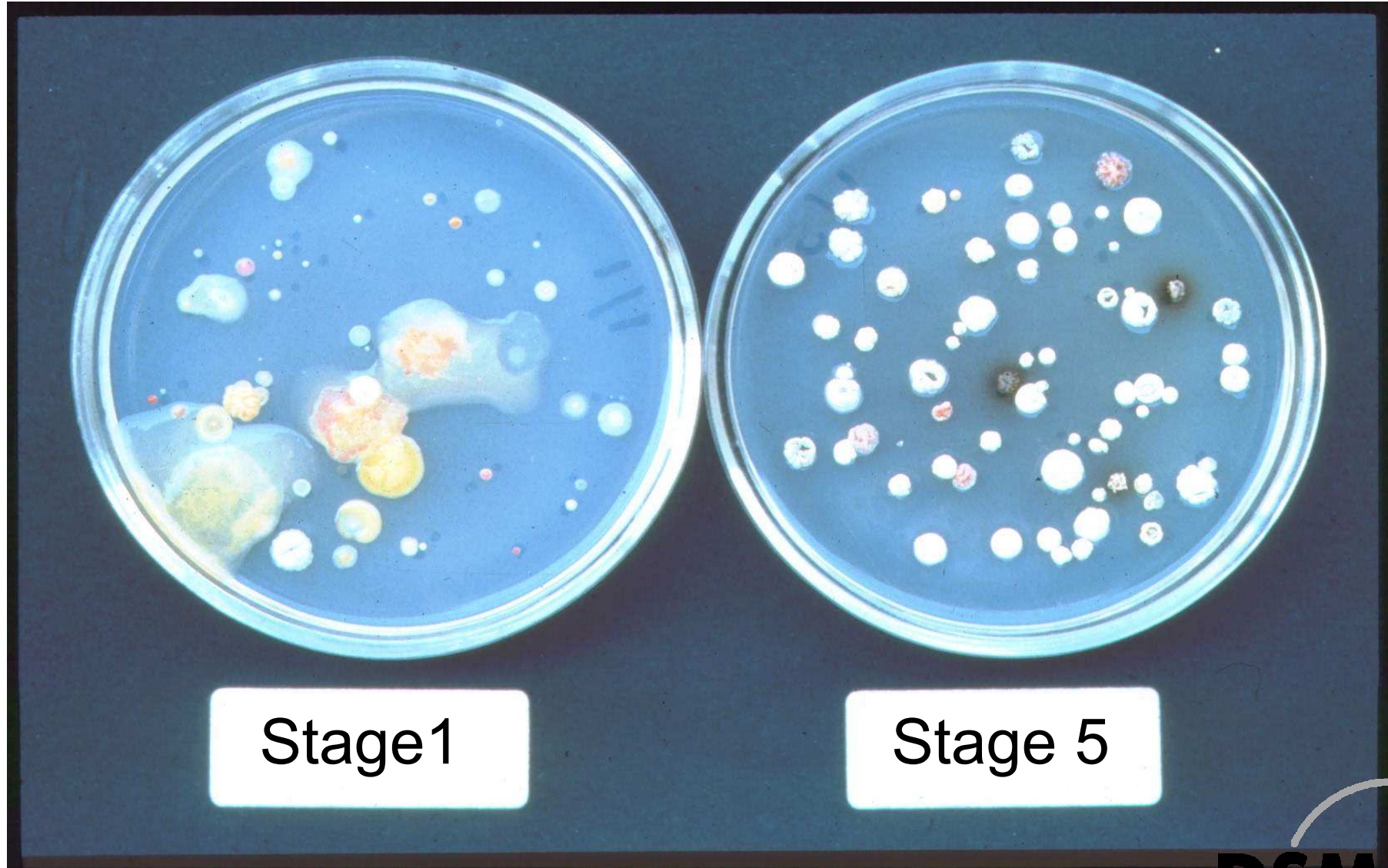


Schematic drawing of a Section of an Impactor Stage

Microorganisms



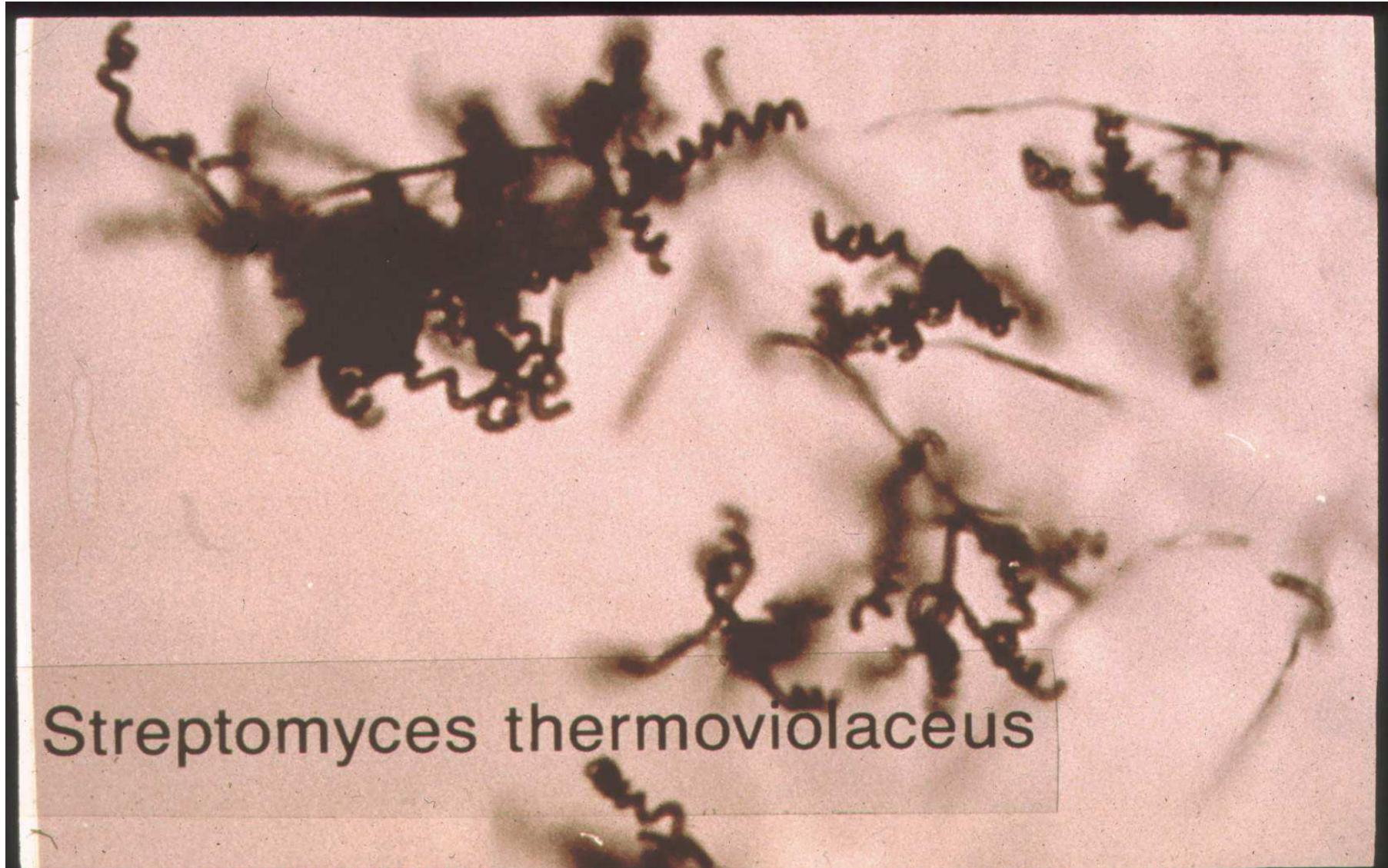
Petri Dish of Stage 1 and 5 of an Andersen Sampler



Stage 1

Stage 5

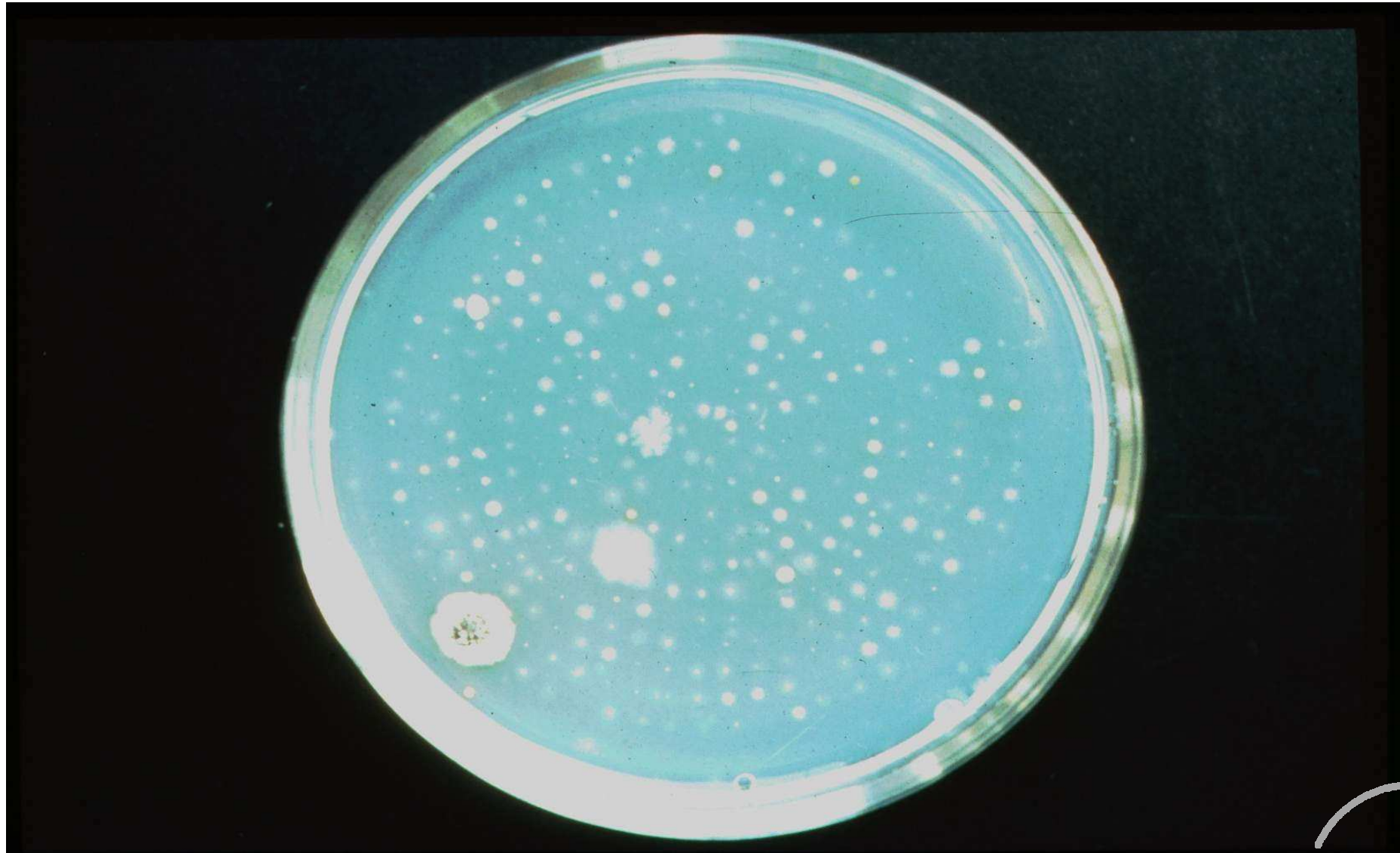
Light micrograph of a thermophilic Actinomycete



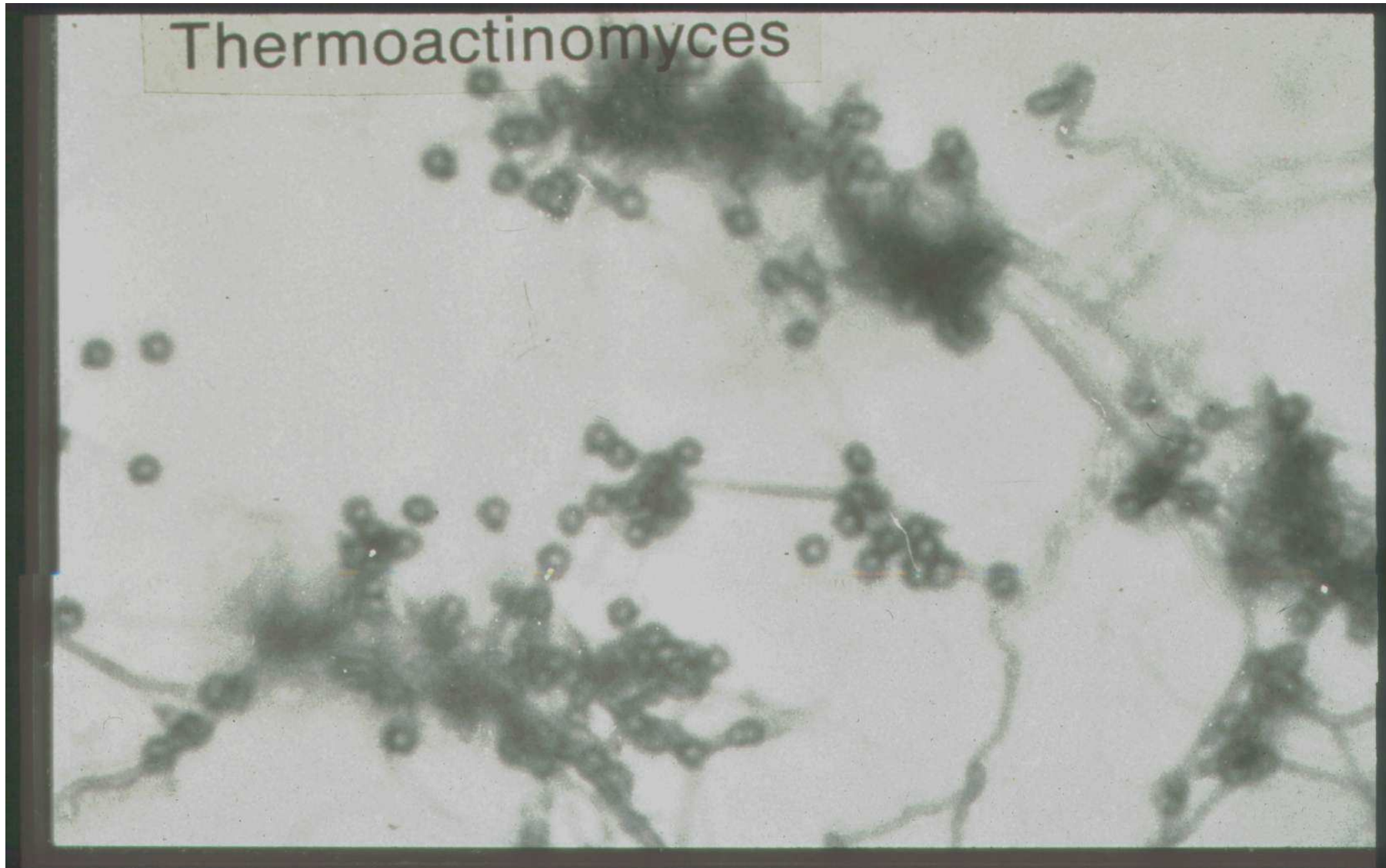
***Streptomyces thermoviolaceus* DSM 40443^T**



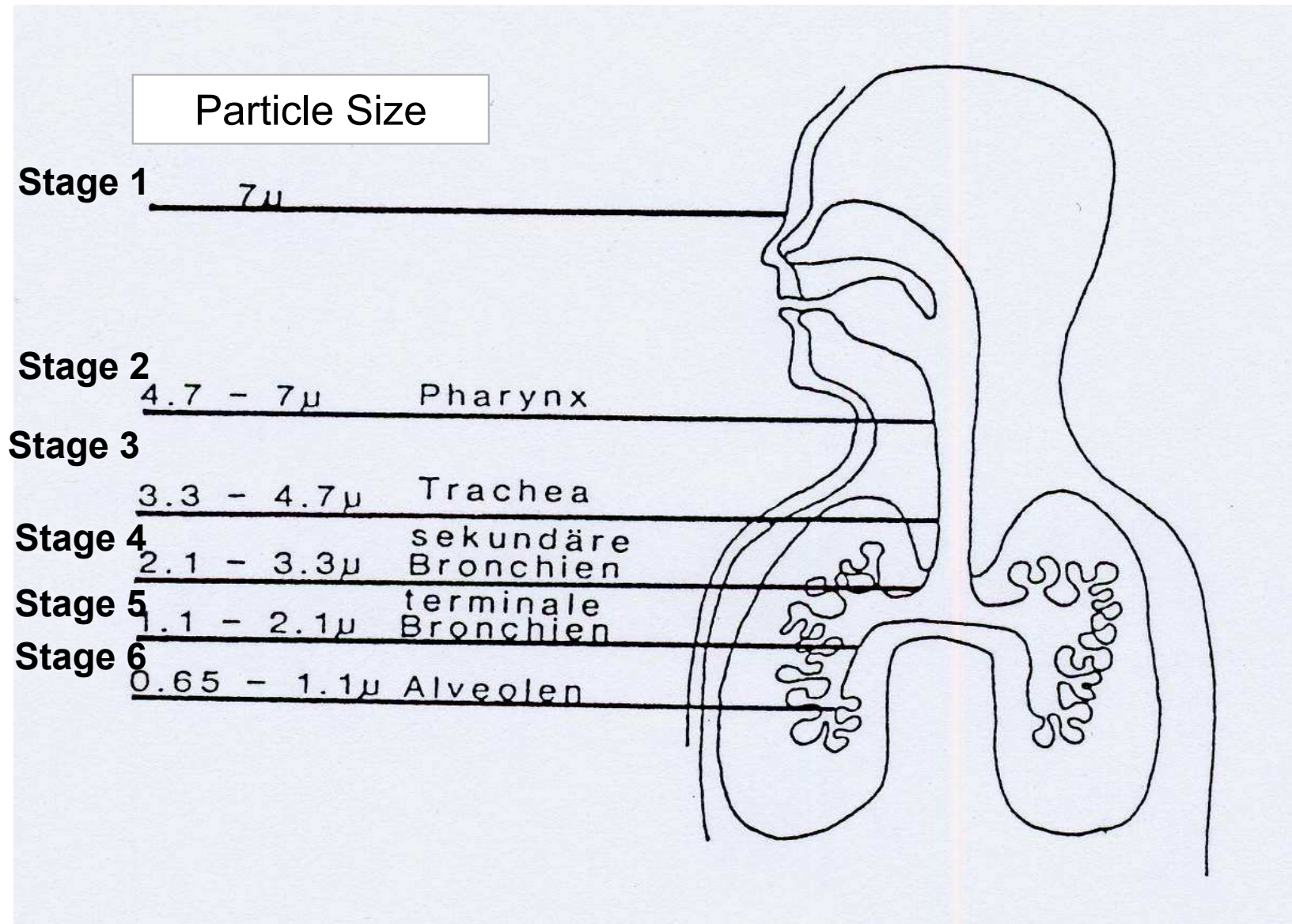
Petri Dish of Stage 6 of an Andersen Samplers



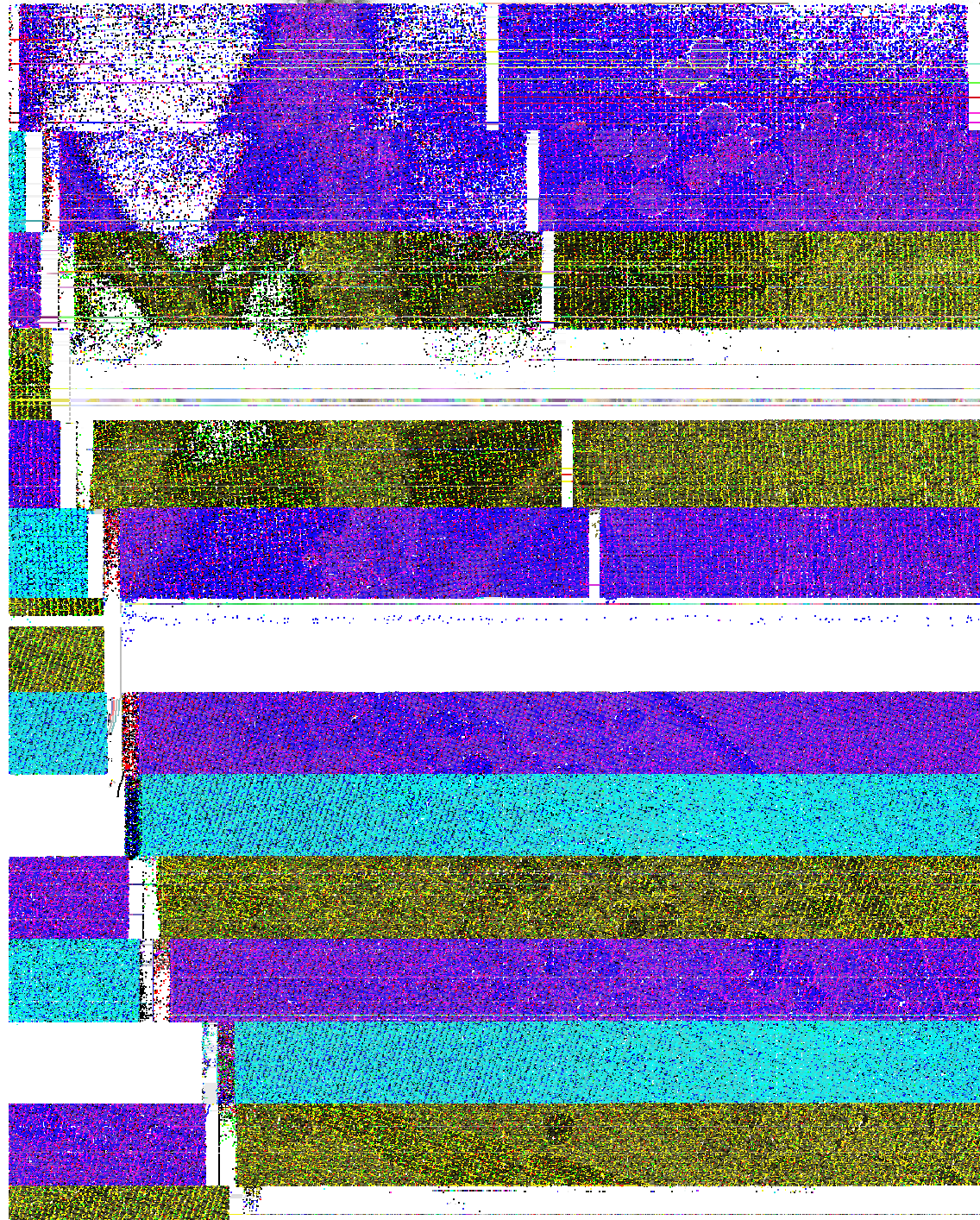
Light Micrograph of *Thermoactinomyces* spec.



Andersen Sampler Simulates Human Respiratory System



Isolation of Actinomycetes by Bait Technique



***Pilimelia
columellifera***
desintegrate the
structure of Hair

Sporangia of ***Pilimelia
columellifera*** DSM 4379
SEM , Bar, 100 µm
(by G. Vobis)
ex Atlas of Actinomycetes
Shinji Miyado 1997

Polyphasic Approach in the Taxonomy of *Actinobacteria*

Microorganisms

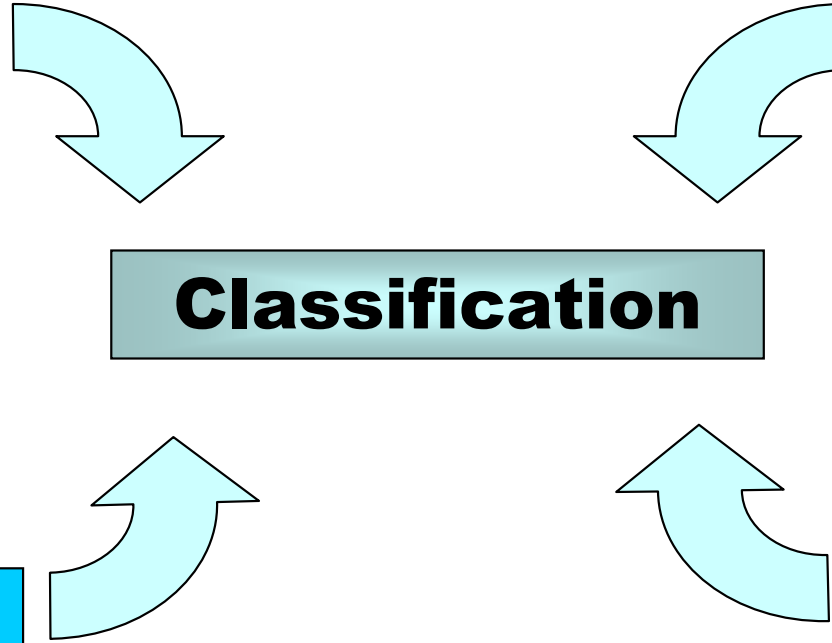
Basic Tests
Morphology

Physiological Data
Biochemical Data

Classification

Molecular Data
Sequencing
Riboprints

Chemotaxonomy
Fatty Acid Pattern
Quinones
Polar Lipids
Cell Wall
Mycolic Acids

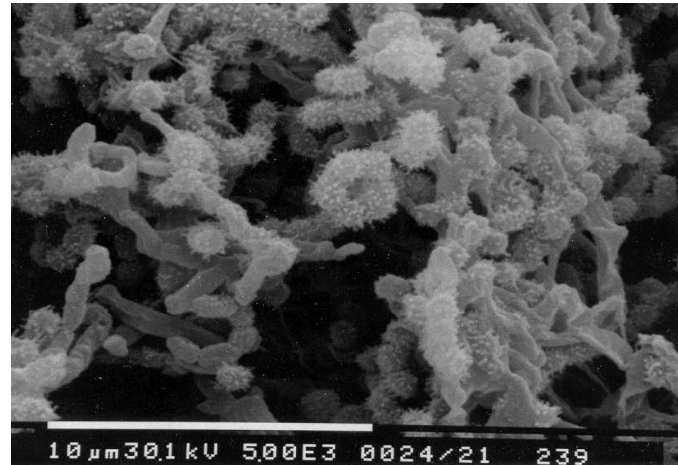


Determination of phenotypic Markers

Macroscopic, microscoscopic Features and physiological Properties



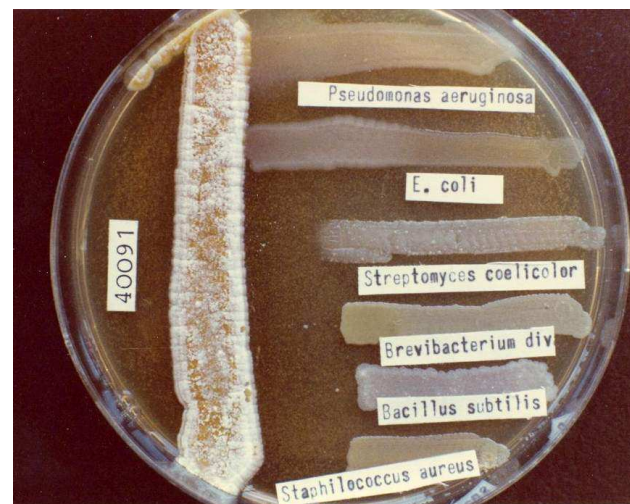
Color and Colony Morphology



Spore Chains and Spore Ornamentation

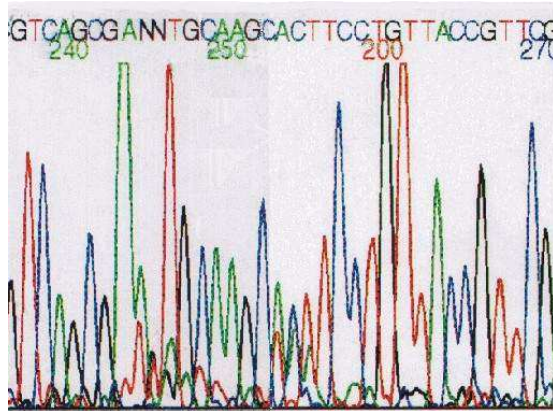


Physiological Tests

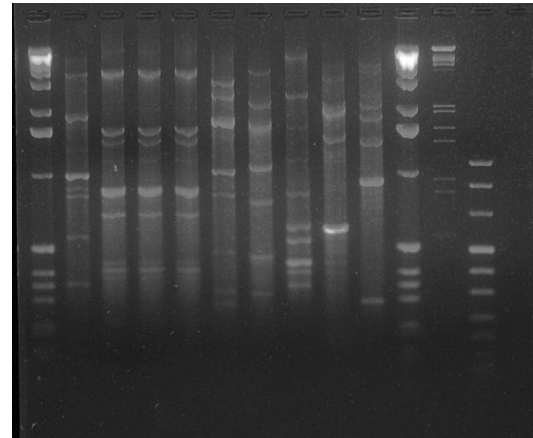


Antibiotic Activity

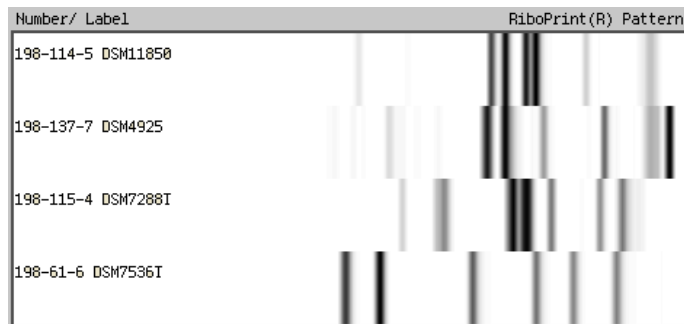
Molecular biological Methods used in the Taxonomy of Actinomycetes



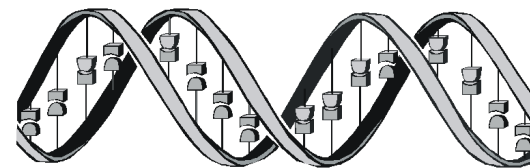
**Sequence Analyses,
Determination of phylo-
genetic Neighbours**



**Amplification of
repetitive Elements**



Automated Ribotyping



DNA/DNA Hybridization

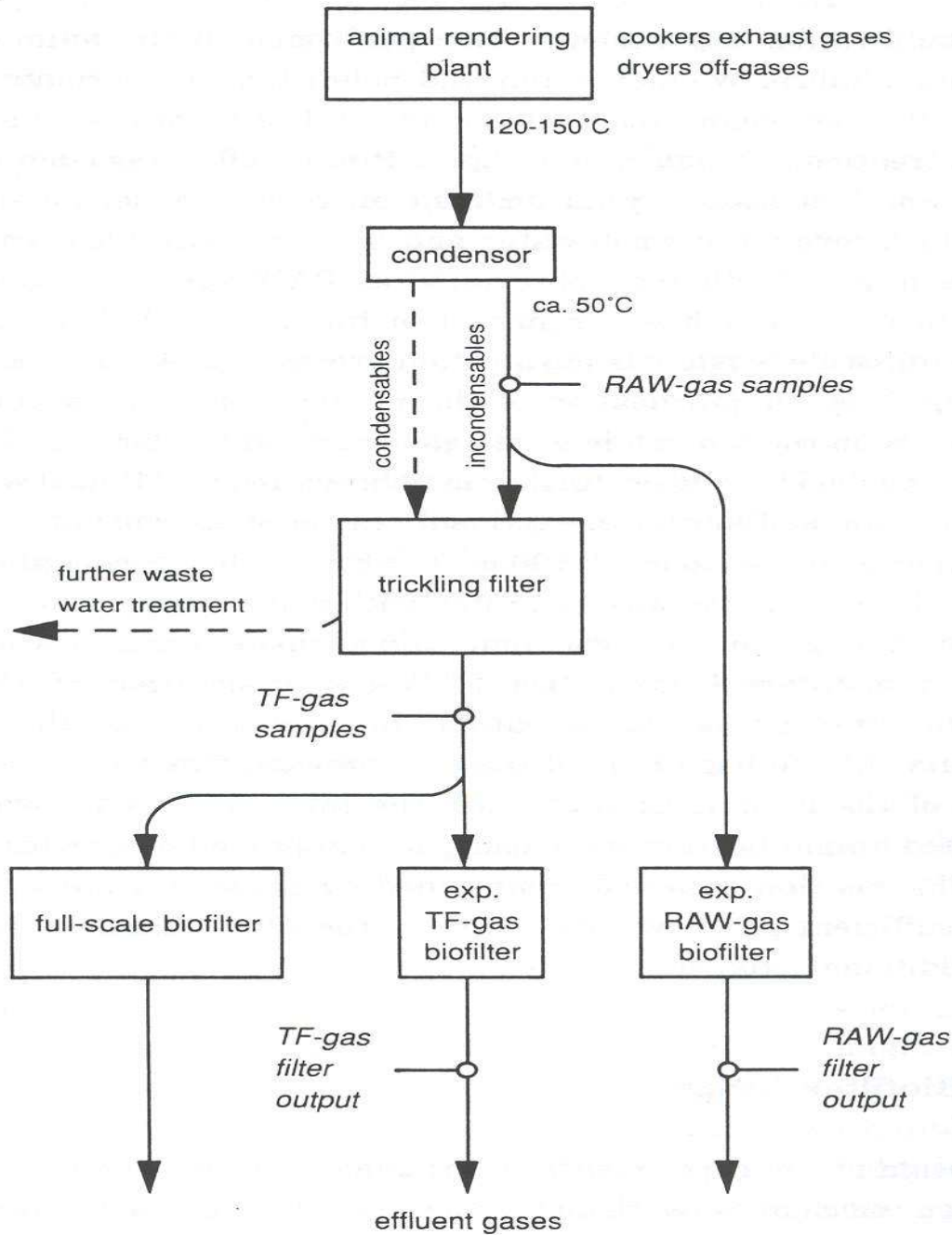
Isolation and Identification of Actinomycetes from different Habitats

- 1. Filling Material of biological Air Filters
Degrader of volatile malodorous Components**
- 2. Micro-organisms causing the Scumming Sludge in
Sewage Plants**
- 3. Composting- and Garbage Combustion Plants
viable air born Micro-organisms**
- 4. Indoor of „Moldy Houses (Air and Walls)
toxigenic Micro-organisms**
- 5. Plastic Garbage, Study of BTA Degradation
BTA, poly-(Butylenadipat-co-Butylenterephthalat)**

Microbiology of Biofilters used for the Treatment of Animal-Rendering Plant Emmissions

1. Pre-treatment of Waste Gases
2. Isolation of Micro-organisms
3. Clustering of Isolates by chemotaxonomic methods
4. Final Identification of Representatives by molecular biological Methods

Scheme Demonstrating the Origin and Pretreatment of Waste Gases used for experimental Biofilters



Schematic Drawing of an experimental biological Air Filter for trapping volatile substances

Microorganisms



Designed to clean the process off-gases of a animal-rendering plant

Experimental Biological Air Filter Chambers

Microorganisms



The two chambers at the left (hight 1m)
were loaded with TF-Gas

The Chamber at the right (hight 0.5 m)
was loaded with RAW-Gas

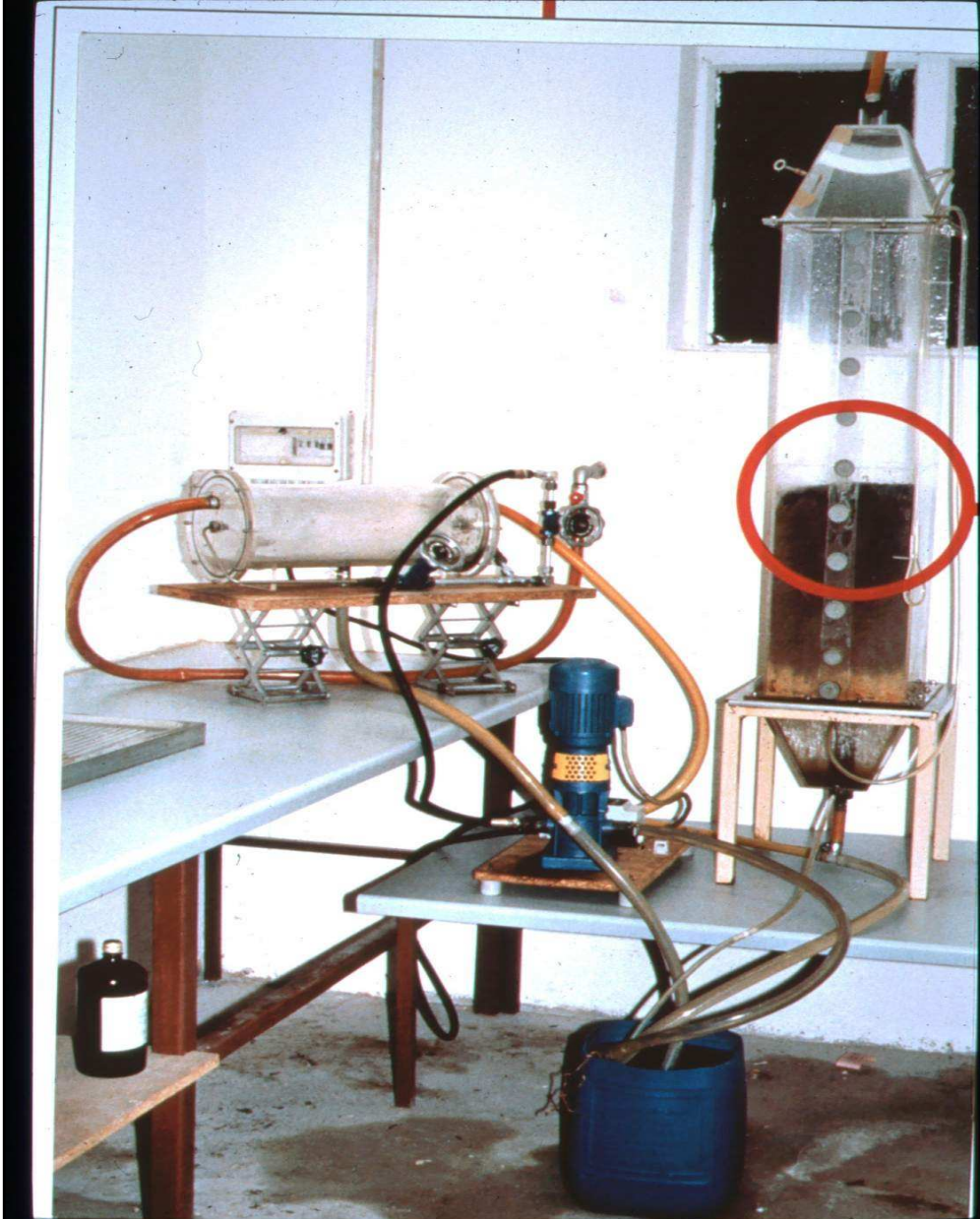
to full scale filter



Coniferous Tree Bark
Compost (Air-dried) was used
as packing Material in the
Filters

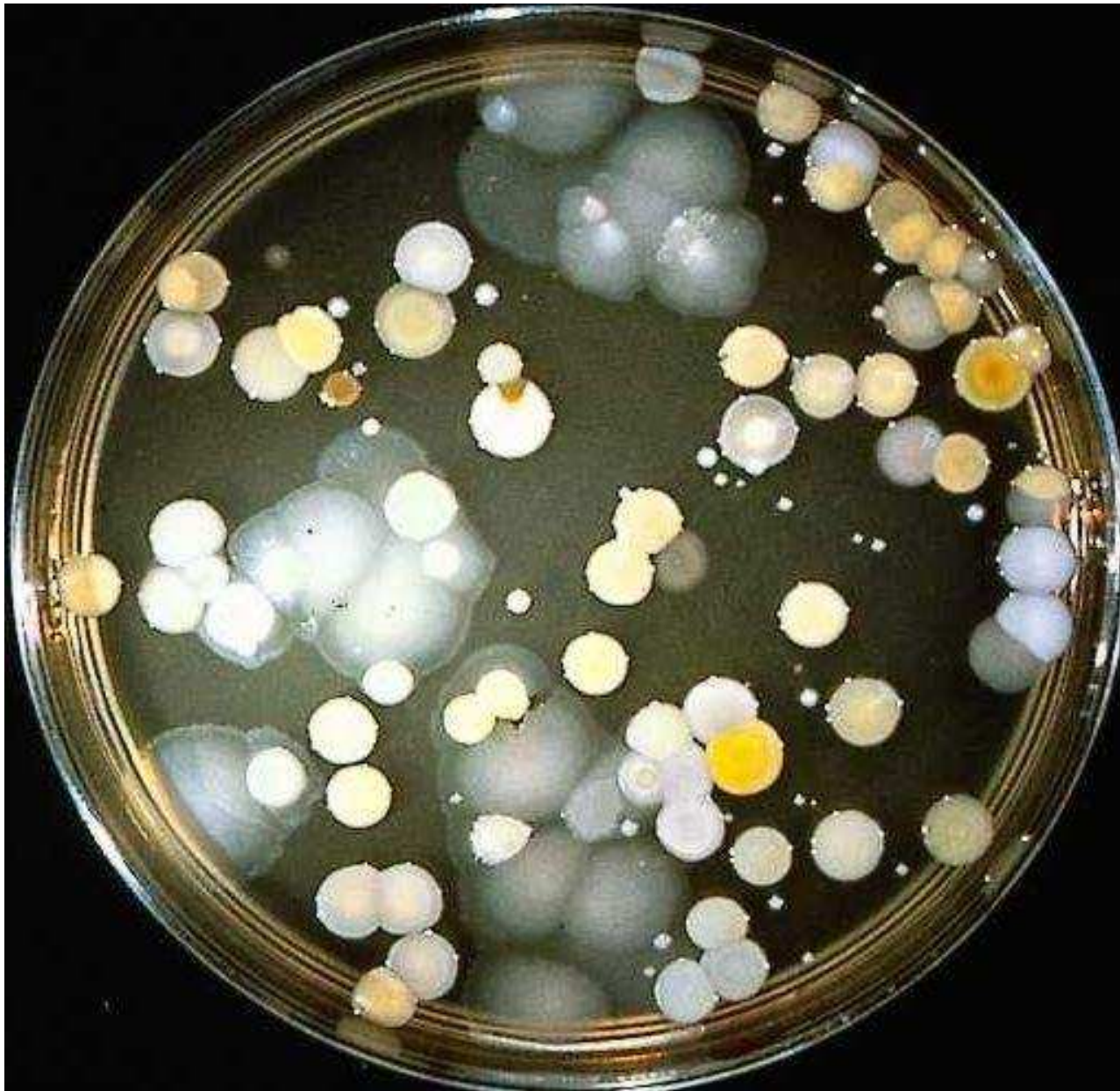
Malodorous volatile Substances are adsorbed by Bark and then degraded and assimilated by Microorganisms

Carbonyls, Furans, Thiophenes, Akylsulfides

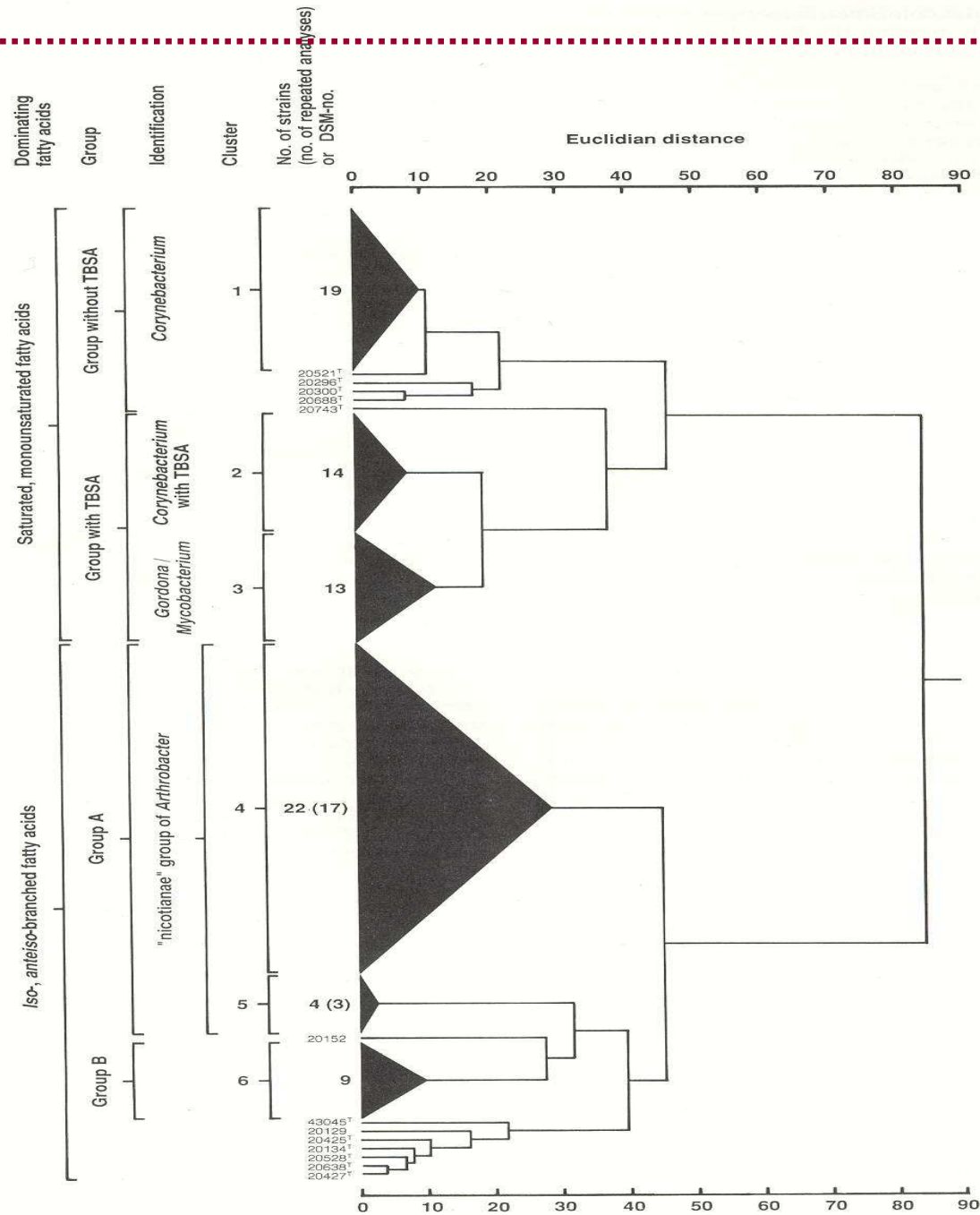


Colonies of microorganisms washed from colonized Filter Material of a biological Air Filter

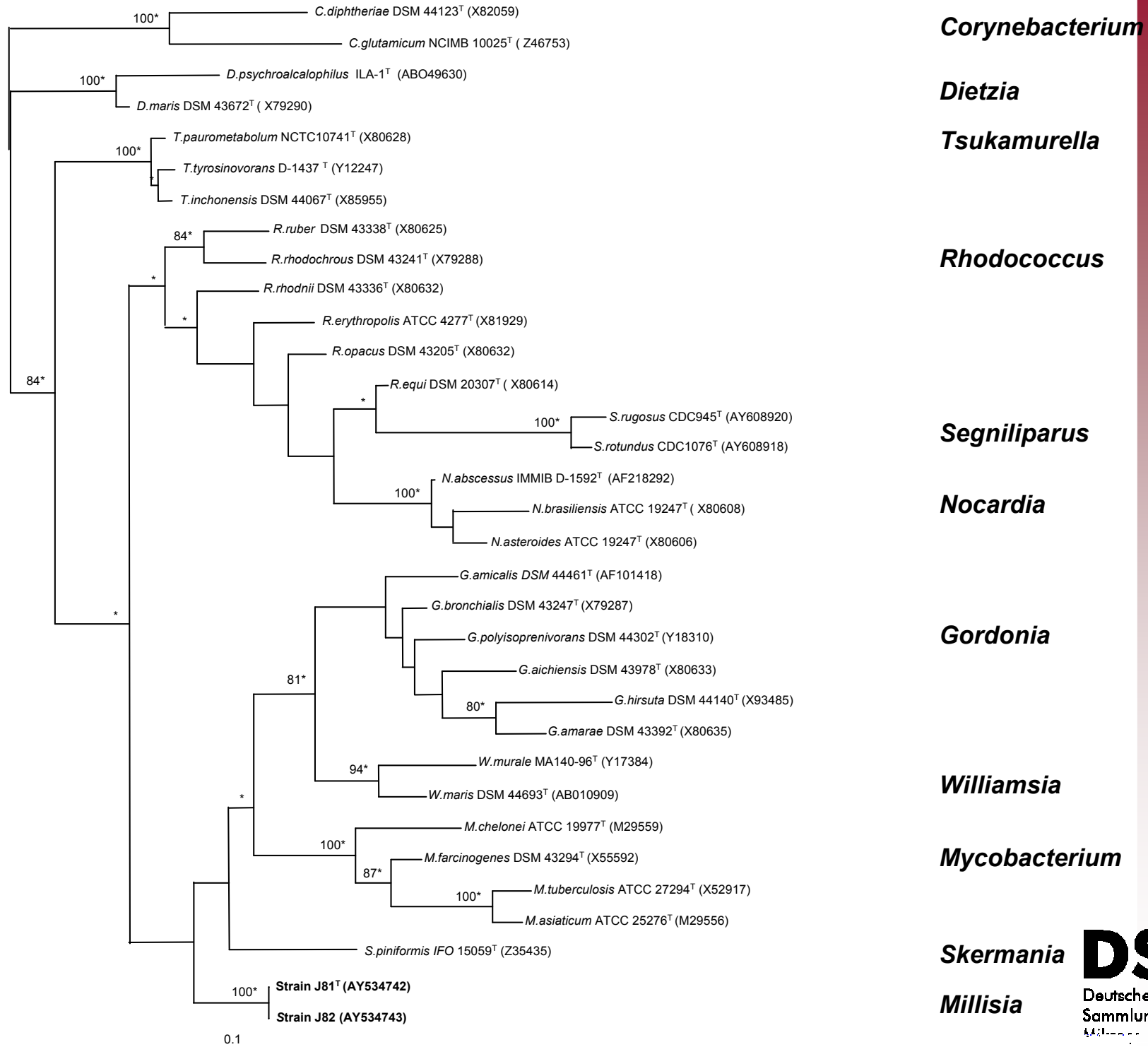
Microorganisms



Differentiation of Biofilter Isolates on their Fatty Acid Patterns



Neighbour-joining Tree based on nearly complete 16S rRNA Gene Sequences



Minimal Standards for Genera of *Corynebacterineae* one of the Suborder of the *Actinomycetales*

Microorganisms

Genotypic Criteria

Phylogenetic position based on 16S rRNA gene sequence

DNA base composition

Phenotypic Criteria

Cultural and biochemical criteria

General morphology

Acid fastness

Relation to oxygen and oxidative or fermentative metabolism

Catalase

Chemotaxonomic criteria

Amino acids + sugars from whole cell hydrolyzates

Acyl type of peptidoglycan

Mycolic acids

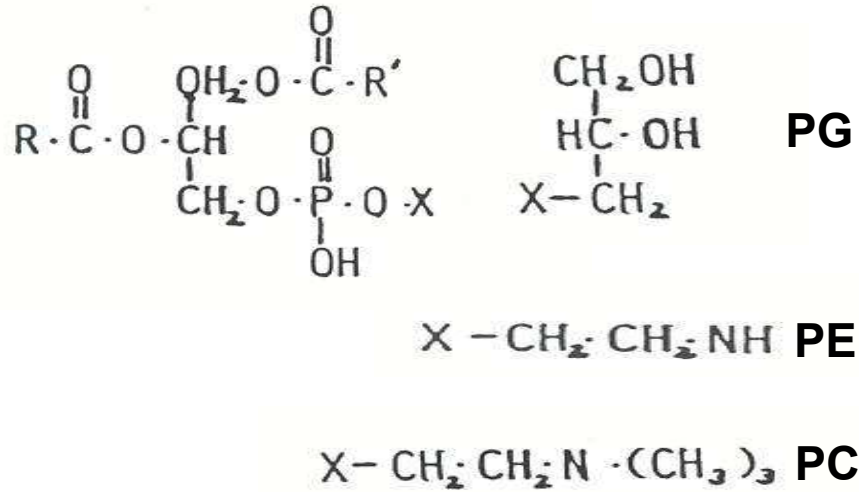
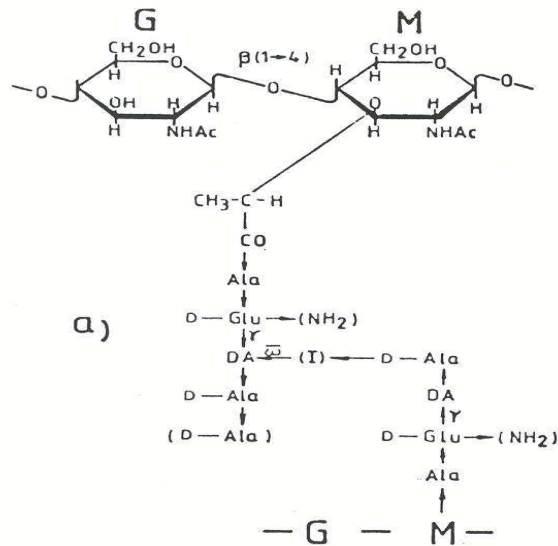
Menaquinones

Polar lipids

Cellular fatty acids

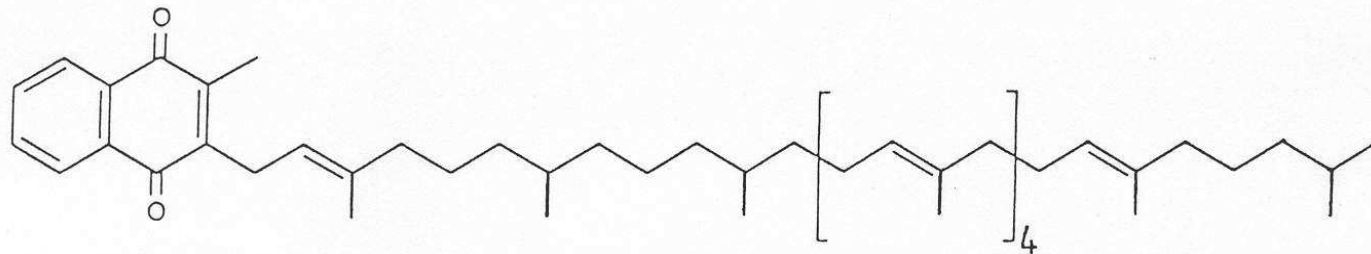
Determination of phenotypic Markers

Chemotaxonomic Markers determined by TLC, GLC and HPLC

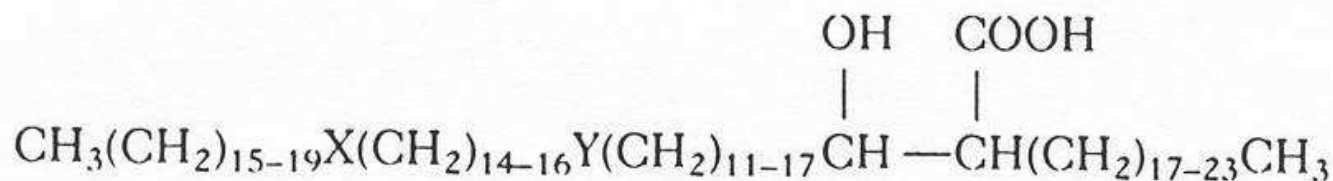


Petidoglycan Composition

Pospholipids Types



Structure of menaquinone MK-9 (II,III,IX-H₆), *Streptomyces*



Mycolic Acids sensu stricto

Polar Lipids of *Dietzia kumjensis* DSM 44709

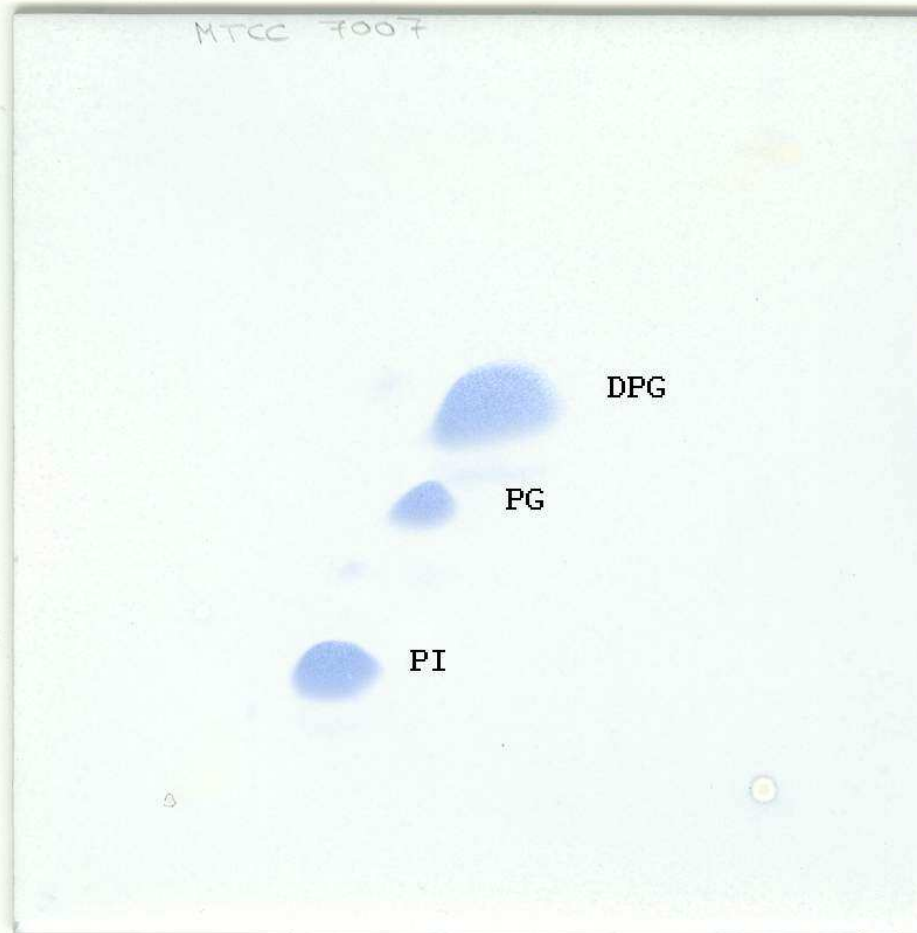
Polar lipids of 44 907= MTCC 7007



Sprayed with ninhydrin

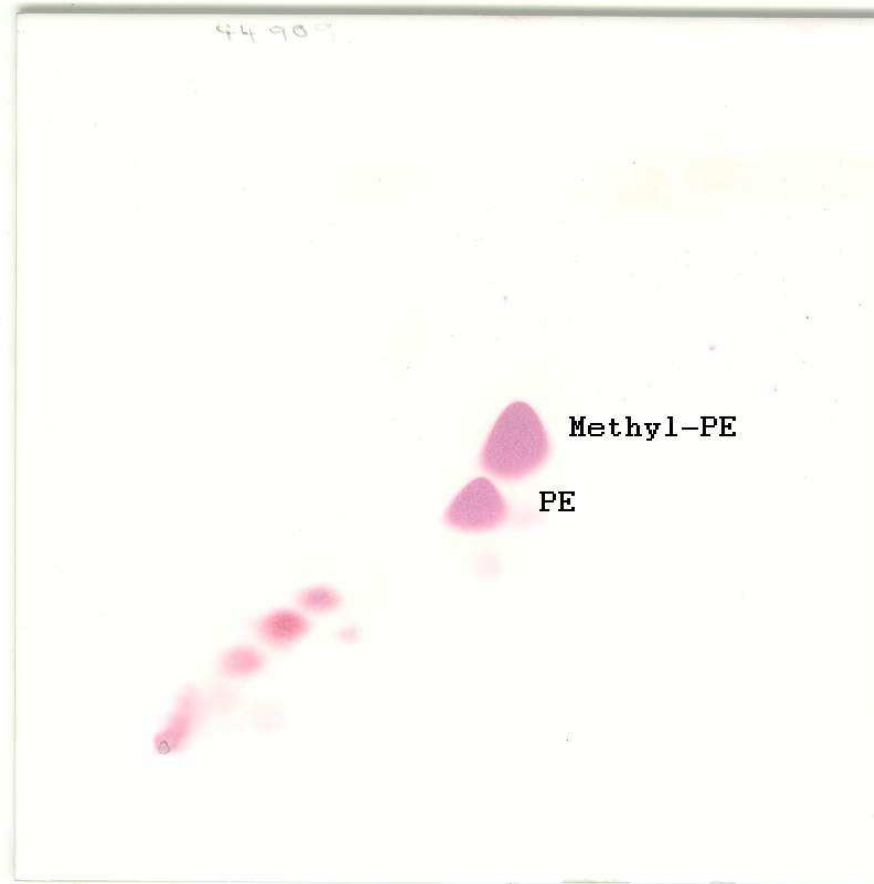
Polar Lipids of *Dietzia kumjensis* DSM 44709

Polar lipids of 44 907 = MTCC 7007



Sprayed with molybdenum blue

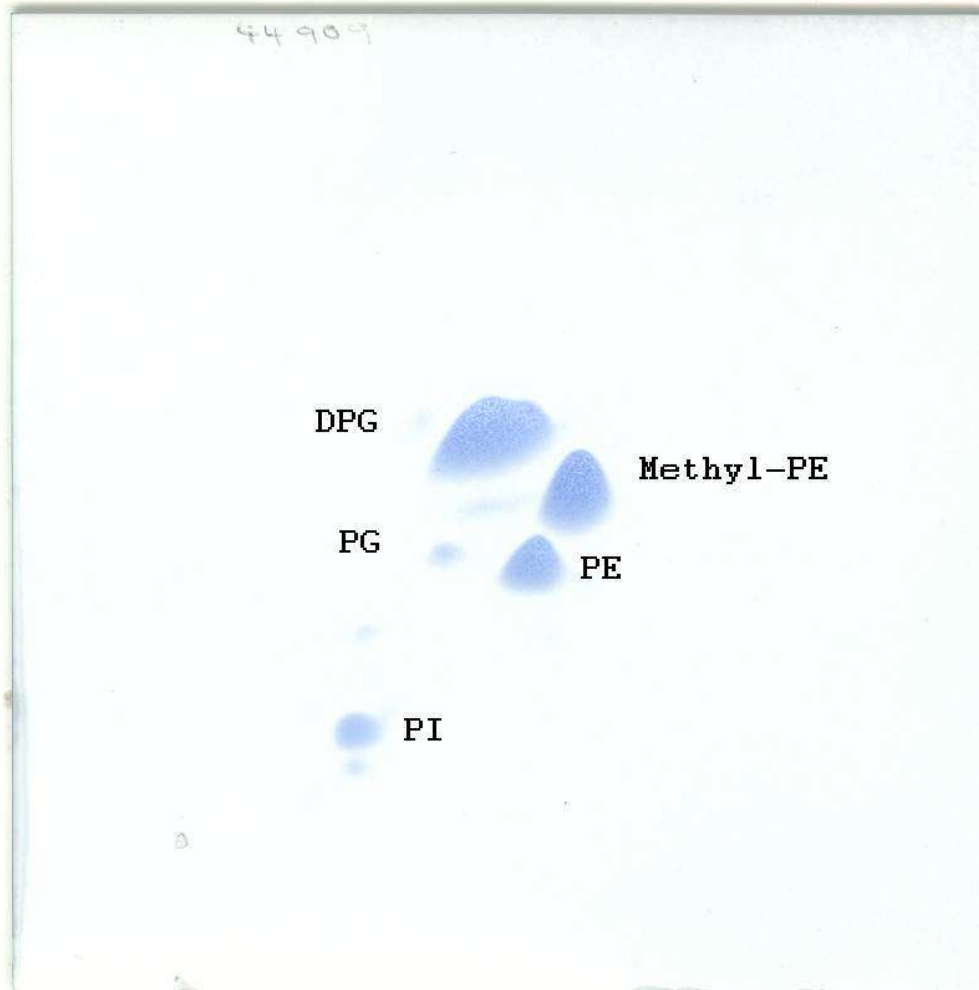
Polar lipids of 44 909 = NRRL B-24416



Sprayed with ninhydrin

Polar Lipids of *Lentzea kentuckyensis* DSM 44909

Polar lipids of 44 909

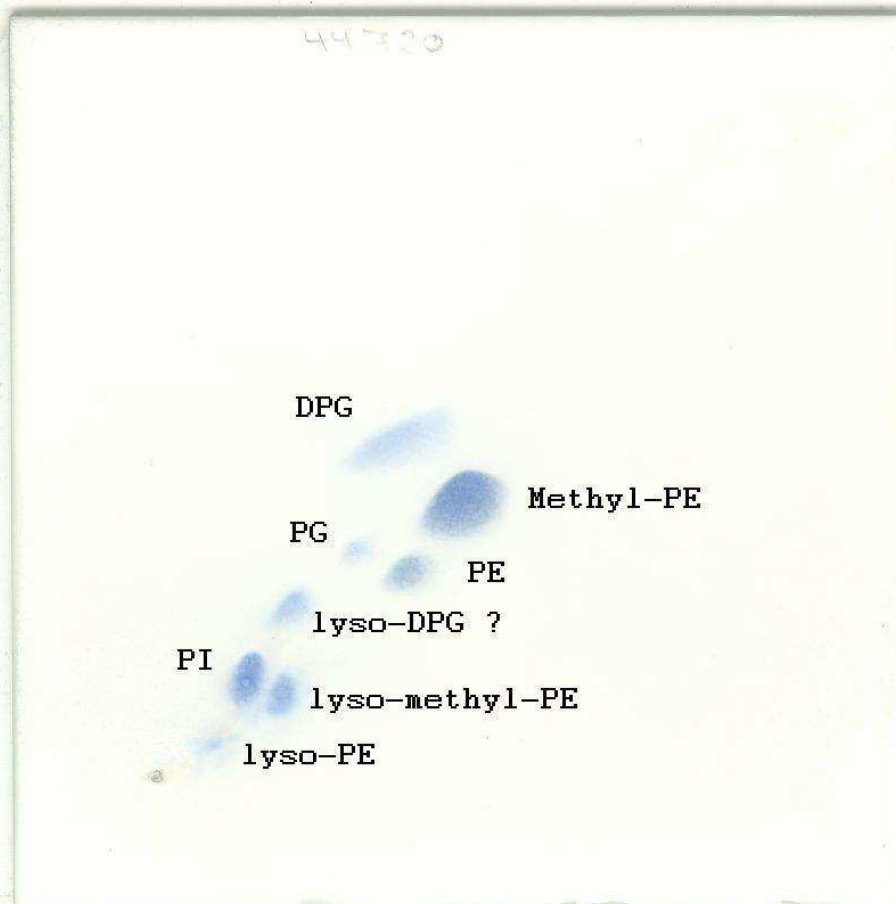


Sprayed with molybdenum blue

Polar Lipids of *Saccharothrix tangerinus* DSM 44720

Microorganisms

Polar lipids of 44 720



Sprayed with molybdenum blue 07.12.06
auf Glasplatte

Polar Lipids of *Jiangella gansuensis* DSM 44835

Microorganisms

Polar lipids of 44 835/2

Polar lipids of 44 835/2



Sprayed with molybdenum blue

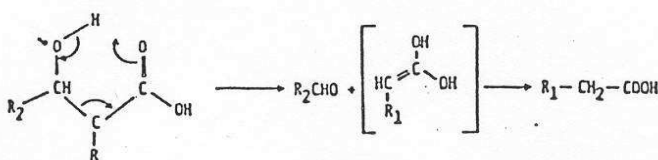
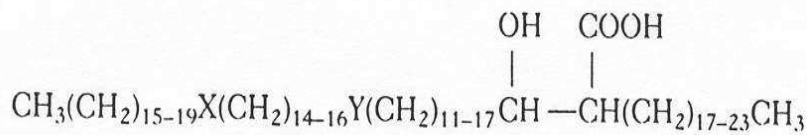
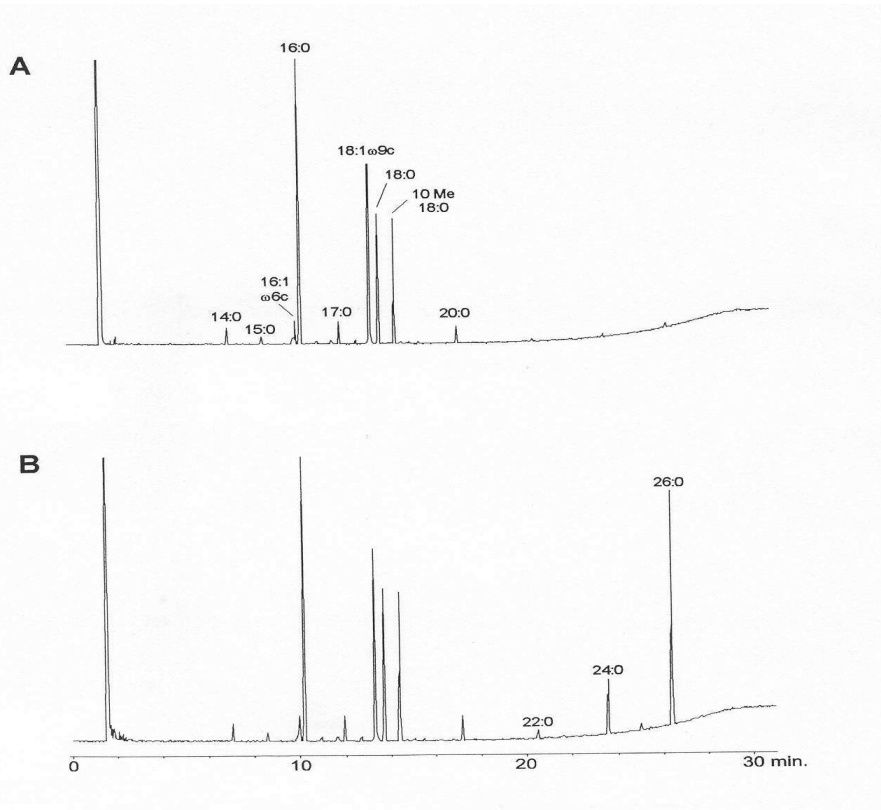


Sprayed with Anisaldehyde

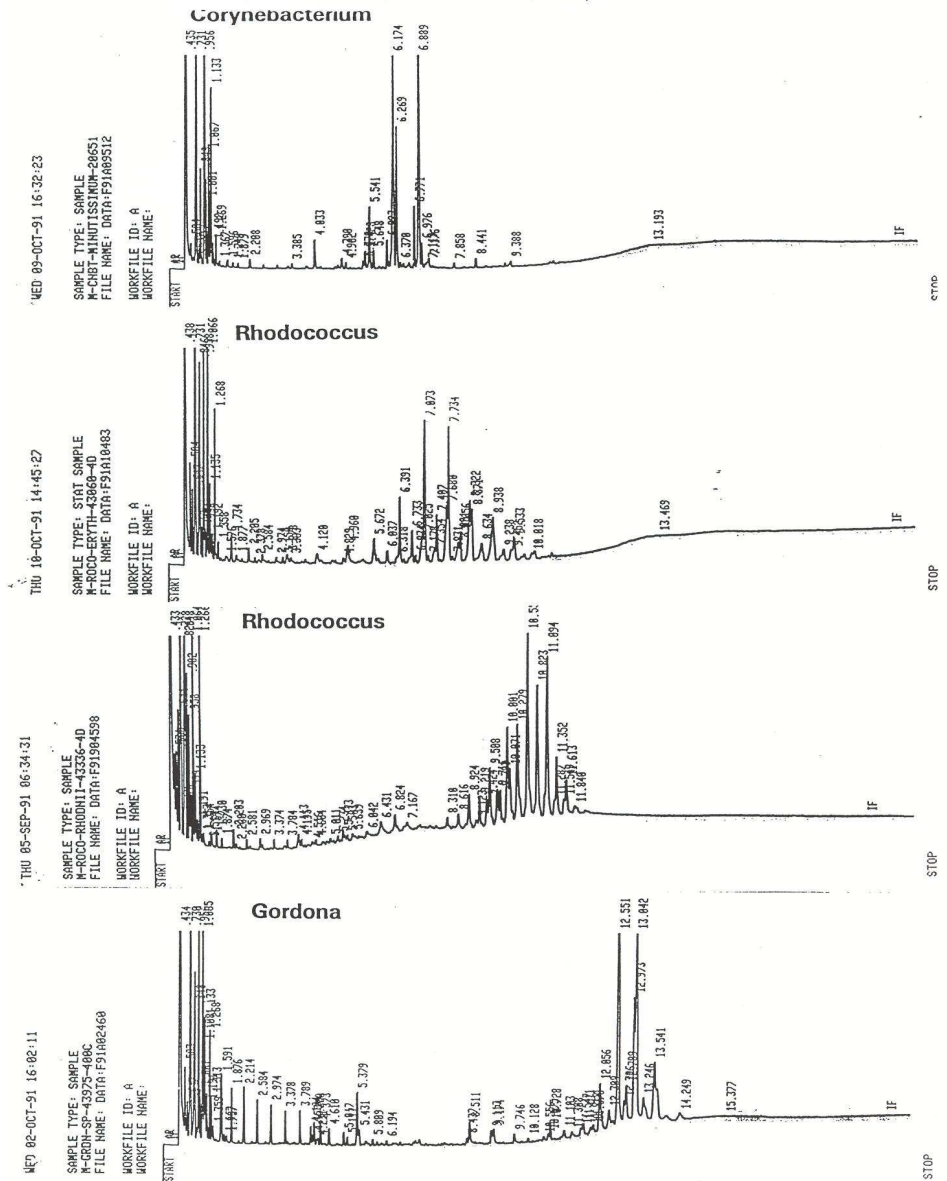
Further Differentiation of Biofilter Isolates by Chemotaxonomic Methods

Microorganisms

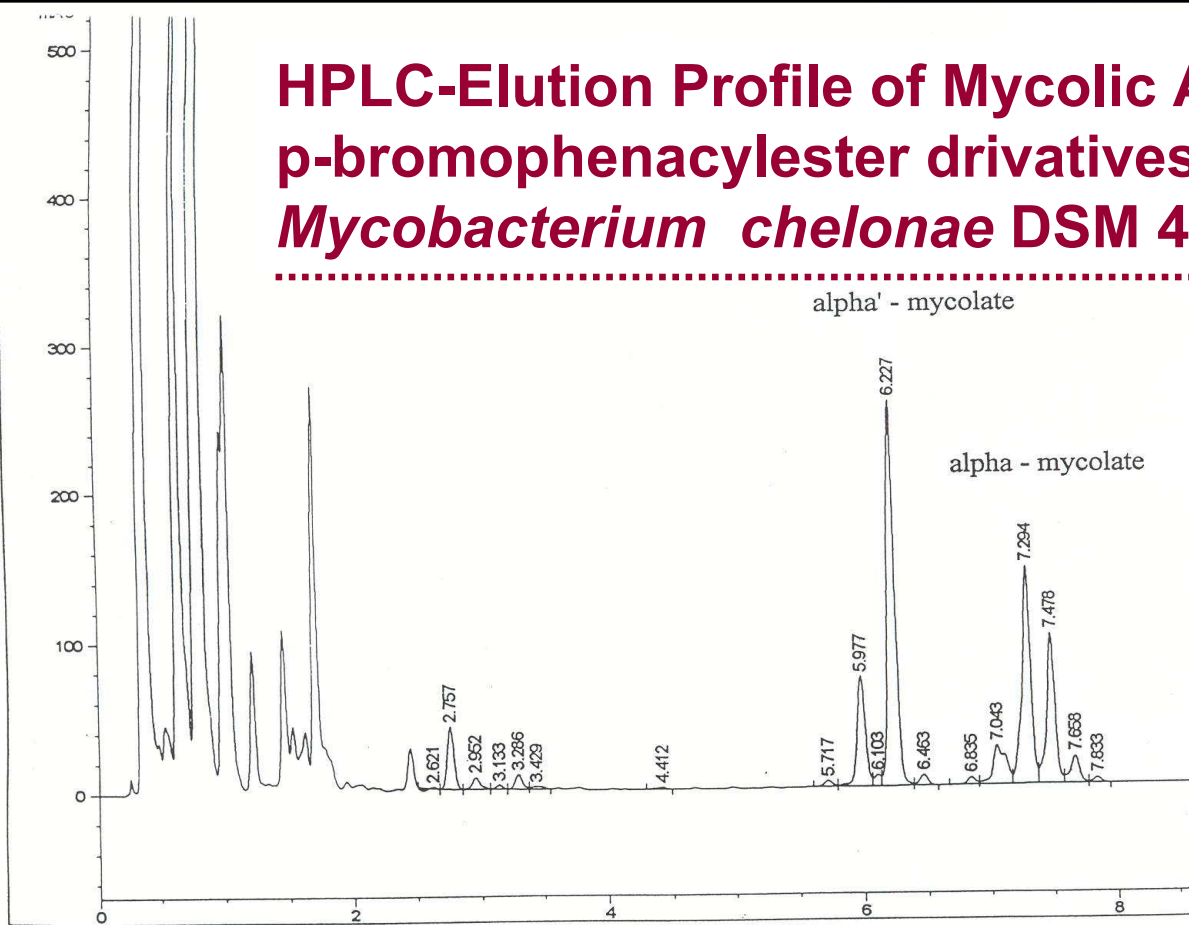
Fatty Acid Pattern of *Mycobacterium*
 (A) without treatment
 (B) after adding TMSH



Mycolic Acid Analyses by high Temperature Gas Chromatography as Trimethylsilyl Derivatives



HPLC-Elution Profile of Mycolic Acid p-bromophenacyl ester derivatives from *Mycobacterium chelonae* DSM 43804^T



Sherlock Version: 2.95

DATA: C97930656A 30-SEP-97 19:47:25

ID: 1026 MS-MYBT-CHELON-43804^T Date of run: 30-SEP-97 19:18:19
 Bottle: 7 SAMPLE [MYCOUV]

RT	Area	Ar/Ht	Respon	ECL	Name	%	Comment 1	Comment 2
2.200	20000000	0.020		24.741	SOLVENT PEAK		< min rt	
2.621	9856			29.870			< min rt	
2.757	414256	0.056		31.522			< min rt	
2.952	78513	0.066	1.000	33.898	ECL 34.000		ECL deviates -0.102	Reference -0.280
3.133	29353	0.060		36.102				
3.286	98910	0.065	1.000	37.961	ECL 37.497	2.43	ECL deviates 0.464	
3.429	21047	0.095	1.000	39.706	ECL 40.092	0.52	ECL deviates -0.386	
4.412	12341	0.080	1.000	51.683	ECL 51.874	0.30	ECL deviates -0.191	
5.717	41154	0.074	1.000	67.125	ECL 67.378	1.01	ECL deviates -0.253	
5.977	730315	0.068	1.000	70.030	ECL 69.996	17.95	ECL deviates 0.034	
6.103	75582	0.051		71.439				
6.227	2577566	0.065	1.000	72.815	ECL 72.841	63.35	ECL deviates -0.026	
6.463	71383	0.069	1.000	75.456	ECL 75.080	1.75	ECL deviates 0.376	
6.835	48330	0.068	1.000	79.596	ECL 79.157	1.19	ECL deviates 0.439	
7.043	254804	0.039	1.000	81.919	ECL 81.801	6.26	ECL deviates 0.118	
7.294	1448140	0.071		84.718				
7.478	997063	0.068		86.779				
7.658	177860	0.074	1.000	88.784	ECL 88.477	4.37	ECL deviates 0.307	
7.833	35043	0.067	1.000	90.752	ECL 90.346	0.86	ECL deviates 0.406	Reference 0.059
9.022	158400	0.058	1.000	109.695	ECL 110.000		ECL deviates -0.305	Reference -0.708

Solvent	Ar	Total Area	Named Area	% Named	Total Amnt	Nbr Ref	ECL Deviation	Ref ECL Shift
20000000		6855805	4068754	59.35	4068754	3	0.301	0.441

MYCOUV [Rev 3.10] Mycobacterium 0.532
 M. abscessus/chelonae 0.532

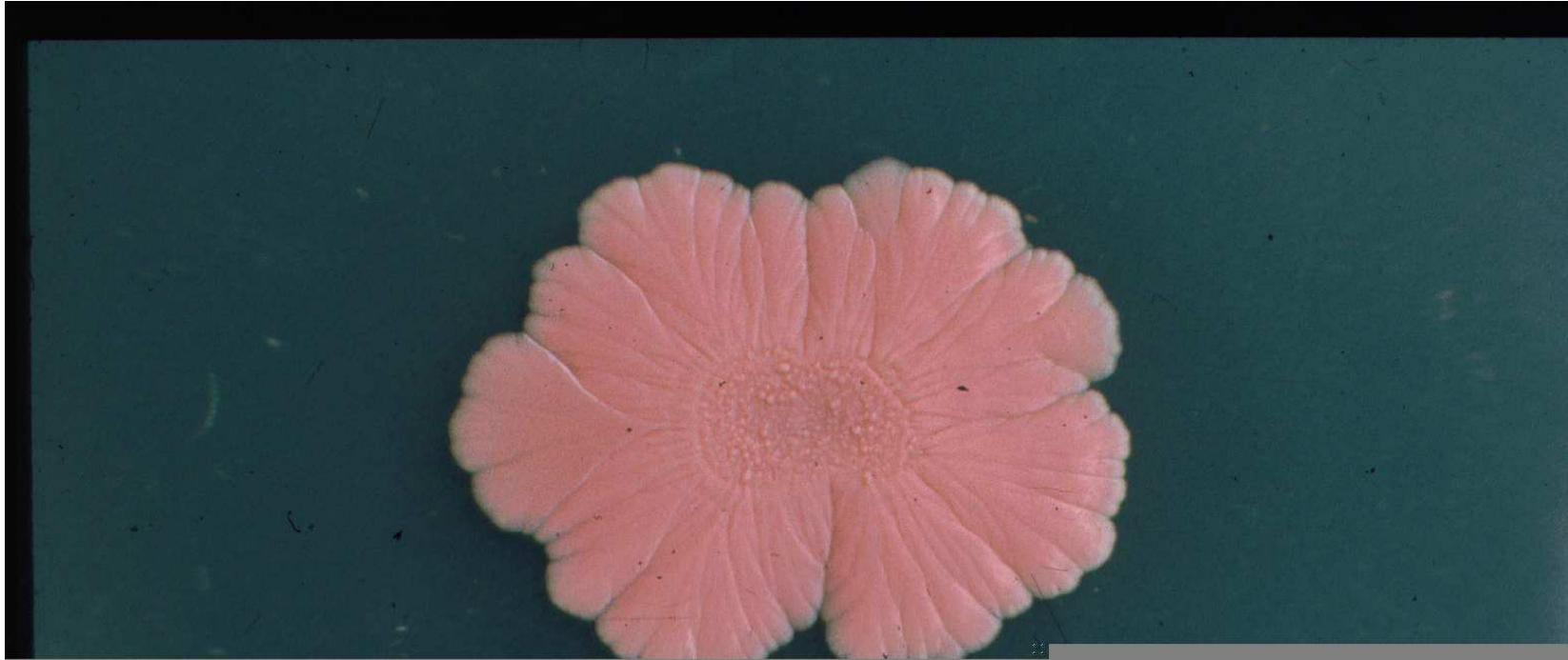


Chemotaxonomic Markers of Genera of the Suborder *Corynebacterineae*

	Acyl type	Major menaquinone	PE	Fatty acid composition	Mycolate size (no. of carbons)	G+C content (mol%)
<i>Smaragdicoccus</i>	G	MK-8(H ₄ ,w-cycl*), MK-8(H ₄ ,dicycl)	+	S, U	43-49	64
<i>Nocardia</i>	G	MK-8(H ₄ ,w-cycl)	+	S, U, T	50-62	64-72
<i>Skermania</i>	G	MK-8(H ₄ ,w-cycl)	+	S, U, T	58-64	68
<i>Millisia</i>	G	MK-8(H ₂)	+	S, U, T	44-52	65
<i>Rhodococcus</i>	G	MK-8(H ₂)	+	S, U, T	34-54	63-73
<i>Gordonia</i>	G	MK-9(H ₂)	+	S, U, T	54-66	63-69
<i>Williamsia</i>	G	MK-9(H ₂)	+	S, U, T	50-56	64-65
<i>Mycobacterium</i>	G	MK-9(H ₂)	+	S, U, T	70-90	70-72
<i>Segniliparus</i>	ND	ND	ND	S, U, T	ND	68-72
<i>Tsukamurella</i>	G	MK-9	+	S, U, T	64-78	67-68
<i>Turicella</i>	ND	MK-10, MK-11	ND	S, U, T	-	65-72
<i>Dietzia</i>	A	MK-8(H ₂)	-	S, U, T	34-38	73
<i>Corynebacterium</i>	A	MK-8(H ₂)	-*	S, U*	22-36	51-67
<i>C. amycolatum</i>	ND	MK-9	ND	S, U	-	ND

Actinomycete isolated from Bark Compost of a biological Air Filter

Microorganisms



New species: *Gordonia hydrophobica*, *G. hirsuta*

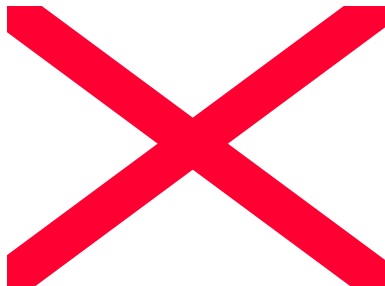
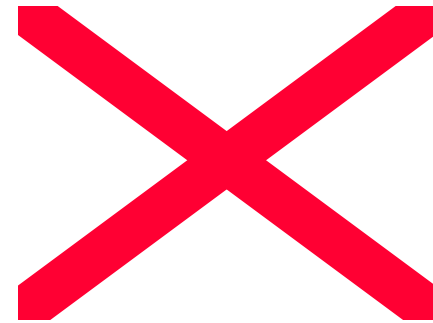
Microbiology of Scumming Sludge

Secondary Clarifier of a biological Sewage treatment Plant

Development of Scumming Sludge



Filamentous Gram-negative Bacteria the Cause of Sludge Bulking in the aeration Tank



**Filamentous Actinomycetes isolated from activated
sludge foams of the settling Tank
(*Gordonia*, *Nocardia*, *Rhodococcus*, *Tsukamurella*,
Millisia)**



Section of a closed Scumming Sludge Cover in a secondary Clarifier of a Sewage Plant



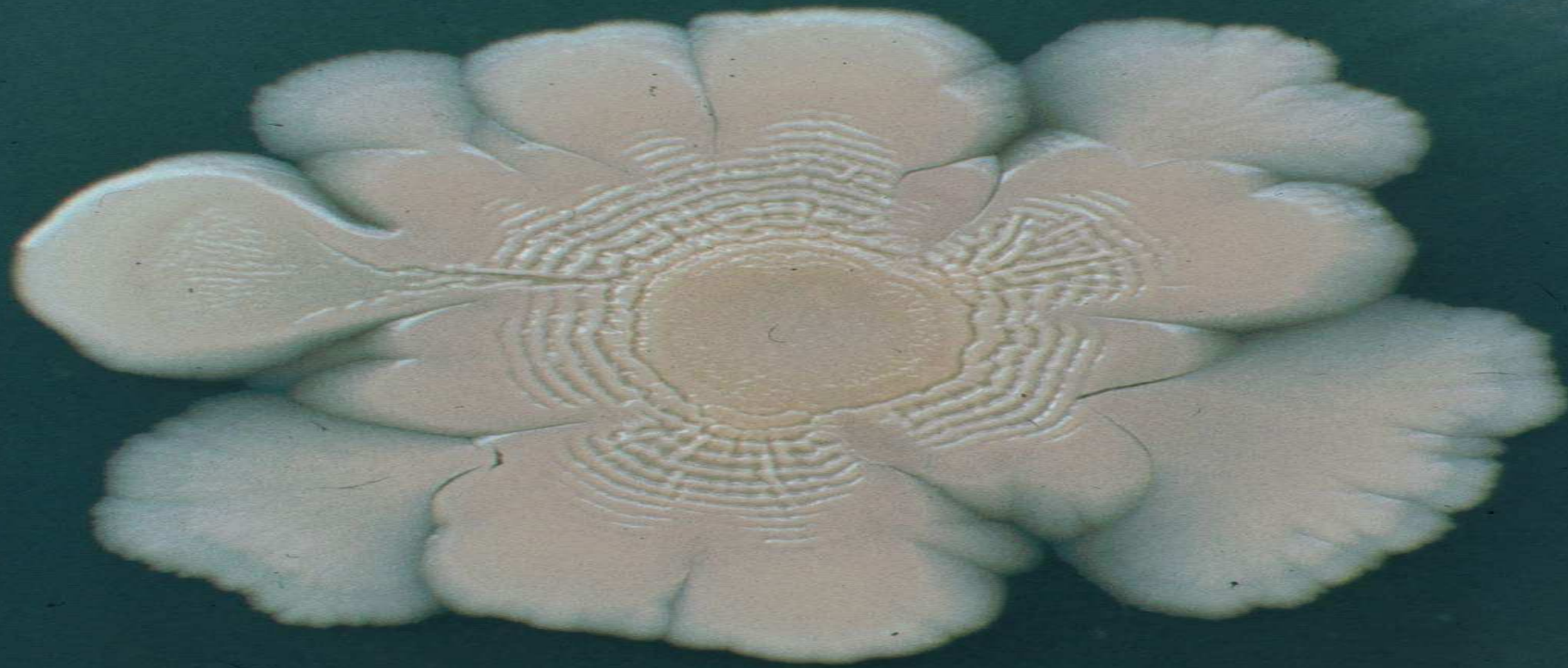
Clear Water-Effluent of the secondary Clarifier



Recycling of activated Sludge from the secondary Clarifier by a mechanical Device



**Actinomycete isolated from scumming sludge of a
municipal wastewater treatment plant**



Rh. erythropolis
43 066

System Appl Microbiol 1984 5, 124-135

**Chemotaxonomy and Physiology of Some Actinomycetes Isolated
from Scumming Activated Sludge**

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Int J Syst Evol Microbiol 2006 56, 739-744

***Millisia brevis* gen. nov., sp. nov., an Actinomycete Isolated from Activated
Sludge Foam**

**Jacques A. Soddell, Fiona M. Stainsby, Kathryn L. Eales,
Reiner M. Kroppenstedt,
Robert J. Seviour and Michael Goodfellow**

**Banking and Accounting Studies, Identification and
Controlling, scientific Approach. GIT Labor-Fachzeitschrift
1096, 12/2004**



Studies on the Micro-Flora of Composting Plants of Garden- and Household-Garbag

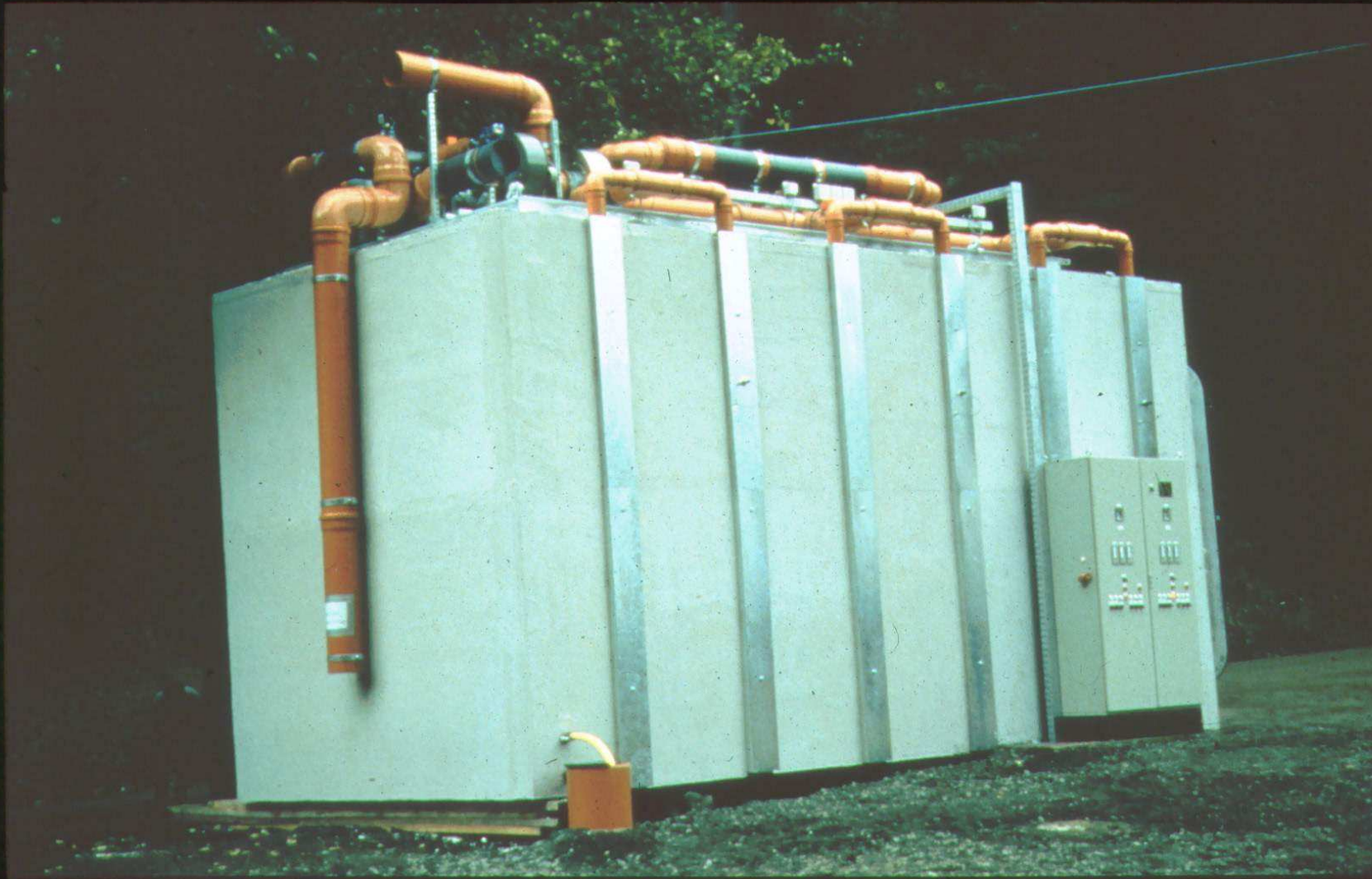
Microorganisms



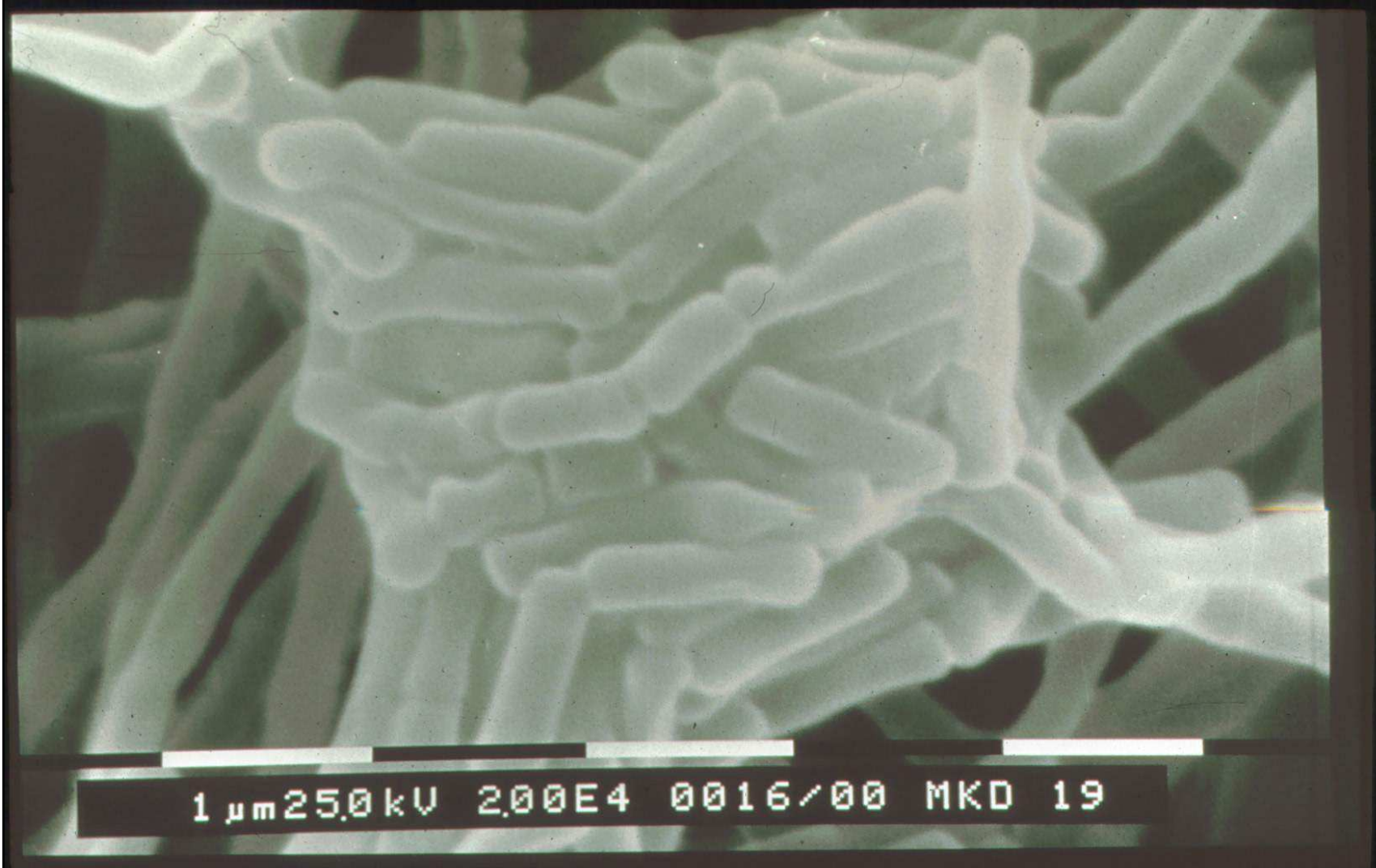
Mesophilic Actinomycetes $1,3 \times 10^5/\text{m}^3$ CFU/Air, thermophilic Actinomycetes CFU, 2.3×10^5 CFU/ m^3 Air, *Thermoactinomycetaceae* 1.4×10^5 CFU / m^3 Air.

Closed computer controlled composting system

Microorganisms



Thermocrispum municipale DSM 44069^T
(Air Isolate from a refuse incineration Plant)



Thermocrispum agreste isolated from rural compost

Microbiology of Moldy Houses

APPLIED AND ENVIRONMENTAL MICROBIOLOGY,
0099-2240/01/\$04.0010 DOI: 10.1128/AEM.67.7.3269-3274.2001
July 2001, p. 3269-3274 Vol. 67, No. 7
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Toxic-Metabolite-Producing Bacteria and Fungus in an Indoor Environment

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3740 AG Baarn, The Netherlands⁵*

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Toxic-metabolite-emitting microbes were isolated from the indoor environment of a building where the occupant was suffering serious building-related ill-health symptoms. Toxic substances soluble in methanol and inhibitory to spermatozoa at <10 mg (dry weight) ml⁻¹ were found from six bacterial isolates and one fungus. The substances from isolates of *Bacillus simplex* and from isolates belonging to the actinobacterial genera *Streptomyces* and *Nocardiosis* were mitochondriotoxic. These substances dissipated the mitochondrial membrane potential (Dc) of boar spermatozoa. The substances from the *Streptomyces* isolates also swelled the mitochondria. The substances from isolates of *Trichoderma harzianum* Rifai and *Bacillus pumilus* damaged the cell membrane barrier function of sperm cells.

Microbiology of Mouldy Houses

Wood infested with Moulds



Antique desk infested by
Aspergillus versicolor

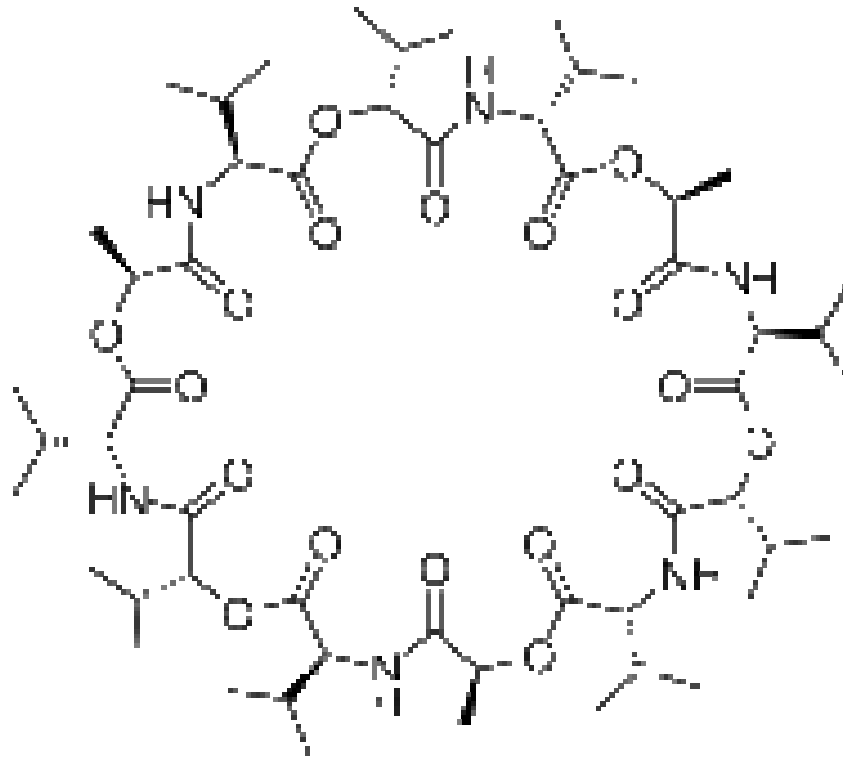


Beam infested by dry rot
Serpula himantoides

Toxin Producers e. g. *Stachybotrys chartarum*
Stachybotroxicosis (Toxin is Satratoxin)

Species	Place where is was found /Material	Suborder/ Family
<i>Arthrobacter ramosus</i> , <i>Arthrobacter</i> sp.	dust ^{1c} plaster board ^{1a}	<i>Micrococcineae</i> / <i>Micrococcaceae</i>
<i>Cellulomonas</i> sp. nov.	plaster board ^{1a}	<i>Micrococcineae</i> / <i>Cellulomonadaceae</i>
<i>Corynebacterium</i> sp. nov.	dust ^{1c}	<i>Corynebacterineae</i> / <i>Corynebacteriaceae</i>
<i>Dietzia</i> sp. nov.	plaster board ^{1d}	<i>Corynebacterineae</i> / <i>Dietziaceae</i>
<i>Micrococcus</i> sp. nov.	plaster board ^{1a}	<i>Micrococcineae</i>
<i>Mycobacterium murale</i> sp. nov.	Plaster board ³	<i>Corynebacterineae</i> / <i>Mycobacteriaceae</i>
<i>Nocardia carnea</i>	wallpaper ⁶	<i>Corynebacterineae</i> / <i>Nocardiaceae</i>
<i>Nocardiopsis exhalans</i> sp. nov.	air ⁴ , loam plaster ⁷	<i>Streptosporangineae</i> / <i>Nocardiopsaceae</i>
<i>Nocardiopsis umidischolae</i> sp. nov.	dust ⁴	<i>Streptosporangineae</i> / <i>Nocardiopsaceae</i>
<i>Nocardiopsis</i> sp.nov	plaster ⁶	<i>Streptosporangineae</i> / <i>Nocardiopsaceae</i>
<i>Nocardiopsis alba</i>	plaster board ^{1d}	<i>Streptosporangineae</i> / <i>Streptomycetaceae</i>
<i>Promicromonospora</i> sp.nov	wallpaper ⁶	<i>Micrococcineae</i> / <i>Promicromonosporaceae</i>
<i>Pseudonocardia</i> sp. nov	plaster and wallpaper ⁷	<i>Pseudonocardineae</i> / <i>Pseudonocardiaceae</i>
<i>Rhodococcus fascians</i>	dust ^{1c}	<i>Corynebacterineae</i> / <i>Nocardiaceae</i>
<i>Streptomyces coelicolor</i>	plaster board ⁵	<i>Streptomycineae</i> / <i>Streptomycetaceae</i>
<i>Streptomyces griseus</i>	dust and air ^{1b} plastic foil ⁷	<i>Streptomycineae</i> / <i>Streptomycetaceae</i>
<i>Streptomyces violaceoruber</i>	dust ⁷	<i>Streptomycineae</i> / <i>Streptomycetaceae</i>
<i>Streptomyces</i> sp.	plaster board ⁷	<i>Streptomycineae</i> / <i>Streptomycetaceae</i>
<i>Williamsia muralis</i> gen nov. sp. nov.	plaster board ²	<i>Corynebacterineae</i> / <i>Williamsiaceae</i>

The Mitochondrial Toxin Produced by *Streptomyces griseus* Strains Isolated from an Indoor Environment Is Valinomycin

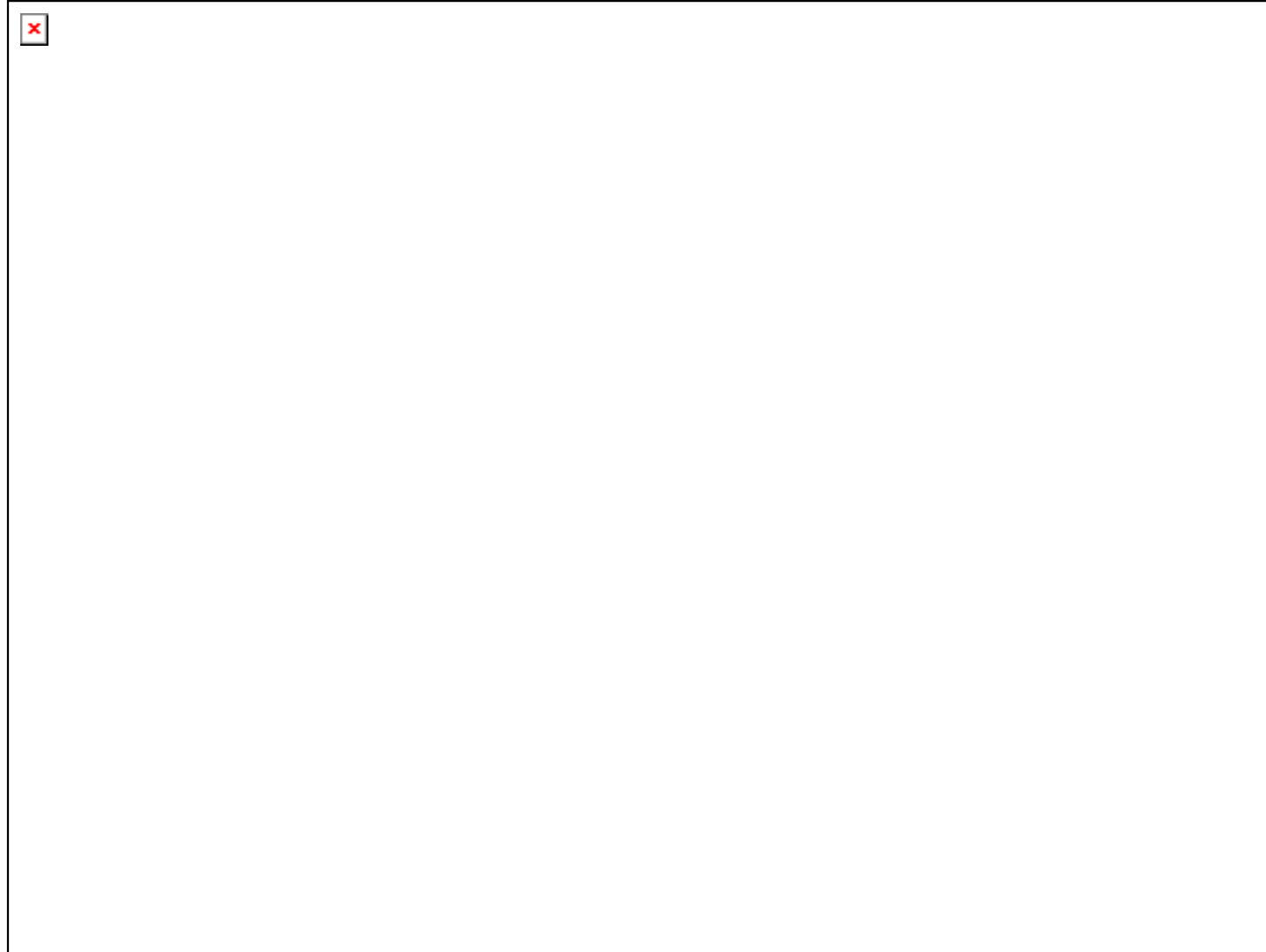


Valinomycin is an Ionophor that selectively transport potassium ions via the cell membrane of mitochondria. It was isolated from *Streptomyces fuvissimus* for the first time.

Streptomyces sp. isolated from Air of an Mouldy House



Unspecified Isolate from Plaster of a Moldy House



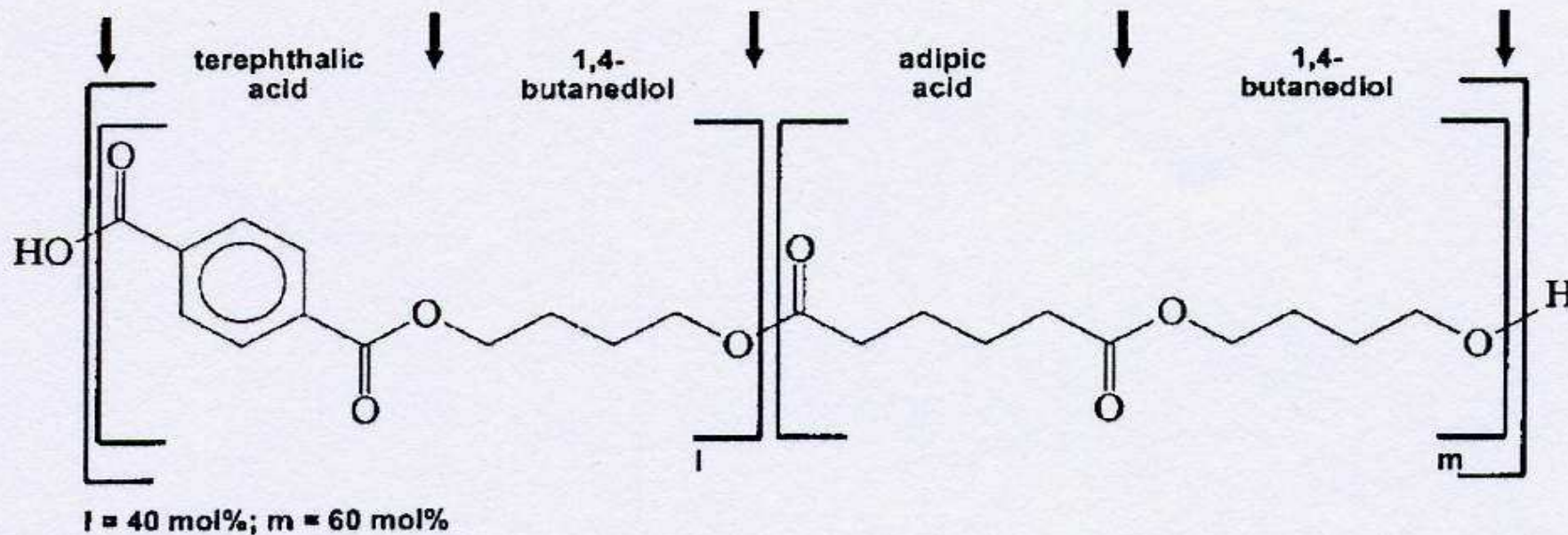
Isolation and Identification of Xenobiotic-degrading Actinomycetes (Plastic Material)

Requirements of synthetic material to pass the assessment of environmental impact

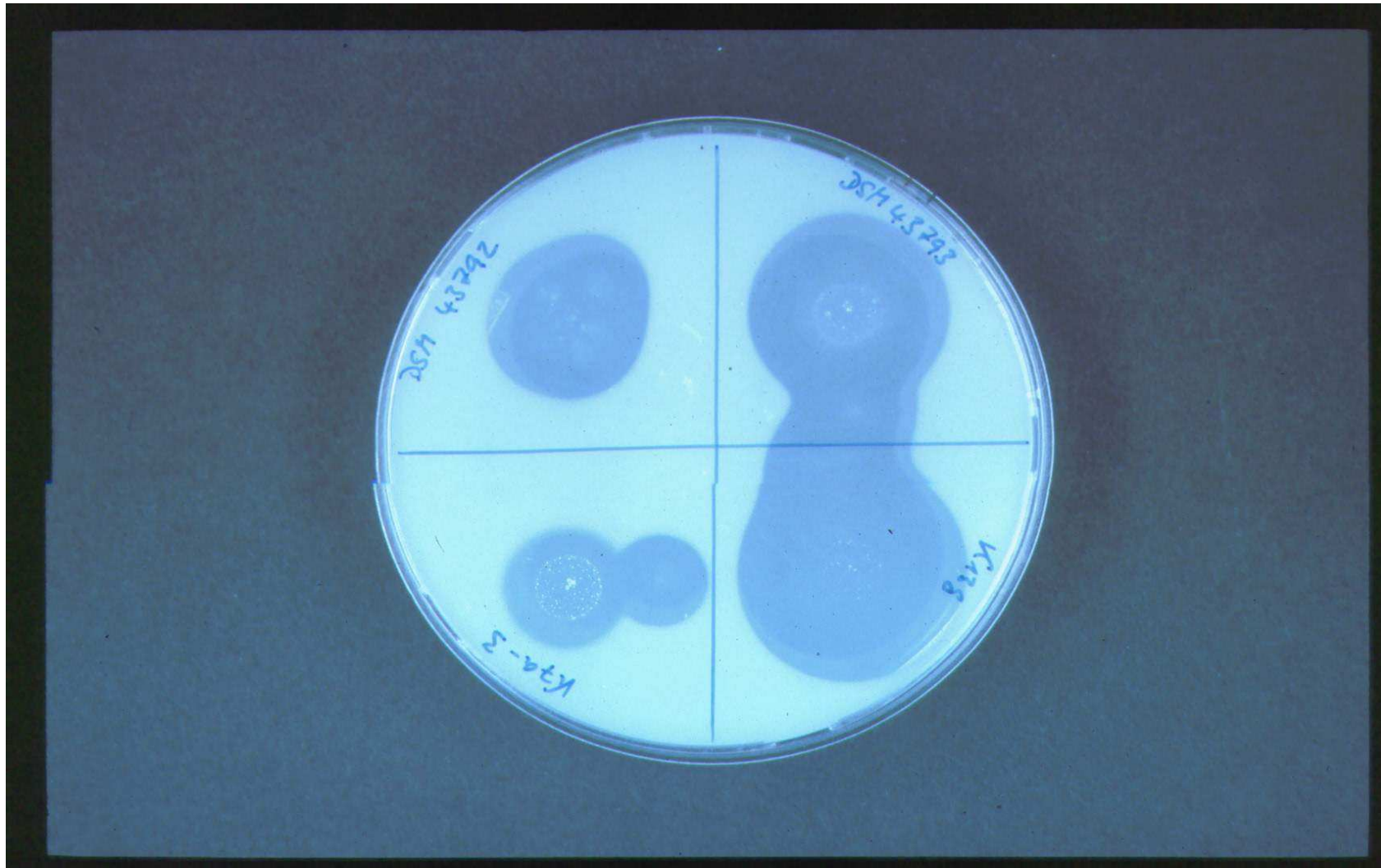


poly(butylendipate-co-butylenterephthalate) = BTA

Poly(Butylenadipat-co-Butylenterephthalat) BTA



Degradation of BTA (suspension) by two strains isolated from compost using BTA foils as bait



Degradation of BTA Foils

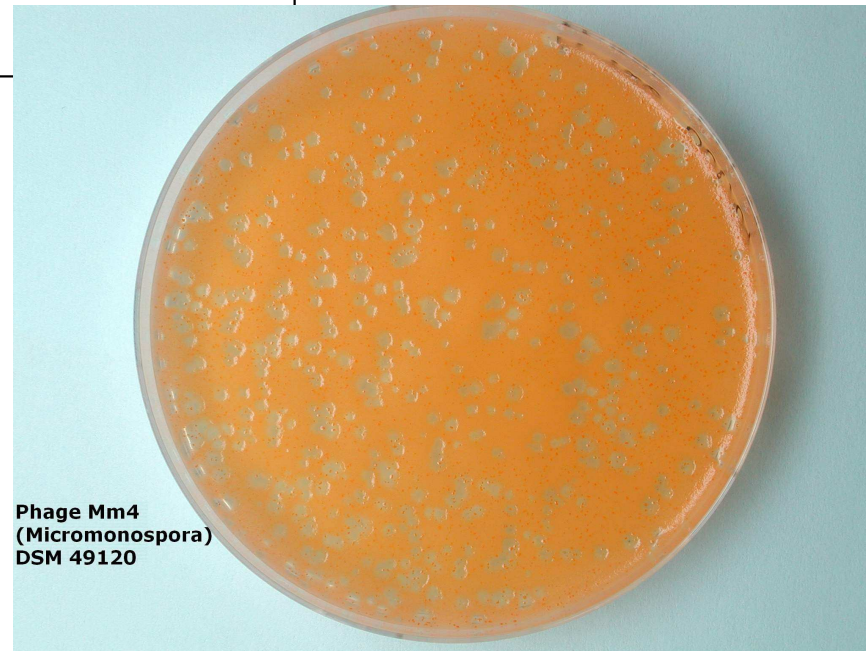
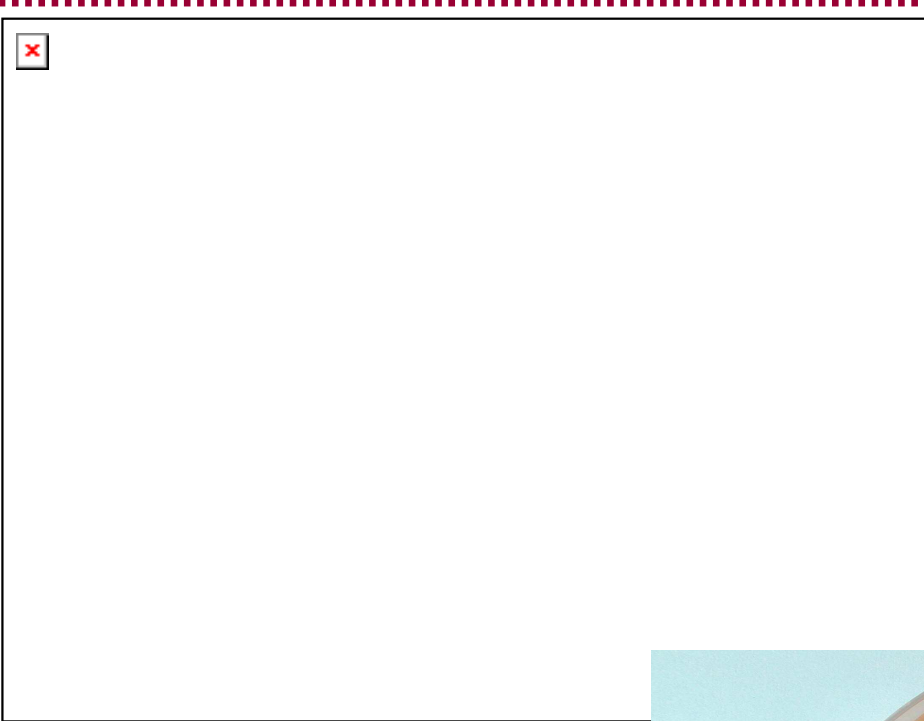


Thermophilic Actinomycetes isolated from Compost

Microorganisms



Bacteriophages



Phage Mm4
(Micromonospora)
DSM 49120

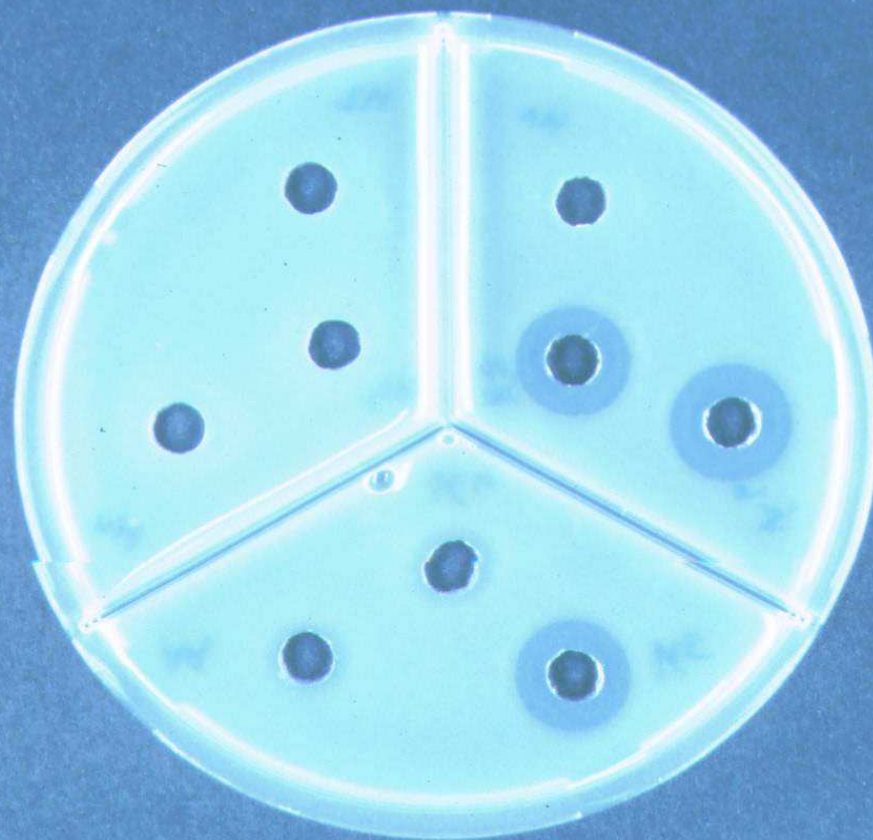
Degradation of BTA (Suspension) by the two Isolates and

Microorganisms

Thermobifida fusca DSM 43792^T und DSM 43793



Proof of Presence of BTA degrading Enzymes in the Fermentation Broth of *Thermobifida fusca* DSM 43792^T visualized by clearing Zones in the BTA Suspension Agar



The Actinomycetales Group

Microorganisms

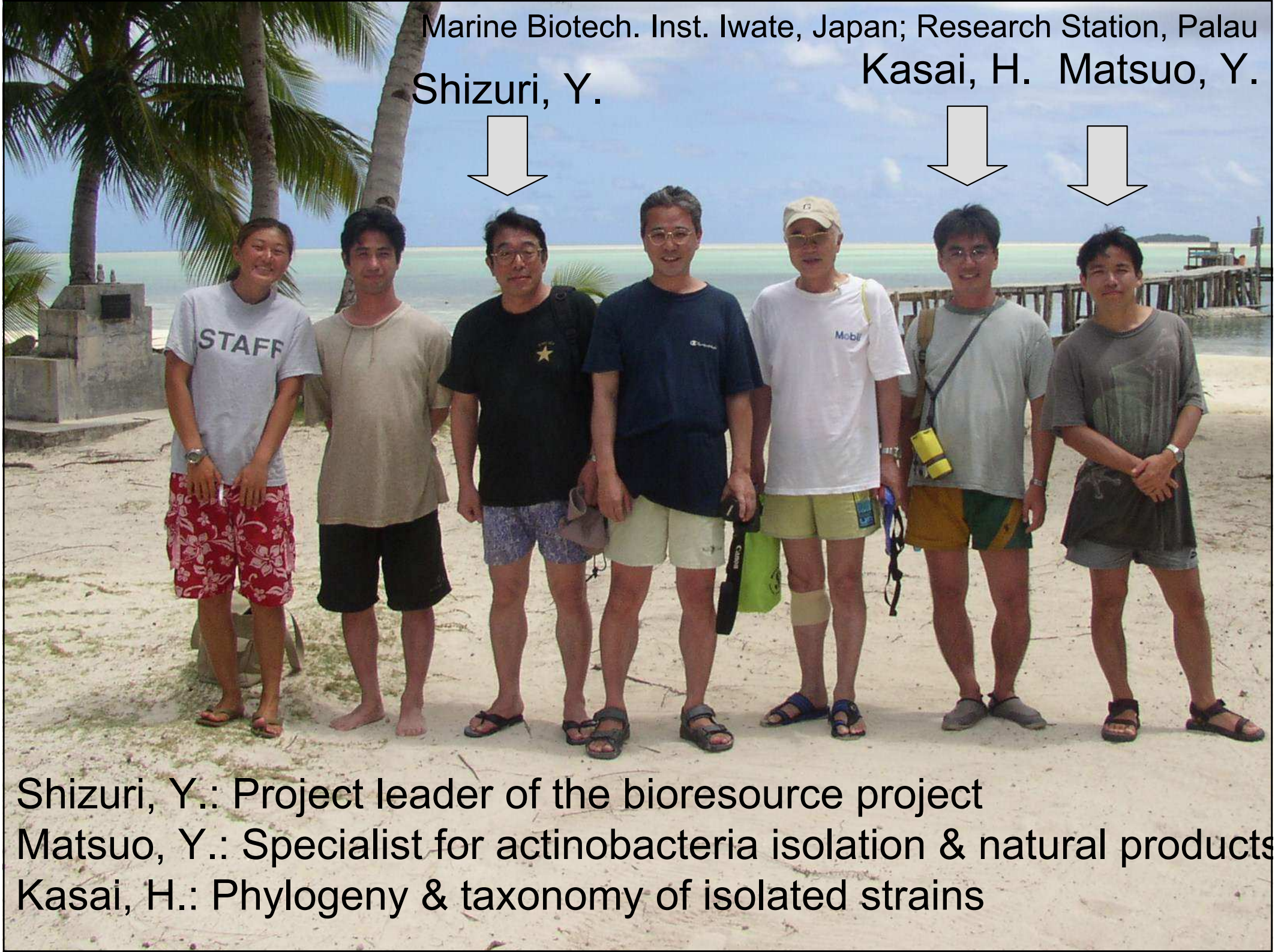
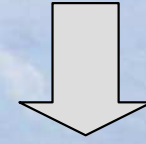
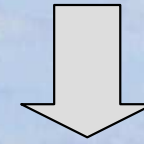
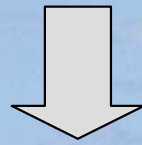


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Matsuo, Y.: Specialist for actinobacteria isolation & natural products

Kasai, H.: Phylogeny & taxonomy of isolated strains