RECIPE

Reconciling Commercial Exploitation of Peat with Biodiversity in Peatland Ecosystems

BACTERIAL DIVERSITY

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Scientific Objectives

Gaining knowledge about the development of diversity and function of bacterial communities regarding the effect of study sites, peat land vegetation and restoration stages on microbial communities.

Approach: Sample Overview

Site		Vegetation	Horizon
Finland	A	*Eriophorum vaginata*, wet	2/**3/4/6/8**
	B	*Eriophorum vaginata*, dry	2/3/4/6/8
	C	*Carex rostrata*, wet	2/**3/4/6/8**
	D	*Sphagnum fallax* (+others), wet	**3/4**/5/**6/8**
	E	bare peat	2/**3/4/6/8**
France (Le Russey)	A	bare peat	**3/4/6/8**
	B	early regeneration	**3/4/6/8**
	C	advanced regeneration	**3/4/6/8**
	D	intact reference	3/4/6/8
Switzerland	A	bare peat	**3/4/6/8**
	B	early regeneration	**3/4/6/8**
	C	advanced regeneration	**3/4/6/8**
	D	intact reference	3/4/6/8
Scotland	A	bare peat, no recolonisation after ca. 5 years of abandonment	**3/4/6/8**
	B	peat recolon. with *Sphagnum ssp*. after 5-10 years of abandonment	3/4/6/8
	C	peat recolon. with *Eriophorum angustifolium* a. 5-10 years of abandonment	3/4/6/8
	D	peat recolon. with *Sphagnum spp*. after 50 years of abandonment	3/4/6/8
France (Baupte)	A	bare peat	**3/4/6/8**
	B	early regeneration	3/4/6/8

Sampling Autumn 2003

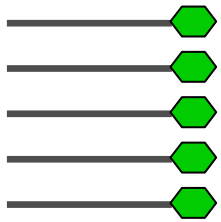
1
2
3 0-5 cm
4 5-10 cm
5 12,5-17,5 cm
6 22,5-27,5 cm
8 42,5-47,5 cm

Approach

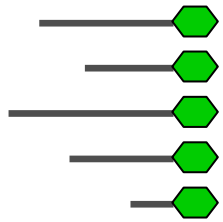
environmental sample

DNA extraction

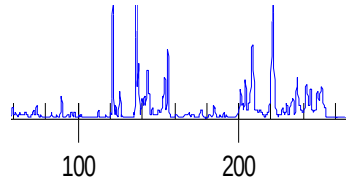
PCR amplification using labeled primers



restriction digestion



detection of labeled fragments using t-RFLP method



Primers and Restriction Enzymes

Primer	Target	Sequence
B27cy5-f	universal bacteria	agagtttgatcctggctcag
1401r	universal bacteria	cggtgtgtacaagaccc

Restriction Enzyme	Restriction site
AluI	ag|ct

Data analysis

- Graphical output (Fragmentogram) and tables of peak area and fragment size using CEQ 8000 Software
- Data converted into binary code
- Data transfered to SPSS (statistical evaluation)
- Hierarchical Clusteranalysis

Specific Questions

1. **Influence of site**
2. **Influence of vegetation**
3. **Influence of regeneration**

on bacterial communities

4. **Fragmentogram: vegetation/ regeneration specific fragments?**

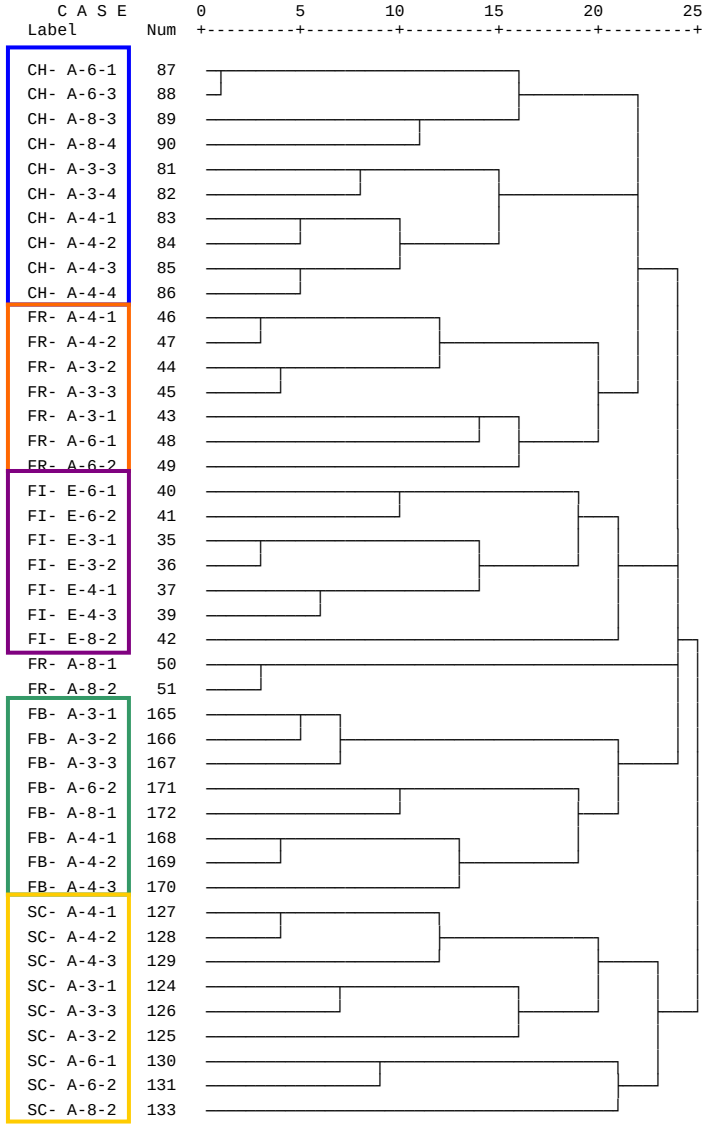
Site		Vegetation
Finland	A	*Eriophorum vaginatum*, wet ←
	B	*Eriophorum vaginatum*, dry
	C	*Carex rostrata*, wet ←
	D	*Sphagnum fallax* (+others), wet ←
	E	bare peat ←
France (Le Russey)	A	bare peat ←
	B	early regeneration ←
	C	advanced regeneration ←
	D	intact reference
Switzerland	A	bare peat ←
	B	early regeneration ←
	C	advanced regeneration ←
	D	intact reference
Scotland	A	bare peat ←
	B	peat recolon. with *Sphagnum ssp*.
	C	peat recolon. with *Eriophorum angustifolium*
	D	peat recolon. with *Sphagnum spp*.
France (Baupte)	A	bare peat ←
	B	early regeneration

Results: Statistical evaluation

(SPSS: Hierarchical Clusteranalysis, Cluster-Method: Linkage between Groups, Binary: Jaccard)

1. **Influence of site (country, bare peat)**

- **distinct geographical clustering of bacterial communities independent from vegetation**
- **two main clusters: SC marks off distinctly from other sites**
- **peat samples from CH and FR form a big joint cluster (close vicinity, similar climatic conditions and plant vegetation)**
- **within main cluster grouping of replicates**



Results: Statistical evaluation

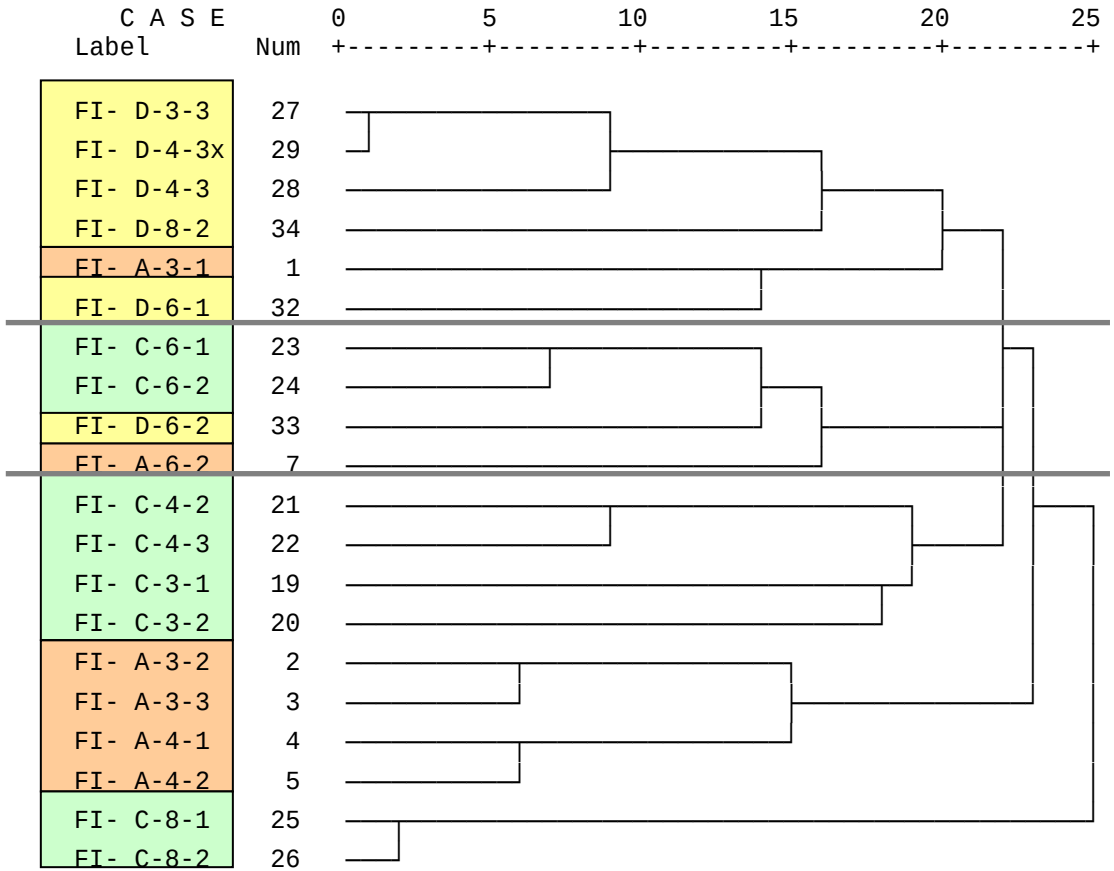
2. Influence of vegetation (FI)

- ***Sphagnum*** **peat samples appear in a common cluster**

⇒ **vegetation effect on bact. communities under *Sphagnum***

- ***Carex* + *Eriophorum*: influence of vegetation on bacterial communities residing in upper depth gradients**

- **vegetation effect becomes less apparent with increasing soil depth (see depth 6)**



Sphagnum fallax (Moss)

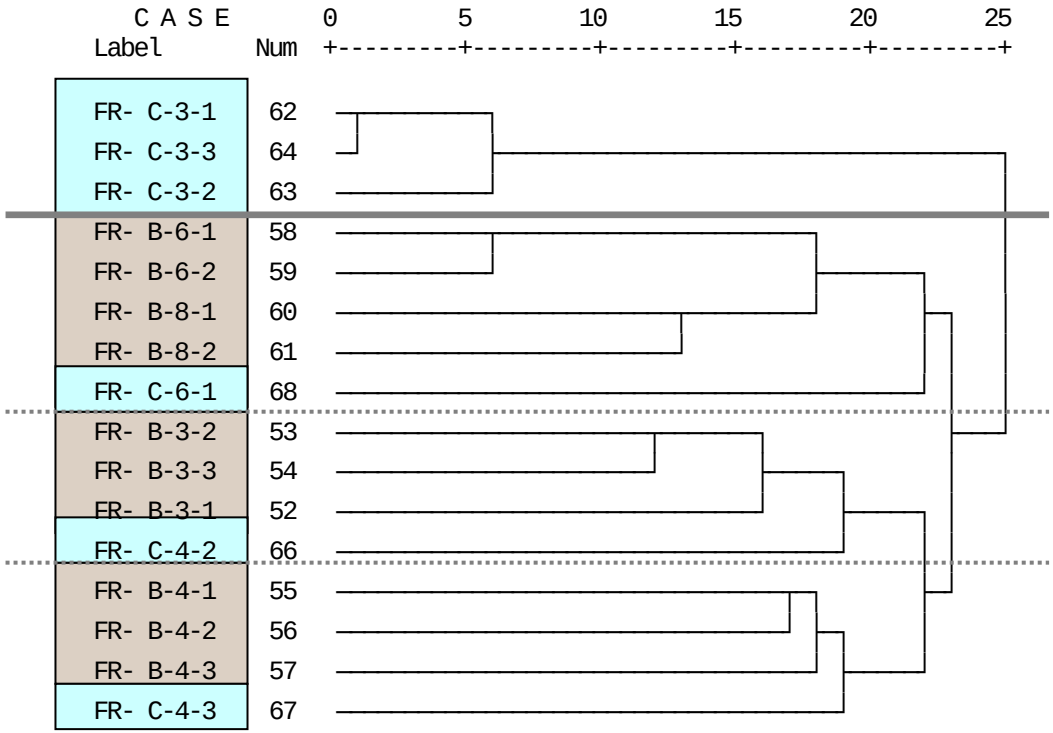
Carex rostrata (grass)

Eriophorum vaginatum (grass)

Results: Statistical evaluation

3.1 Influence of regeneration in France (Le Russey)

- ➢ **two main clusters: depth 3 of advanced regenerated peat marks off distinctly from rest**
- ⇒ **bacterial communities in advanced regenerated peat are influenced by the regeneration process**
- ➢ **effect becomes less apparent with increasing soil depth**
- ➢ **second cluster: depth effect predominant**



Advanced Regeneration

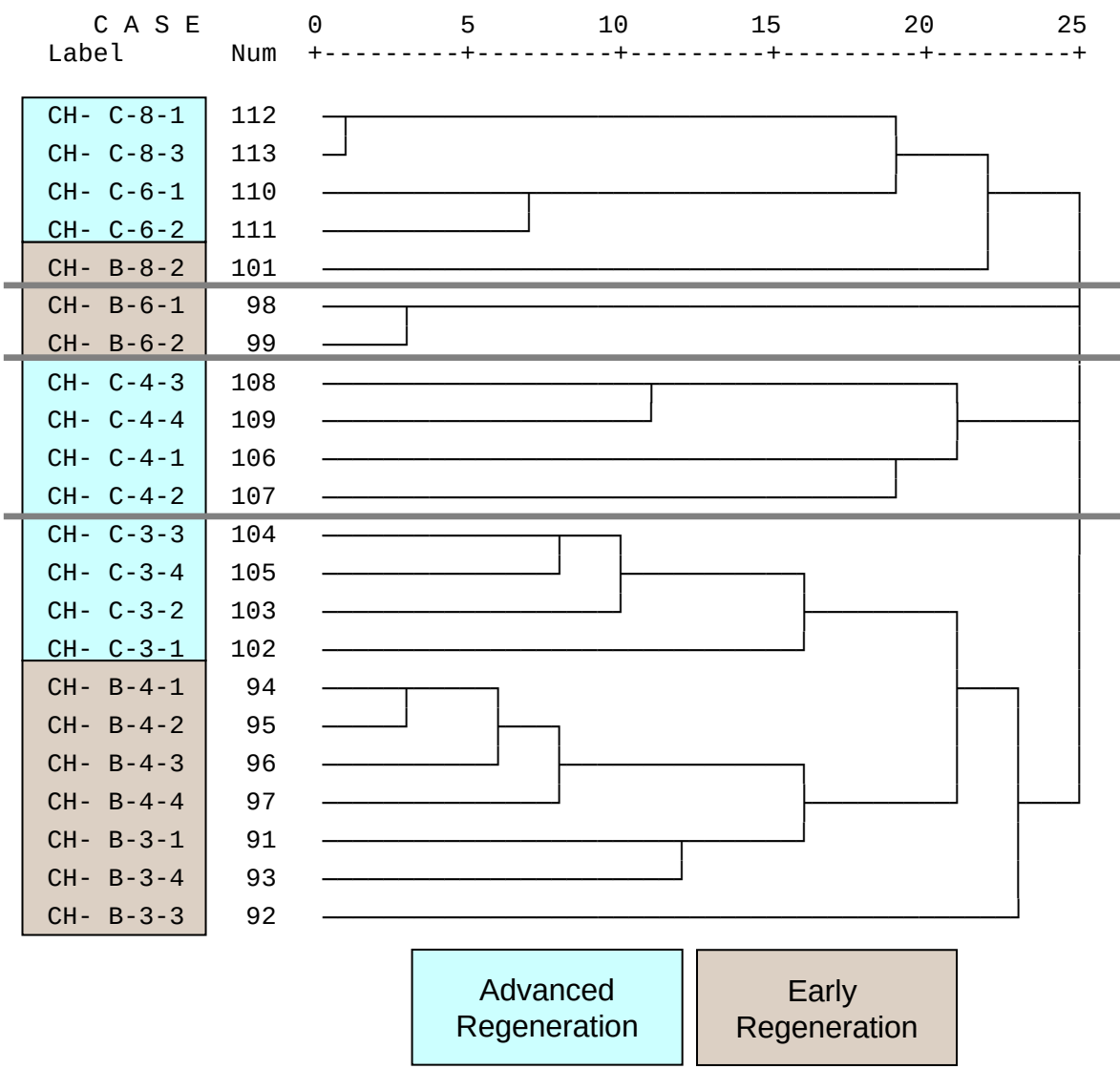
Early Regeneration

Results: Statistical evaluation

3.2 Influence of regeneration in Switzerland

- four main cluster
- primary effect of depth
- secondary effect of regeneration
- early regeneration: upper depth gradients distinctly separates from the lower depth gradients

⇒ no predominating influence of regeneration on bacterial communities



Conclusion

site effect	✓	**geographical clustering independent from vegetation**	**++++**
vegetation effect	✓	**bacterial communities under grass more similar to each other than bacterial communities under moss**	**+++**
regeneration effect	✓	**France (Le Russey): regeneration processes affect bacterial communities**	**++**
	X	**Switzerland: primary depth effect**	

Scientific Objectives

1. **Influence of country** ✓
2. **Influence of vegetation** ✓
3. **Influence of regeneration** ✓

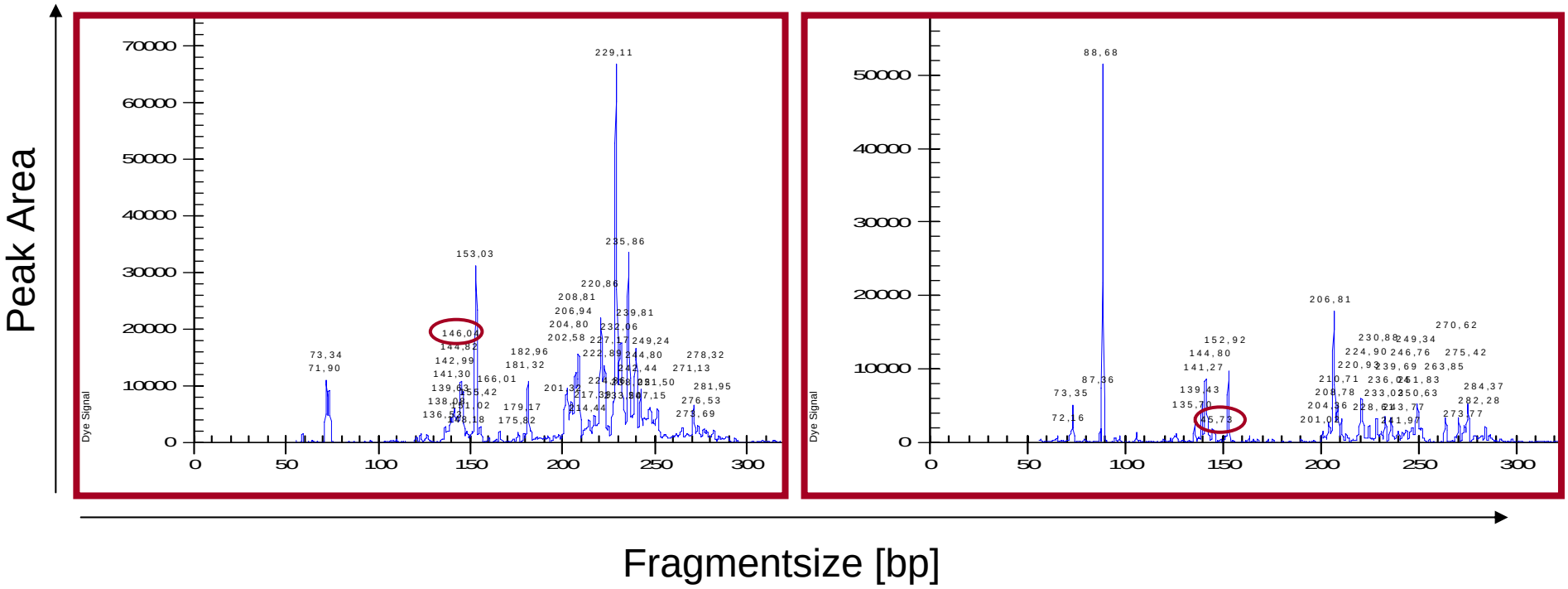
on bacterial communities

4. **Fragmentogram: vegetation/regeneration specific fragments?**

Fragmentogram of advanced regenerated peat samples (Le Russey, FR)

Fragment: 146 bp

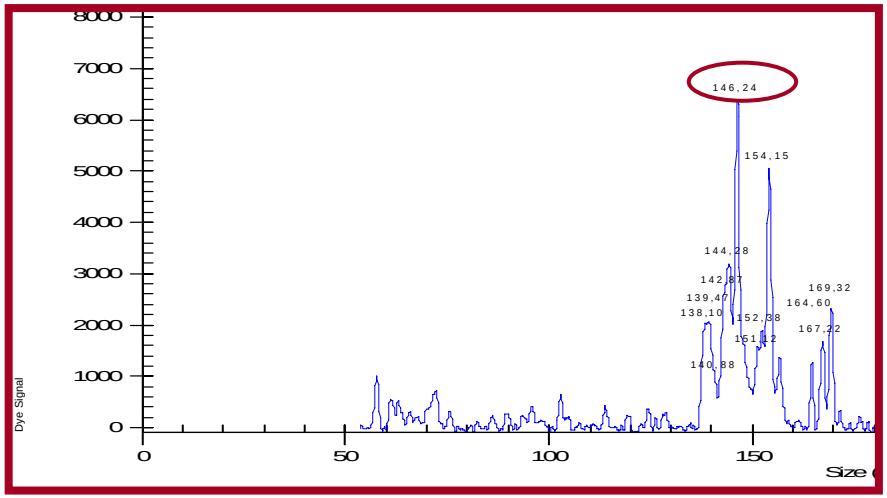
FR-C-4-1 **FR-C-4-2**



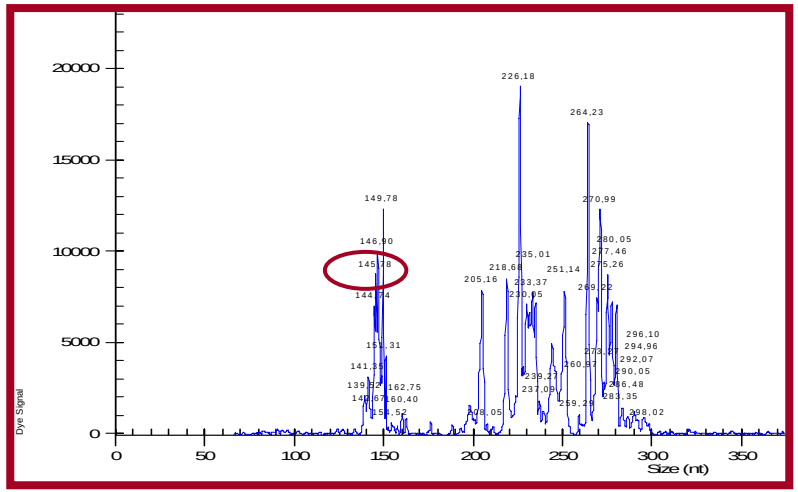
Fragmentogram of advanced regenerated peat (Chaux d'Abel, CH)

Fragment: 146 bp

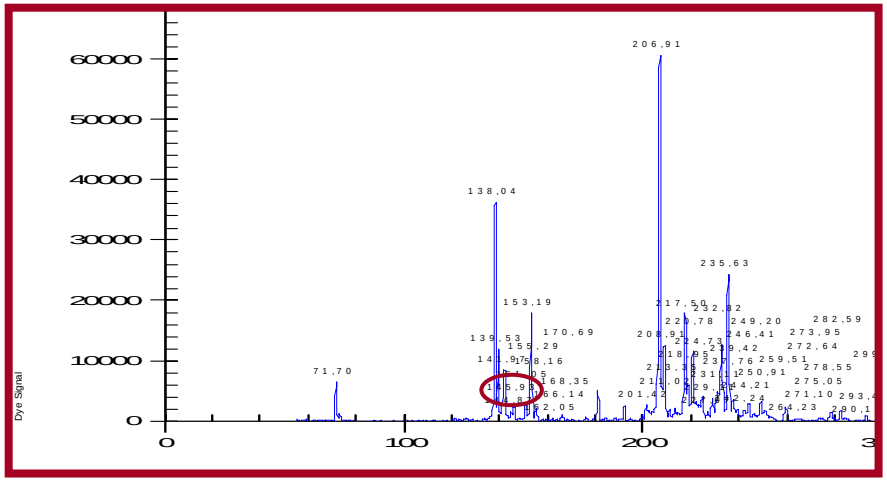
CH-C-4-1



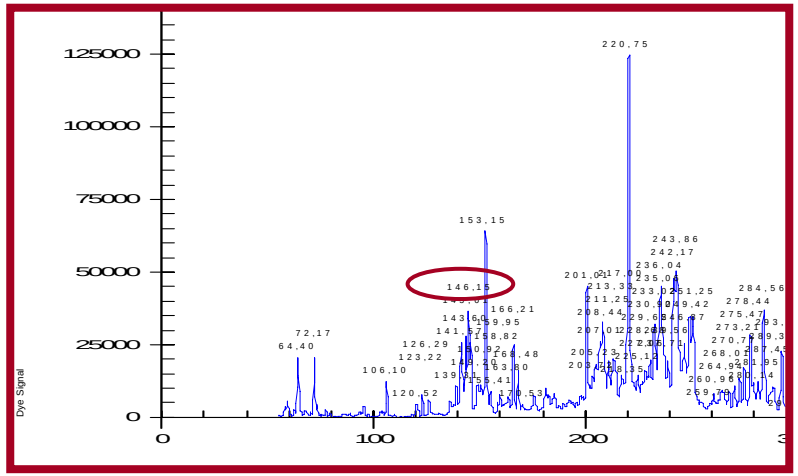
CH-C-4-2



CH-C-4-3

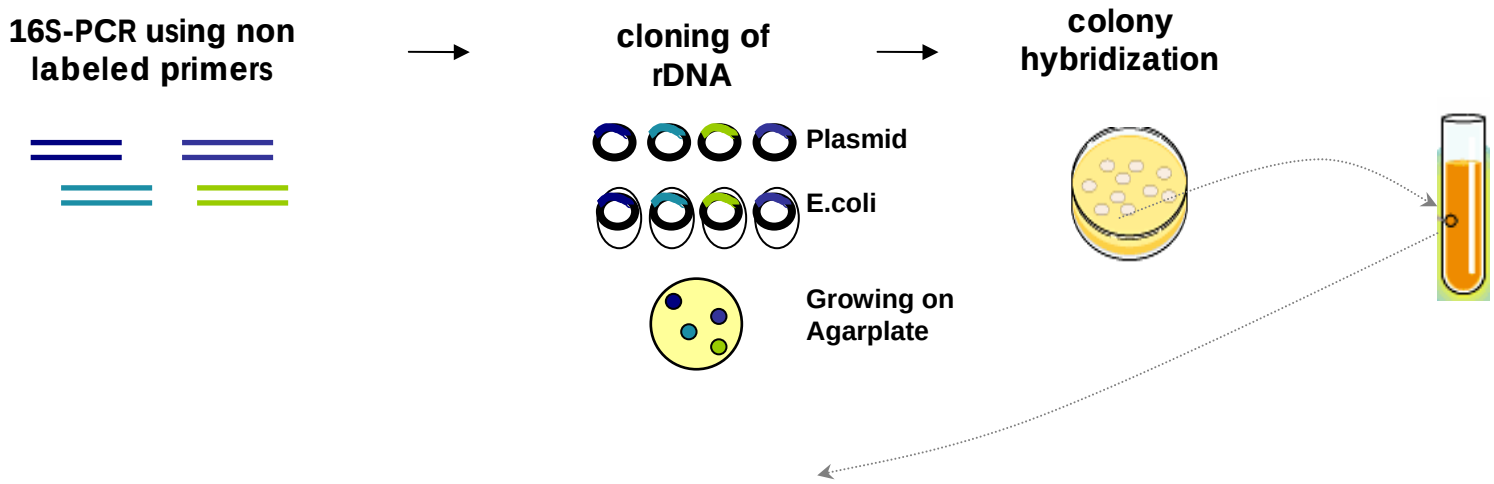


CH-C-4-4



Identification of Fragments of Interest

Approach



- t-RF Database
- which probes to use
- what are the hybridization conditions

Alignment with Database

Alignment with database: http://rdp8.cme.msu.edu/html/

TAP-TRFLP permits the user to perform in silico T-RFLP experiments on the RDP alignments (Marsh et. al, 2000), by assigning the sequences of

- primers
- restriction enzymes

that have been used. The output can be sorted and viewed either phylogenetically or by size.

=> Reduce organisms to a common level: phylum ‚**FIRMICUTES**‘

Hybridization Probes

http://www.microbial-ecology.de/probebase/

Probe	Sequence	T_M	Labeling 5'
LGC 354 A	tgg aag att ccc tac tgc	44.3 °C	DIG
LGC 354 B	cgg aag att ccc tac tgc	46.8 °C	DIG
LGC 354 C	ccg aag att ccc tac tgc	46.8 °C	DIG

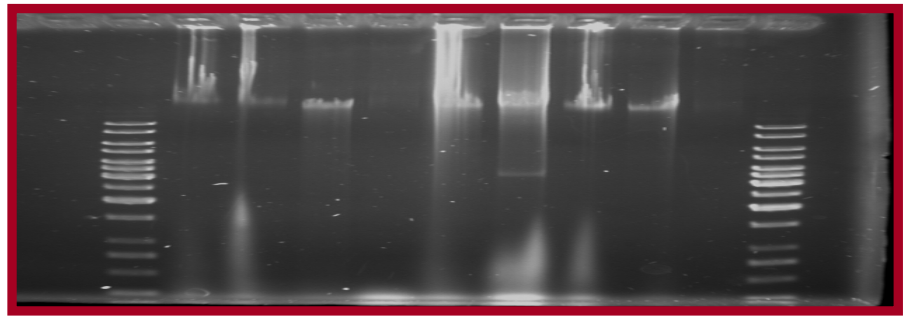
To test the specificity of the probe several bacterial isolates were chosen to serve as positive controls:

LGC 354 A:	*Leuconostoc fallax*	DSM 20189/ Medium 11, 30°C
	Lactobacillus suebicus	DSM 5008/ Medium 11, 30°C
LGC 354 B:	*Bacillus lichenoformis*	DSM 13/ Medium 1, 37°C
	Bacillus subtilis	DSM 10/ Medium 1, 30°C
	Bacillus alcalophilus	DSM 485/ Medium 31, 37°C
LGC 354 C:	*Enterococcus hirae*	DSM 20160/ Medium 53, 37°C
	Streptococcus thermophilus	DSM 20617/ Medium 53, 37°C

Determination of Hybridization-Conditions: Approach

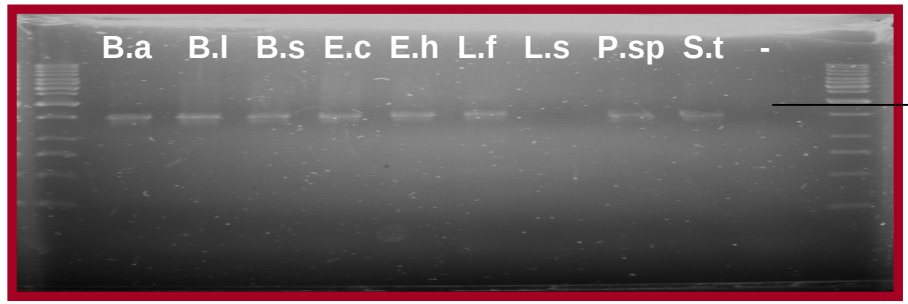
- DNA Isolation

Phenol-Chloroform-Extraction of control strains



- Amplification of 16S rDNA

using unlabelled primers

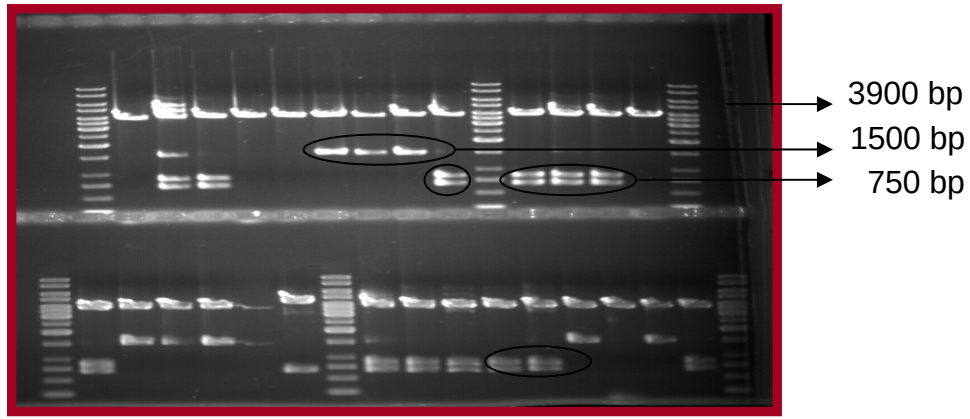


- Cloning + Transformation into E.coli Strains

using TA Cloning Kit (Invitrogen)

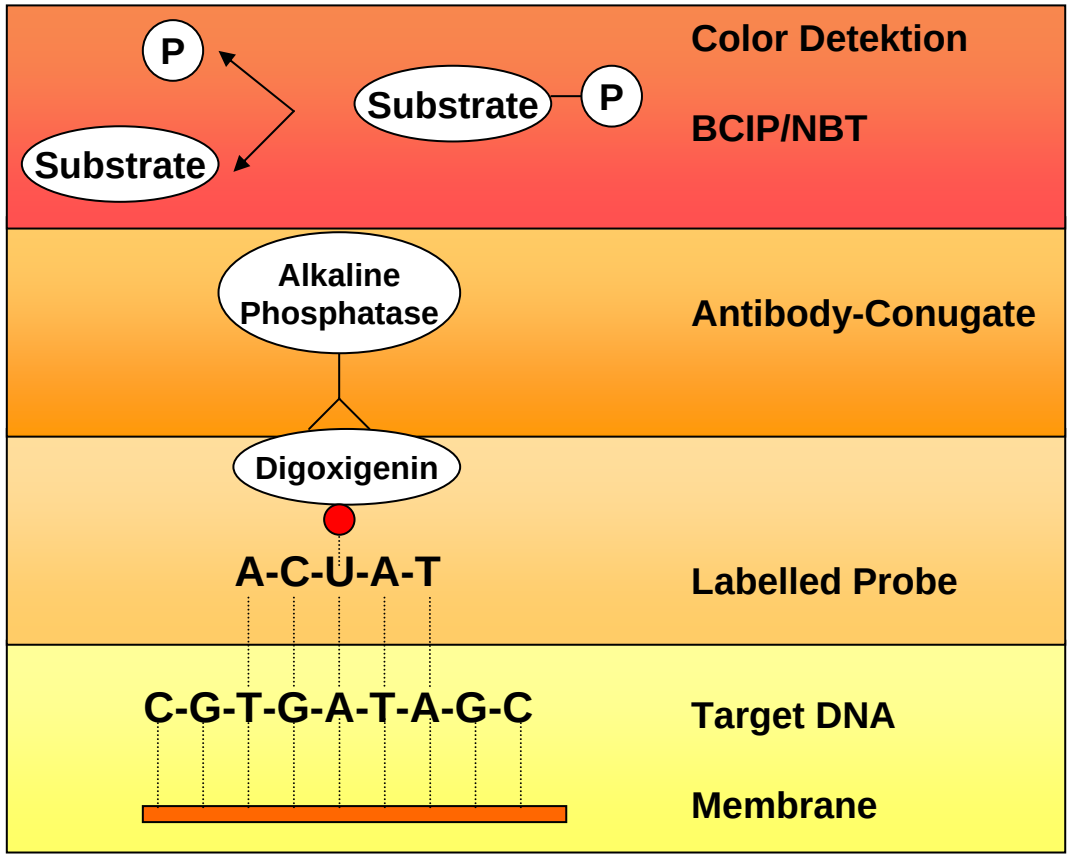
- Enzymatic Digest

using restriction enzyme EcoRI



Determination of Hybridization-Conditions: Approach

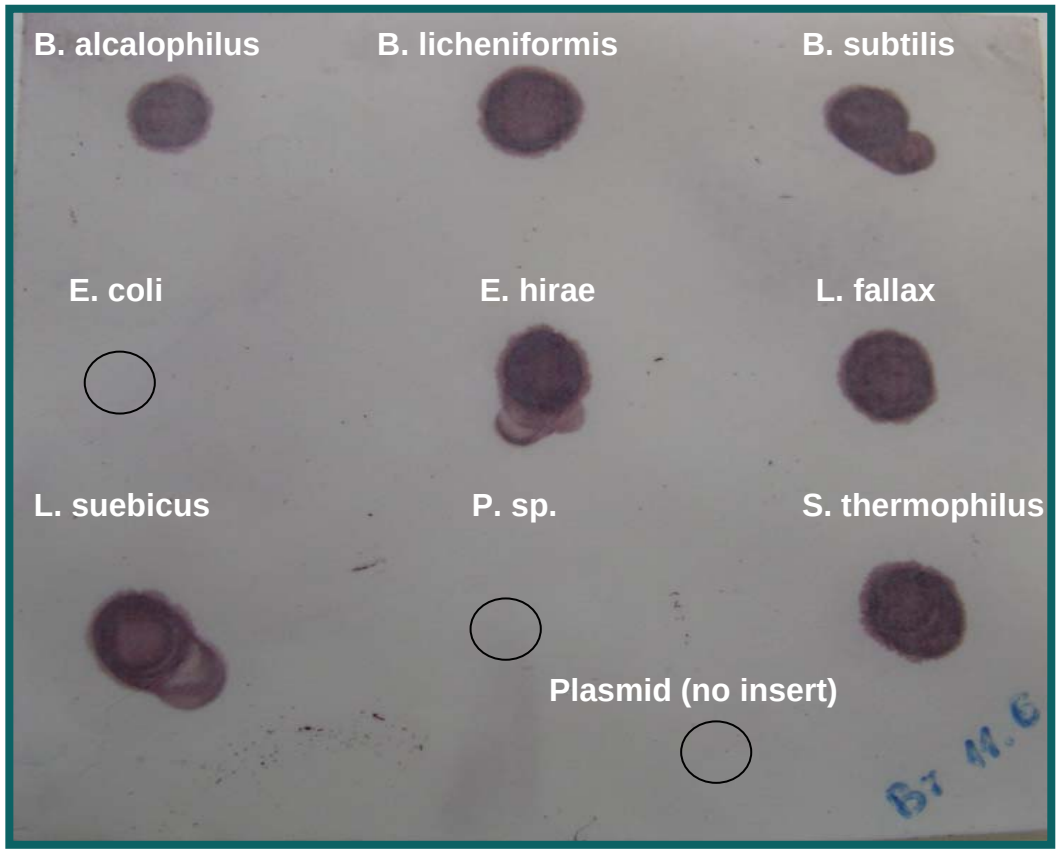
- Digoxigenin mediated Hybridization



Results

Determination of hybridization conditions

T_D=44.6°C



…to be done

- **colony hybridization with samples of interest at determined hybridization conditions**
- **sequencing of positive clones**
- **confirmation of concept by applying t-RFLP**

Thanks!

Michael Schloter

Alexandra Hagn

Andreas Gattinger

Stephen Chapman

Rebekka Artz