Experiment	**Enrichment and Isolation of Bacteria**
Advisor	NN.
Reading	Chapters1.6, 4.2, 4.3, 5.8, 16.4 in BBOM 9th Madigan M.T., J.M. Martinko and J. Parker: "Brock - Biology of Microorganisms", 9th Edition, (BBOM, International Edition), Prentice Hall, 1999. ISBN: 0-13-085264-3.
Objectives	• Isolate bacteria from an environmental sample • Learn how to define media composition and growth conditions • Define selection parameters
Background	In natural **habitats**, microbes live in **communities**. For studies on the role of individual **populations** in an **ecosystem**, however, microbiologists often study **pure cultures**. They **enrich** in defined or undefined **liquid media** or on **solid agar media** in petri dishes and **isolate clones** from individual **colonies**. A **petri dish** is a sterile „box" with a lid. It contains a solid medium (nutrient agar) on which microorganisms are allowed to grow. The medium is solidified by agar which can only be degraded by a few marine microbes. **Agar media** are heated until the agar has melted, they are then allowed to cool to about 45˚C before aliquotes of molten agar are poured into petri dishes. The medium is then allowed to solidify under **steril conditions** in the laminar flow hood. Prepared plates are sealed in plastic bags and stored at room temperature. No **contaminants** should appear on the plates if they are prepared properly. Specific **supplements** (vitamins, trace elements) or **inhibitors** (cycloheximide, ampicillin etc.) added to the medium increase selectivity.
Literature	
www. Links	
Practical Work	**Isolation and enrichment** steps to be carried out under *in situ* temperature conditions: • Get samples from a habitat where the desired organism is present • Choose medium: defined or undefined, low nutrient concentration or high nutrient concentration (for media composition see appendix) • Allow some of the organisms to grow a in a liquid medium which is *selectiv*e for the organisms you would like to isolate (= liquid enrichment culture) and b on a nutrient medium solidified with agar in a petri dish • Streak 100µl of the liquid enrichment culture onto agar plates and let grow under temperature conditions close to the ones *in situ* • Pick single colonies from the plates, and transfer them onto fresh ones (= isolation of strains) • Check purity by microscopy or with molecular techniques • Let the isolated strains grow in liquid culture for further biochemical and physiological analyses

	Each pair of students will receive 8 culture tubes and 2 agar plates containing one of several media. Please label tubes and plates with the media designation, the inoculum, the date and the group designation ! Liquid media and agar plates are inoculated with samples from different ecosystems (sediments of high mountain oligotrophic lakes, compost heaps, soil and water from the tropical show house of the Botanical Garden, etc.). The original cell suspensions are diluted up to 10^7 times and 100µl of each dilution are spread on a set of agar plates. Perform the following steps **Week 1**: **Dilution series and plating** • Transfer 0.6 ml of the original cell suspension into dilution tube 1 containing 5.4 ml of medium. • Repeat transfer 7 times. • Do not inoculate tube number 8 (control) • Streak 100µl aliquotes of each dilution tube onto one quarter of an agar plate (work aseptically !). You will need 2 agar plates for the 8 dilution samples. • Measure turbidity of the dilution tubes. • Let dilution tubes and agar plates grow at the appropriate temperature until next week's course. **Week 2**: **Analysis of growth and transfer** • Quantify growth by measuring turbidity and by counting colonies • Determine shapes of organisms in the phase contrast microscope • Pick single colonies of organisms which you would like to isolate from the agar plates and transfer them onto fresh agar plates • Incubate for a few days and isolate pure strains
Materials and Experimental Protocols	The following equipment is supplied for this experiment*:* • Undefined microbial communities in liquid cultures • 8 dilution tubes containing 5.4 ml of medium • 2 nutrient agar plates • pipettes and spreaders Procedures: • Making dilution series • Inocluating agar plates • Picking colonies asceptically • Comparing organisms under the microscope The composition of the media is listed in the appendix
Laboratory Rules & Precautions	Since it cannot be excluded that the bacterial communities which we use might contain pathogenic organisms, it is necessary to work cautiously and aseptically. Use **good laboratory practice**! Do not contaminate yourself, others or the laboratory environment. All waste must be sterilized before disposal. Please wash your hands before you leave the room and desinfect bench surfaces with 70 % ethanol.
Goals & Experiences gained	Enrich and isolate organisms from an environmental sample Learn how to design bacterial diets Examine bacteria under the microscope

<table>
<tr><td>Timing</td><td colspan="5">90 minutes</td></tr>
<tr><td rowspan="3">Reporting</td><td>Sample</td><td colspan="4">Medium (see appendix)</td></tr>
<tr><td></td><td>DLN 0.1x</td><td>DRN 10x</td><td>URN 1x</td><td>ULN 0.01x</td></tr>
<tr><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Questions to be answered</td><td colspan="5">Why is enrichment and isolation of bacteria done?
What alternative methods can be used for enrichment and isolation?
How are nutrient agar plates prepared and what are they used for?
What are the essential components in a culture medium for bacteria and why are they essential?
Design a general medium suitable for the enrichment of Cyanobacteria.</td></tr>
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Appendix: Media composition

Table 1: Low- and rich-nutrient, defined and undefined media

Medium Components	Defined low-nutrient medium DLN 0.1x		Defined rich-nutrient medium (RN) DRN 10x		Undefined rich- and low-nutrient medium	
	Amount	Conc.	Amount	Conc.	URN 1x Amount	ULN 0.01x Amount
$MgSO_4$ * 7 H_2O	1.48 mg/l	6 µM	150 mg/l	0.6 mM		
CaCl * 2 H_2O	1.47 mg/l	10 µM	150 mg/l	1 mM		
$NaHCO_3$	5.04 mg/l	60 µM	500 mg/l	6 mM		
NH_4Cl	0.54 mg/l	10 µM	54 mg/l	1 mM		
$NaNO_3$	0.85 mg/l	10 µM	85 mg/l	1 mM		
KH_2PO_4	0.36 mg/l	3 µM	36 mg/l	0.3 mM		
NaCl					5 g/l	5 g/l
Trace element solution 5000x	0.2 ml/l	1x	0.2 ml/l	1x	0.2 ml/l	0.2 ml/l
Vitamine solution 1000x	1 ml/l	1x	1 ml/l	1x		
Cycloheximide	50 mg/l	50 ppm	50 mg/l	50 ppm		
Ampicillin					(50 mg/l)	(50 mg/l)
Glucose	22.6 mg/l	120 µM	2.26 g/l	12 mM		
Tryptone					10 g/l	0.1 g/l
Yeast extract	0.5 mg/l		0.5 mg/l		5 g/l	0.05 g/l
NaOH 1 M					1 ml/l	
pH	7.2		7.2		7.2	7.2

LB-Medium ("Luria Bertani broth") amounts per liter. Solid media: 15 g agar per liter of liquid medium
Cycloheximide inhibits eukaryotes. Ampicillin selects for Amp^R

Table 2: Trace elements stock solution (5000x concentrated)

Components	Amount [g/l]	Final concentration [mM] in stock solution (5000x)
Fe(III)citrate monohydrate	10.52	40
$ZnSO_4$ * 7 H_2O	0.222	0.850
$MnCl_2$ * 4 H_2O	1.400	7.100
$Co(NO_3)_2$ * 6 H_2O	0.025	0.086
Na_2MoO_4 * 2 H_2O	0.390	1.600
Citric acid monohydrate chelator	6.250	30

Table 3: Vitamin stock solution (1000x concentrated)

Component	Amount [mg/l]	Final concentration [µM] in stock solution (1000x)
p-Aminobenzoic acid	10	73
Biotin	10	41
Cyanocobalamine	20	15
D-(+)-Ca-Pantothenate	30	65
Folic acid	10	23
α-Lipoic acid	20	97
Nicotinic acid	50	406
Pyrodoxine HCl	20	97
Riboflavine	30	80
Thiamine HCl	20	59