Project Title	**Survival strategies of microorganisms:** **The formation of reserve polymers in *Bacillus megaterium***
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Textbook Chapters	Madigan, M.T., J.M. Martinko and J. BROCK, BIOLOGY OF MICROORGANISMS, 9th ed. 1999, Prentice Hall. Chapters 3.11, 3.12, 3.13, and 14.20 Dawes E.A. (1986) Microbial Energetics. Blackie, Glasgow Chapters 11 and 12 Ebert G. (1992) Biopolymere. Teubner, Stuttgart, Chapter 5 Rehm H.J., Reed G. (Eds.) Biotechnology. Volume 6: Products of Primary Metabolism. Wiley-VCH, Weinheim, 1996, Chapter 13, pp. 403-464
Objectives and Research Questions	Cultivation of *Bacillus megaterium* in a air-lift bioreactor Observation of growth responses (spore formation, reserve polymer formation) on environmental stress, e.g. presence of heavy metals, excess of carbon substrate
Background	A wide variety microorganisms are known to form polymeric energy and carbon storage products. These polymers belong to the family of polyhydroxyalkanoates (PHA), with polyhydroxybutyrate (PHB) representing the best known member of this family. All polymers are formed intracellularly as granular inclusion bodies under unbalanced ("stressed") growth conditions, i.e. an excess of carbon substrates on one hand and a limiting compound (e.g. nitrogen) on the other. Polymer formation acts as microbial survival strategy. Because of these unbalanced growth conditions, reduction equivalents which originate from metabolic oxidation processes are stored in a water-insoluble, chemically and osmotically inert form. *Bacillus megaterium*, a gram-positive, spore-forming bacterium, is able to form PHA in large quantities, up to 50% of its cell dry weight. The composition of the polymer depends on the growth and carbon substrates present in the medium. PHA can be easily identified by fluorescence microscopy following staining with Nile Blue. Additionally, the composition of the polymer can be determined by high pressure liquid chromatography. PHA can be processed by plastic-manufacturing industries and applied as biodegradable plastics for a wide range of technical applications.
Selected Literature	Omar S., Rayes A., Eqaab A., Voss I., Steinbüchel A. (2001) Optimization of cell growth and poly(3-hydroxybutyrate) accumulation on date syrup by a Bacillus megaterium strain. Biotechnology Letters 23:1119-1123 Page W.J., Tenove C.J. (1996) Quantification of poly-beta-hydroxybutyrate by fluorescence of bacteria and granules stained with Nile Blue A. Biotechnology Techniques 10(4):215-220 Chen G.Q., König K.H., Lafferty R.M. (1991) Occurrence of poly-D(-)-3-hydroxyalkanotes in the genus Bacillus. FEMS Microbiology Letters 84:173-176

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Practical Work	Standard microbiological techniques will be used for the cultivation and growth optimization of *Bacillus megaterium*. From these results, an air-lift bioreactor (3L working volume) will be set up and operated. Growth, polymer formation and physiological reactions will be followed by optical and chromatographic methods. Reserve polymers can be obtained from the cells by solvent extraction.
Materials and experimental Protocols	Cultivation of *Bacillus megaterium*; operation and maintenance of an air-lift bioreactor; spectrophotometric techniques; high pressure liquid chromatography; fluorescence microscopy; solvent extraction
Goals and Experiences gained	Familiarity with • literature on microbial formation of reserve materials. • cell cultivation techniques (operation of an air lift bioreactor) • analytical techniques (fluorescence microscopy, high pressure liquid chromatography)
Timing	Cultivation and analyses can be performed during the duration of the course.
Reporting	Oral presentation, written report
Questions to be answered	How much reserve polymer is formed by *Bacillus megaterium* under "unbalanced" growth conditions and what is its composition? How can reserve polymer formation be followed in *Bacillus megaterium* by fluorescence microscopy?
Laboratory Rules and Precautions	Standard microbiological working techniques and precautions are required.