Experiment	**Fermentation of lactose by lactic acid producing bacteria: Yoghurt**
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Textbook Chapters	Chapter in BBOM 9th: 13.18 BBOM 9th: Madigan M.T., J.M. Martinko and J. Parker: "Brock - Biology of Microorganisms", 9th Edition, Prentice Hall, 1999. ISBN: 0-13-085264-3 or ISBN: 0-13-081922-0 (hard cover version) Chapter 14 in: White David: "The Physiology and Biochemistry of Prokaryotes", 2nd ed. Oxford, University Press, 2000. ISBN: 0-19-508439-X. Chapter 8.2 in: Schlegel, Hans Günter und Zaborosch, Christiane: "Allgemeine Mikrobiologie", 7. Auflage, Thieme Verlag 1992.ISBN: 3-13-444607-3
Objectives	Use of microbes to make yoghurt: Evaluate the effect of different **temperatures** on **metabolic activity**. Are "yoghurt bacteria" **thermophiles** ? Derive microbial metabolic activities that lead to the **coagulation** of milk proteins.
Background	Two bacteria are involved in making yoghurt: *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Both are **homolactic** (**homofermentative**); that is, they use **lactose** as an energy source and produce **lactic acid** as their **sole fermentation product**. Initially, the *Streptococcus thermophilus* is dominant, then it is inhibited by the produced acid. In a second phase, the *Lactobacillus bulgaricus* continues to ferment the remaining lactose; the pH drops from 6.5 to about 4.5. (Fermentation will be discussed in more detail in Biochemistry II.)  **Fig. 1:** Bacilli and cocci enriched from yoghurt Description of the *Streptococcus thermophilus* summarized from "**Bergey's Manual** of Determinative Bacteriology": • Gram positive spherical or ovoid cells, 0.7-0.9 µm in diameter in pairs to long chains. Final pH range in glucose broth is 4.0-4.5. The preferential fermentation of the disaccharides, sucrose and lactose, may result in a lower pH value as compared to glucose fermentation. Acid is produced from glucose, fructose, lactose and sucrose; no acid from trehalose, maltose, inulin, glycerol, mannitol, sorbitol or salicin and rarely from raffinose, xylose or arabinose. Optimum temperature is between 40°C and 45°C. Growth occurs at 50°C but not at 53°C. No growth at temperatures below 20°C. Heat tolerance: survives 65°C for 30 min. Source: milk and milk products such as cheese and yoghurt. Often used as a starter culture for these products. This species is easily recognized by its thermal tolerance; unable to ferment maltose and unable to grow in media containing ≥2.0% sodium chloride.

	Description of *Lactobacillus bulgaricus* according to Rogosa M. and P. A. Hansen (1971): • Gram positive rod, width <1 µm, contains aldolase, is negative for catalase, indole, nitrate reductase, oxidase, and benzidine reactions, attacks glucose and produces lactic acid as the major product. Does not ferment adonitol, dulcitol, erythritol, glycerol, glycogen, inositol, inulin, sorbose, starch. Does not produce gas from ribose, gluconate or glucose. Requires niacin, riboflavine and pantothenate. Produces up to 1.7% acid in milk, does not produce NH_3 from arginine. Utilizes lactose, and weakly also fructose, galactose, mannose. Does not utilize aesculin, amygdalin, arabinose, cellobiose, maltose, mannitol, melezitose, melibiose, raffinose, salicin, sucrose, trehalose, and xylose. In contrast to *Lactobacillus plantarum*, *Lactobacillus buchneri*, and *Lactobacillus brevis*, *Lactobacillus bulgaricus* grows at 45°C but not at 15°C. At least 19 strains identical with Orla-Jensen's strain (designated neotype strain; ATCC 11842, Orla-Jensen's *Thermobacterium bulgaricum* number 14, which he isolated from Bulgarian yoghurt) have been isolated from Bulgarian, Russian, Greek, Syrian, and Armenian yoghurts.
Literature	• Bergey's Manual of Determinative Bacteriology, Buchanan & Gibbons co-eds., 8th ed. [reprinted], The Williams & Wilkins Company, Baltimore 1975. • Rogosa M. and P. A. Hansen. 1971. Nomenclatural considerations of certain species of *Lactobacillus* Beijerinck. International Journal of Systematic Bacteriology 21 (2): 177-186.
www. Links	http://healthcastle.com/herb_lact.shtml http://www.reaseheath.ac.uk/%20/WidePart/ http://www.uni-hohenheim.de/i3ve/00217110/00612941.htm http://www.epub.org.br/bjmbr/year1998/v31n12/3137c.htm
Experimental Protocol	1. Mark 5 sterile glass beakers 1: 4°C, 2: 37°C, 3: 37°C boiled, 4: 60°C and 5: control, respectively. The beakers are covered with aluminium foil. 2. Aseptically add 20 ml of milk to each beaker. 3. The milk in beaker 3 should be boiled before adding the inoculum (keep on a wire mesh above a Bunsen burner (100°C) until the milk is cooking but not boiling over). A folded piece of paper towel can be used to hold the hot beaker. 4. Inoculate each beaker (except # 5) with about 0.5 ml of fresh yoghurt "nature" using a sterile teaspoon. Mix with the spoon. 5. Cover all beakers with the same aluminium foil again and incubate at the appropriate temperatures: beaker 1 in a refrigerator (+4°C), beakers 2, 3 and 5 at 37°C and beaker 4 at 60°C. 6. After 24 hours, all beakers will be stored in the refrigerator until the next course period when we will taste our "self-made" products. 7. Measure the pH of the whey.

Homemade Yoghurt (a receipt for your own kitchen)	1. Inoculum is a tenth of the volume (with one 2 dl cup of white yoghurt you can inoculate 2 l of milk). 2. Either boil the milk shortly or better keep it at 85°C for 30 min. Then let it cool down to 45°C. 3. Add the inoculum while stirring the milk. 4. Fill milk-yoghurt mixture into clean glasses, cover with aluminium foil. 5. Heat water to 45°C in a big pan, take pan from the hot plate, put glasses inside and cover the pan with a heat insulation (a woolen blanket for instance), or incubate the glasses in a 45°C preheated oven. 6. Leave at 45°C over night, then put into the refrigerator. Remark: The initial heating to 85°C during 30 minutes denatures some of the milk proteins which afterwards leads to a yoghurt of a more stable and finer consistency. At 45°C, the yoghurt formation is finished after about 4 hours, longer incubation times will lead to a more solid and more acidic tasting yoghurt.
Material	Each group gets: - 1 teaspoon - 5 clean, sterile (autoclaved) glass beakers, 100 ml, covered with aluminium foil - 100 ml of milk Available: - plain yoghurt bought in a food store - tripod, wire mesh, Bunsen burner - paper towels - 4°C, 37°C (or 45°C) and 60°C incubating rooms
Laboratory Rules & Precautions	No microbial risks involved. Keep the inoculum uncontaminated. Watch milk carefully while heating it.
Experiences gained	Taste a little bit of microbiology !
Timing	30 minutes
Reporting	Explain the purpose of the 8 steps outlined in the Experimental Protocol. Take notes on the experiment in your laboratory journal and report the results in class.

Protocol	**LABORATORY REPORT: Lactic Acid Bacteria** **Date:** **PURPOSE of assay steps**: **1.** . . . . **2.** . . . . **3.** . . . . **4.** . . . . **5.** . . . . **6.** . . . . **7.** . . . . **8.** . . . .

Assay No.	1	2	3	4	5
Treatment of medium					
Inoculum					
Incubation					
pH beginning pH end					
Coagulation					
Taste					
Remarks					

Questions to be answered

1. What is the effect of **different temperatures** on microorganisms ?
2. List bacteria which use the **heterofermetative** pathway to produce lactic acid and mention others which do it **homofermentatively** (BBOM 9th Table 13.25).
3. Outline the homofermentative **pathway** for lactose breakdown to lactic acid and find the differences between homofermentation and heterofermentation (BBOM 9th Figure 13.54).
4. Write the **stoichiometrically correct equation** for the formation of lactate from a **disaccharide** (e.g. lactose $C_{12}H_{22}O_{11}$) by a homofermeter.
5. Why is it <u>not</u> recommended to use a heterofermentative lactic acid bacterium to produce a typical yoghurt ?
6. How would you **test** whether or not your yoghurt contains heterolactic bacteria ?

Observations