

Lab 5. Bioassay Investigation of Lysine Content in Foods.

A **bioassay** (or biological assay) refers to an assay of a substance based on the growth or physiological response of a living organism. Bioassays commonly utilize a microbial species whose growth requires a particular limiting nutrient. Microbial growth can then be used as a quantitative measure of the limiting nutrient when it is present at extremely low concentrations in a mixture of substances, such as a food or bodily fluid. For example, the antibiotic nisin, used as a food preservative, has been assayed in many types of foods using the bacterium *Lactococcus lactis* (Hakovirta et al, 2006). Another example of a bioassay is the use of baker's yeast as an indicator organism for a trichothecene toxin in contaminated soil (Abolmaali et al, 2008).

Our investigation focuses on the assay of lysine content in foods, using the bacterium *Pediococcus acidilactici* ATCC 8042 (Difco & BBL Manual). Lysine is one of the nine essential amino acids required by humans in the diet, because the human body lacks the enzymes for its biosynthesis. The World Health Organization (WHO) recommends daily lysine consumption of 12 mg/kg body weight.

In our investigation, we will (1) construct a standard curve to test the effectiveness of the Difco bioassay for lysine; (2) use the bioassay to investigate food quality by measuring the lysine content of various foods. The course of this investigation will generate further insights into the nature of bacterial growth curves (introduce in Lab 3) and demonstrate an application of principles of bacterial metabolism (Lab 4).

Week 1. Bioassay: Lysine Standard Curve and Analysis of Powdered Milk

Media provided for Week 1; preparation is described

Before media preparation, all glassware must be autoclaved for 1 hour.

***Pediococcus acidilactici* ATCC 8042 grown overnight** in Difco *Lactobacilli* broth

0.85% NaCl Solution (2.55 g of NaCl in 300 mls of DI water, Autoclave)

L-lysine Stock Solution (2.5 g of L-lysine in 250 mLs of DI water, Filter Sterilize)

L-lysine assay medium (5 ml per tube):

Suspend 10.5 g of L-lysine assay powdered medium in 100 ml of DI water.

Heat with frequent agitation and boil for 2-3 minutes.

Dispense 5 ml medium into sterile screw-cap tubes.

Part A. L-lysine Standards

1. Dilute **0.6 ml** of L-lysine Stock Solution into **9.4 mls** of DI H₂O; filter Sterilize, into sterile tube.

2. Make standard solutions by adding an aliquot of Diluted Stock to 5 ml assay medium:

Add volume:	0	500 μ l	1 ml	1.5 ml	2 ml	2.5 ml	3 ml	4 ml	5 ml
Final μg/ml	0	30	60	90	120	150	180	240	300

3. Adjust the volume of each tube to 10 ml using DI water (5 ml minus the lysine aliquot volume). Autoclave for 10 min (total cycle approx. half an hour).

Part B. Lysine Assay using a Microwell Plate

1. Strain ATCC 8042 is grown overnight in 10 mls of *Lactobacilli* broth from 18-24 hours at 37°C. Centrifuge overnight cultures at room temperature for 10 min. Remove supernatant, using micropipet. Add 10 ml of sterile 0.85% NaCl to the pellet.

2. Centrifuge cells 3 more times, and each time add 10 ml of sterile 0.85% NaCl. The final time, resuspend cells using sterile squeeze-pipet.

3. Dilute 1 ml of cell culture into 19 ml of sterile 0.85% NaCl (1/20 dilution). This is your **Diluted Cell Suspension**.

4. Inoculate each lysine standard assay tube (10 ml total) with **50 µl** of Diluted Cell Suspension.

5. Aliquot 200 µl of lysine-cell samples into appropriate wells of microtiter plate. Record the grid pattern of your samples. **Each pair of partners use one row of the plate.**

6. In the Spectramax, take spectral readings at OD_{660nm} every hour for 20 hours at 37 °C.

Data Analysis – Bring plots to lab next week. (No writeup due, but we will compare plots.)

1. Plot your own growth curves. (Try offsetting the X axis values to get several curves on one plot.) What growth phases do you see? Does bacterial growth level off or continue?

2. Plot lysine standard curves (OD_{660nm} as a function of lysine concentration) using Spectramax plate readings at 14 h, 16 h, 18 h, and 20 h. Which portions of your curves appear to parallel the standard lysine concentrations?

3. Plan an investigation of food lysine content for Weeks 2 and 3.

References

Abolmaali, S., R. Mitterbauer, O. Spadiut, M. Peruci, H. Weindorfer, D. Lucyshyn, G. Ellersdorfer, M. Lemmens, W. D. Moll, and G. Adam. 2008. Engineered bakers yeast as a sensitive bioassay indicator organism for the trichothecene toxin deoxynivalenol. *J. Microbiol. Methods*. Jan 9; [Epub ahead of print].

Difco & BBL Manual, Amino acid assay media: lysine assay medium, pp 46-48. BD Diagnostic Systems, <http://www.bd.com/ds/technicalCenter/>

Hakovirta, J., J. Reunanen, and P. E. J. Saris. 2006. Bioassay for nisin in milk, processed cheese, salad dressings, canned tomatoes, and liquid egg products. *Appl. Envir. Microbiol.* **72**:1001-1005.