Data-Analysis Problems for Microbiology Courses

Charles E. Deutch

Microbion Research

8734 E. Indian Hills Rd. Unit J

Orange, CA 92869

Email: Charles.Deutch@asu.edu

Telephone: (602) 703-9770

Key words: data analysis, exams, experimental methods, microbiology, problem sets

Introduction

There is general agreement that scientific literacy in biology should include both information about specific topics and an understanding the process by which this information is obtained. The Vision and Change in Undergraduate Biology Education document produced by the American Association for the Advancement of Science (AAAS) identified the key concepts that students need to understand as well as the set of competencies they need to acquire (AAAS 2009; Woodin et al. 2010). The competencies include the ability to apply the process of science, the ability to use quantitative reasoning, and the ability to use modeling and simulation. Since the publication of this proposal, instructors of a variety of different courses have developed strategies for doing this that go beyond the standard textbooks, class lectures, and lab exercises. These include the careful analysis of graphs (Pechenik and Tashiro 1992; Malamitsa et al. 2008), the reading of news stories and related journal articles (Krontiris-Litowitz 2013; Moisander 2021), the use of course based undergraduate research experiences or CUREs (Bennett et al. 2021; Sun et al. 2022), and extended undergraduate research programs such as SEA-PHAGES (Jordan et al. 2014). These approaches have been linked to Bloom's taxonomy of learning where students move from the acquisition of basic knowledge and comprehension to application and analysis and finally on to evaluation and synthesis (Bloom et al. 1956).

While the goals of undergraduate education have changed, most textbooks have not. Although those in the area of Genetics normally include data analysis problems as the end of each chapter, only a few of those in the areas of Biochemistry, Cell Biology, and Ecology do so. This is particularly a problem in the case of Microbiology, where the primary target audience for textbooks is students in allied health fields such as nursing. The acquisition of basic information and demonstrating its relevance to human disease are the primary goals of the course, not the

scientific process. Even the textbooks for biology or microbiology majors such as those by Willey et al. (2022), Wessner et al. (2021), Madigan et al. (2017), and Slonczewski et al. (2020) contain very little material on this process. In 1997, I published a paper entitled "Using data analysis problems in a large general microbiology course" in The American Biology Teacher (Deutch 1997). This paper described the organization of my course, the structure of the data analysis problems, and the use and student response to these problems. Each problem was based on a single journal article and included five multiple-choice questions. The multiple-choice questions were of three basic types: type A questions were those that asked students to recall basic terms or concepts related to the problem; type B questions were those that asked students to read a table or figure of data; and type C questions were those that asked students to draw conclusions from the data, to make predictions, or integrate the material with other information presented in the course. The paper included three examples dealing with microbial structure, microbial metabolism, and microbial pathogenesis.

The purpose of this manuscript is to provide additional examples of these data-analysis problems and to set up a mechanism by which other instructors might contribute more problems appropriate to their courses and areas of interest. The new examples fall into six general areas (microbial structure, microbial growth, microbial metabolism, microbial genetics, microbial ecology, and microbial pathogenesis), although some problems utilize concepts from several areas. The examples in each area include one problem taken from an older set written in the 1990s and one new problem based on journal articles published from 2010 to 2020. Most have five multiple-choice questions but some have more. The problems are written in Microsoft Word so other instructors can modify them as necessary to meet the needs of students in their courses. As suggested before, the problems may be used as part of problem sets, discussion

boards, or exams. I have found that in-class discussions with groups of students work best, but other instructors may use them in other ways. I encourage other instructors to create similar problems based on their personal areas interest and those of the students in their courses.

References

American Association for the Advancement of Science (AAAS) Conference Homepage. 2009. Vision and Change in Undergraduate Biology Education: A View for the 21st Century. Available at https://www.aps.org/programs/education/undergrad/upload/Revised-Vision-and-Change-Final-Report.pdf

Bennett, K. F., Arriola, P. E., Marsh, T. L., Mineo P. M., Raimondi, S. L, and Shaffer, C. L. 2021. CURE in a box: an online CURE for introductory biology majors that incorporates *Vision and Change*. Journal of Microbiology & Biology Education 22(1): 22.1.20. doi: 10.1128/jmbe.v22i1.2325.

Bloom, B. S., Engelhart, M. D., Furst, E. J., Hill, W. H. and Krathwohl, D. R. 1956. Taxonomy of educational objectives: The classification of educational goals. Handbook I: Cognitive domain. David McKay Company.

Deutch, C. E. 1997. Using data analysis problems in a large general microbiology course. The American Biology Teacher 59: 396-402.

Jordan T. C., Burnett, S. H., Carson, S., Caruso, S. M., Chase, K., DeJong, R. J. et al. 2014. A broadly implementable research course in phage discovery and genomics for first-year undergraduate students. Mbio 5(1): e0151-13. doi: 10.1128/mBio.01051-13.

Krontiris-Litowitz, J. 2013. Using primary literature to teach science literacy to introductory biology students. Journal of Microbiology & Biology Education 14: 66-77. doi: 10.1128/jmbe.v14i1.538

Madigan, M., Bender, K., Buckley, D., Sattley, W., and Stahl, D. 2017. Brock Biology of Microorganisms, 15th edition. Pearson.

Malamitsa, K., Kokkotas, P., and Kasoutas, M. 2008. Graph/chart interpretation and reading comprehension as critical thinking skills. Science Education International 19: 371-384.

Moisander P. H. 2021. Practicing critical thinking in undergraduate microbiology classes by presenting news stories with data evidence. Journal of Microbiology & Biology Education 22: e00171-21. doi: 10.1128/jmbe.00171-21.

Pechenik, J. A., and Tashiro, J. A. 1992. The graphing detective: an exercise in critical reading, experimental design & data analysis. The American Biology Teacher 54: 432-435.

Slonczewski, J. L., Foster, J. W., and Zinser, E. R. 2020. Microbiology: an evolving science, fifth edition. W. W. Norton & Company.

Sun E., König, S. G., Cirstea, M, Hallam, S. J., Graves, M. L., and Oliver, D. C. 2022. Development of a data science CURE in microbiology using publicly available microbiome data sets. Frontiers in Microbiology 13:1018237. doi:10.3389/fmicb.2022.101823

Wessner, D., Dupont, C., Charles, T., and Neufeld, J. 2021. Microbiology, third edition. Wiley.

Willey, J., Sandman, K., and Wood, D. 2022. ISE Prescott's Microbiology, 12th edition. McGraw Hill.

Woodin T., Carter, V. C., and Fletcher, L. 2010. Vision and change in biology undergraduate education, a call for action – initial responses. CBE Life Sciences Education 9: 71-73. doi: 10.1187/cbe.10-03-0044.

Sample Data Analysis Problems

Examples of sample problems are given on pages 8 through 59. Each problem begins on a separate page so they can be individually copied, downloaded, and edited.

Problems in Microbial Structure

Problem 1 focuses on differences in the chemical composition of the cell or plasma membrane and the outer membrane of the Gram-negative bacterium *Paracoccus denitrificans*. Problem 2 concerns the possible functions of the S layer of the pathogenic Gram-negative bacterium *Campylobacter fetus*.

Problems in Microbial Growth

Problem 3 deals with the effect of the compound glycine betaine on the growth of the Gram-positive bacterium *Listeria monocytogenes* in high concentrations of salt at different temperatures. Problem 4 deals with the growth and formation of extracellular polysaccharides by the Gram-positive bacterium *Streptococcus thermophilus.*

Problems in Microbial Metabolism

Problem 5 focuses on the role of the enzyme fructose-1,6-bisphosphate phosphatase in the metabolism of the Gram-negative bacterium *Xanthobacter flavus*. Problem 6 is concerned with the role of the enzyme L-malate dehydrogenase in the metabolism of the autotrophic nitrifying bacterium *Nitrosomonas europeae*.

Problems in Microbial Genetics

Problem 7 deals with the transformation of the pathogenic bacterium *Vibrio vulnificus* in the laboratory. Problem 8 focuses on the use of transposon mutagenesis to identify genes involved in desiccation tolerance in by the food-borne pathogen *Staphylococcus aureus.*

Problems in Microbial Ecology

Problem 9 deals with role of *Rhizobium meliloti* in nitrogen fixation in alfalfa. Problem 10 is concerned with the isolation and potential use of a novel bacterium from Antarctica that has a thermotolerant enzyme.

Problems in Microbial Pathogenesis

Problem 11 deals with the mechanism of pathogenesis and the role of pili in the bacterium *Vibrio cholerae*. Problem 12 is concerned with the exotoxin proteins formed by *Aeromonas trota* that have hemolysin or proteolytic activities.

Problem 1

Area: Microbial Structure
Microorganism: *Paracoccus denitrificans*
Reference: Nauyalis, P. A., Hindahl, M. S., and Wilkinson, B. J. 1985. Isolation, characterization and protein and polar lipid compositions of *Paracoccus denitrificans* outer membrane. Biochimica et Biophysica Acta 840: 297-308.

The cell membrane and the outer membrane of Gram-negative bacteria differ in biochemical structure and function. *Paracoccus denitrificans* is a Gram-negative bacterium that has been extensively studied because it carries out respiration in a way that is very similar to that found in mitochondria. To study the composition of the cell membrane and the outer membrane of *P. denitrificans*, Nauyalis, Hindahl, and Wilkinson (Biochim. Biophys. Acta 840: 297-308, 1985) disrupted the bacteria mechanically and subjected the broken cells to high-speed centrifugation. They collected two membrane fractions and then analyzed their composition.

component	fraction A	fraction B
protein (µg/mg)	623	501
lipid (µg/ml)	515	321
carbohydrate (µg/ml)	11.6	40
KDO (µg/ml)	2.3	5.7
hexosamine (µg/ml)	1.8	12.4
NADH dehydrogenase (units/mg protein)	119	trace
succinate dehydrogenase (units/mg protein)	460	29

1. Which of the following statements best describes the organization of the proteins and lipids in these membranes?

   a. The lipids form a bilayer with the proteins bound to the inner and outer surfaces.
   b. The proteins form an inner layer with the lipids bound to the surfaces.
   c. The lipids form a bilayer with the proteins either inserted into the bilayer or bound to their surfaces.
   d. The lipids and proteins are randomly organized into a continuous layer.

2. The space between the cell membrane and the outer membrane in a Gram-negative bacterium is referred to as:

   a. the cytoplasm
   b. the periplasm
   c. the epiplasm
   d. the extracellular space

3. The compound KDO (2-keto-3-deoxyoctonic acid) is almost exclusively found in the lipopolysaccharides characteristic of Gram-negative bacteria. The enzymes NADH dehydrogenase and succinate dehydrogenase are usually involved in respiration and ATP synthesis. From the results in the table, which of the following statements is most correct?

   a. Fraction A corresponds to the cell membrane.
   b. Fraction A corresponds to the outer membrane.
   c. Fraction B corresponds to the cell membrane.
   d. Neither fraction corresponds to the cell membrane.

4. From the results given in the table, which of the following statements is most correct.

   a. The cell membrane and the outer membrane have the same composition.
   b. The cell membrane and the outer membrane were completely separated in this experiment.
   c. The cell membrane and the outer membrane were partially separated in this experiment, but each fraction was contaminated by small amounts of the other.
   d. The cell membrane and the outer membrane were not separated in this experiment.

When the phospholipid composition of the membrane fractions was measured, the results in the next table were obtained (each phospholipid is given as the percent of total lipid phosphorus).

phospholipid	fraction A	fraction B
phosphatidylglycerol	55	86
phosphatidylethanolamine	16	7
phosphatidylcholine	9	7
cardiolipin	15	1

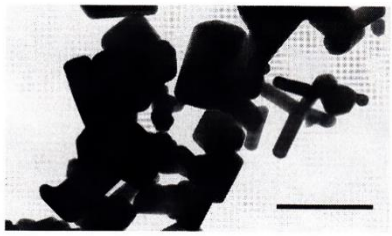
5. The data in the table indicate that:

   a. the two membranes are composed only of phospholipids.
   b. the cell membrane and outer membrane have the same phospholipid composition.
   c. The cell membrane and outer membrane differ in phospholipid composition.
   d. The cell membrane is a bilayer but the outer membrane is a monolayer.

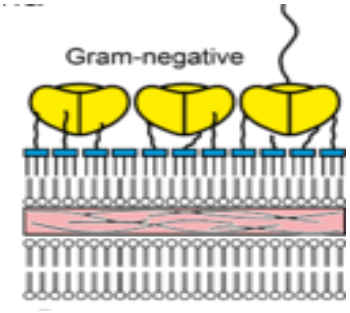
Problem 2

Area: Microbial Structure
Microorganism: *Campylobacter fetus*
Reference: Graham, L. L., and Feero, S. E. 2019. The *Campylobacter fetus* S layer provides resistance to photoactivated zinc oxide nanoparticles. Canadian Journal of Microbiology 65: 450-460.

The genus *Campylobacter* includes more than 26 species of Gram-negative spiral shaped bacteria that are often pathogenic to humans or other animals. *C. fetus* includes several subspecies that can cause venereal disease in cattle, sheep, or goats, leading to premature labor and spontaneous abortion. While antibiotics can be used to treat these infections, an alternative approach involves the use of zinc oxide (ZnO) nanoparticles which can penetrate the cell envelope and lead to the formation of reactive oxygen species that are highly toxic. Graham and Feero (Can. J. Microbiol. 65: 450-460, 2019) investigated the role of the S layer of the cell envelope *C. fetus* in protecting the bacteria from ZnO nanoparticles. The following picture from their paper shown an electron micrograph of some of these particles.



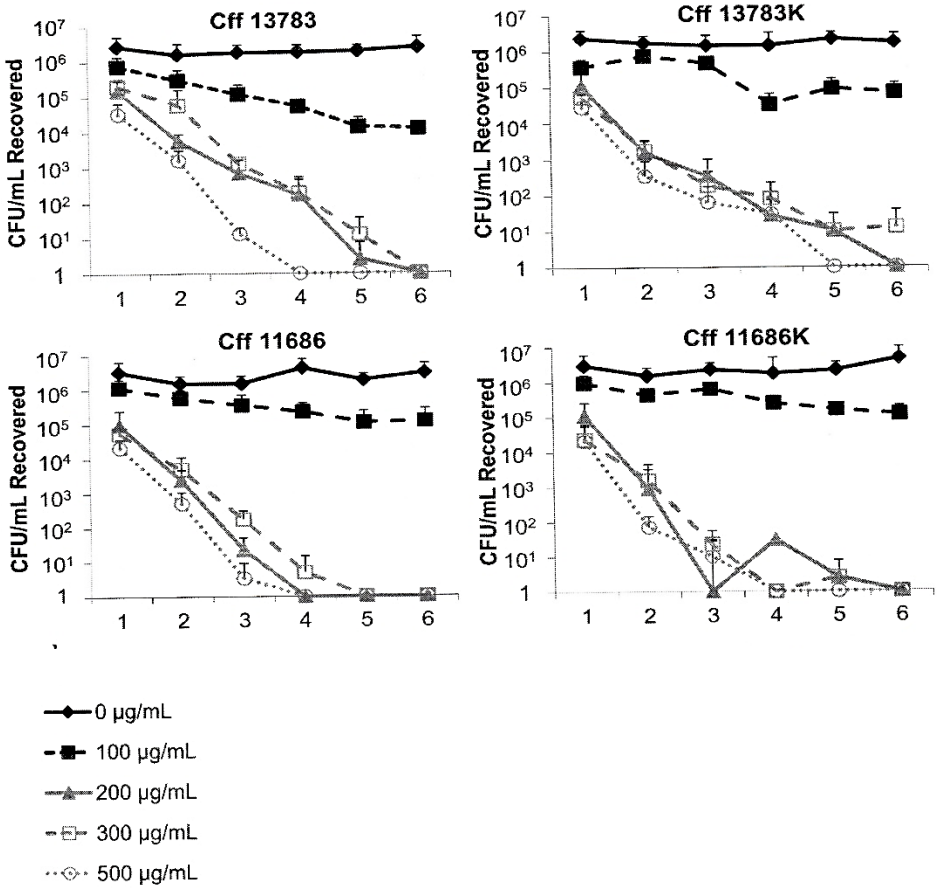
The following figure is a diagram of the envelope of a Gram-negative bacterium.



1. What is the best definition of a bacterial S layer?

   a. It is an organized bilayer of phospholipids and proteins that surrounds the cytoplasm.
   b. It is a multilayered network of peptidoglycan molecules that lies outside of the plasma membrane.
   c. It is a second layer of phospholipids and proteins and surrounds the cell wall.
   d. It is an ordered array of identical protein molecules that lies outside of the outer membrane.

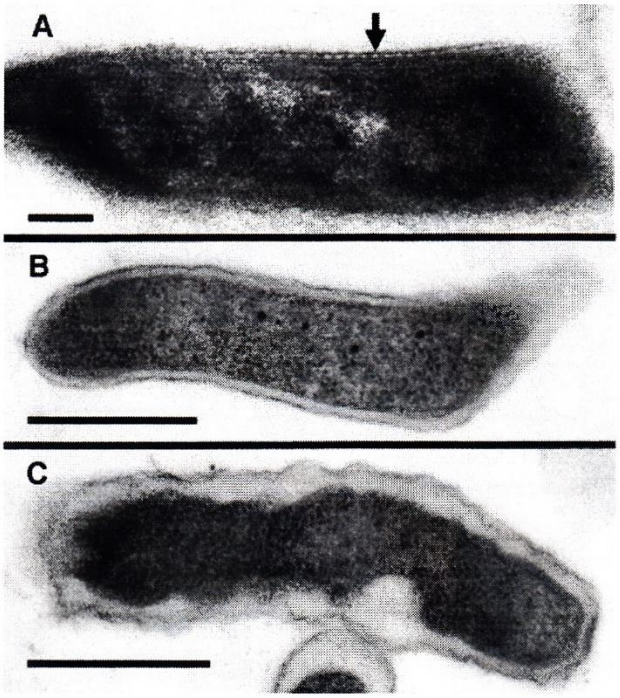
To study the effects of ZnO nanoparticles on *C. fetus*, Graham and Feero used several strains of that different in their expression of the S layer proteins. Cff 13783 and Cff 11686 had high levels of expression and Cff 13783K and Cff 11686K had low levels of expression due to insertion of transposable element carrying a gene for kanamycin resistance. The bacteria were routinely cultured on Columbia agar supplemented with 5% sheep blood without or with 50 µg/ml kanamycin. The next figure shows the number of viable bacteria present in each culture expressed as colony forming units/ml (CFU/ml) following exposure to different concentrations of ZnO nanoparticles for up to 6 hours.



2. What is the best conclusion to be drawn from these data?

a. There was no decrease in viability in the untreated samples or in any of the treated samples.
b. There was no decrease in viability in the untreated samples but viability was reduced in a concentration dependent-fashion in all of the treated samples.
c. There was no decrease in viability in the untreated samples but viability was reduced in a concentration-dependent fashion more in the strains with an S layer than in the ones without an S layer.
d. There was no decrease in viability in the untreated samples but viability was reduced in a concentration-dependent fashion more in the strains without an S layer than in the ones with an S layer.

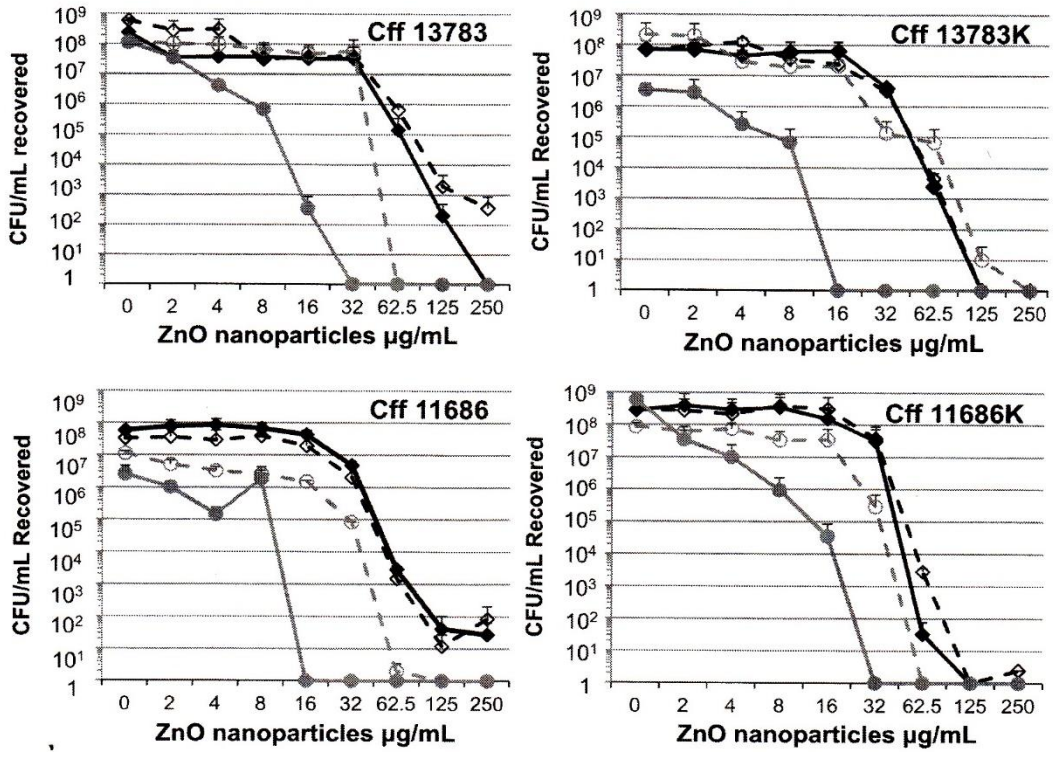
To determine if the ZnO nanoparticles might affect the cell envelope of the bacteria, the authors examined thin sections of the bacteria by electron microscopy. The results are shown in the following figure. Panel A shows an image of freeze substituted *C. fetus*, Panel B shows an image of *C. fetus* not treated with ZnO nanoparticles but processed by conventional EM techniques; and Panel C shows an image of *C. fetus* treated with 500 µg/ml ZnO nanoparticles for four hours and processed by conventional EM techniques.



3. What can you see in these images?

   a. The ZnO treated cells have the same general appearance as the untreated cells.
   b. The bacteria treated with ZnO nanoparticles have extensive gaps in the cell envelope between the cell membrane and the outer membrane.
   c. The bacteria treated with ZnO nanoparticles have no S layer.
   d. The bacteria treated with ZnO nanoparticles have no cell envelope and have undergone extensive lysis.

In previous studies, it was found that bacteria treated with ZnO nanoparticles and then exposed to visible light died at a greater rate than those kept in the dark. To determine if this were true for C. fetus, they inoculated medium containing different concentrations of ZnO nanoparticles in the wells of microtiter plates with the same strains used before. One plate was incubated in the dark and the other exposed to light for 30 min. They then determined the number of viable cells after 24 hours and expressed the results again as CFU/ml. In some cases, the enzyme catalase was added at a concentration of 4 µg/ml to remove reactive oxygen species like hydrogen peroxide. The results are shown in the next figure.



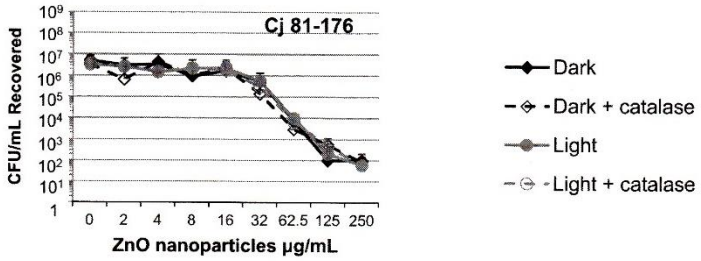
4. Look first at the solid line for the cultures that did not contain catalase but that were treated in the dark or the light. What can you conclude from these data?

   a. All of the strains showed the same sensitivities to different concentrations of ZnO nanoparticles.
   b. The strains that have more S layer proteins (Cff 13783 and Cff 11686) are more sensitive to the ZnO nanoparticles than the strains that have less S layer proteins (Cff 13783K and Cff 11686K).
   c. The strains that have more S layer proteins (Cff 13783 and Cff 11686) are less sensitive to the ZnO nanoparticles than the strains that have less S layer proteins (Cff 13783K and Cff 11686K).
   d. For any particular strain like 11783, the expression of the S layer proteins has no effect.

5. Now look at the dashed lines for the cultures that contained catalase. What can you learn from these data?

   a. Catalase has no effect on the sensitivity of the bacteria ZnO nanoparticles and light.
   b. Catalase makes the bacteria more sensitive to ZnO nanoparticles and light.
   c. Catalase makes the bacteria less sensitive to ZnO nanoparticles and light.
   d. Catalase only affects the bacteria that are kanamycin resistant.

To determine if these results were specific to *C. fetus*, the authors did similar experiments with another species of *Campylobacter* called *C. jejuni*. The results are shown in the next figure.



6. What can you conclude from this experiment?

   a. *C. jejuni* shows the same sensitivities to ZnO nanoparticles and light as *C. fetus*.
   b. *C. jejuni* is sensitive to ZnO nanoparticles but only in the light.
   c. *C. jejuni* has the same sensitivities to ZnO nanoparticles in both the dark and the light.
   d. *C. jejuni* is not sensitive to ZnO nanoparticles.

7. Based on these experiments, what would be the most interesting experiment to do next?

   a. Repeat these experiments with the same concentrations of ZnO nanoparticles.
   b. Repeat these experiments with higher concentration of ZnO nanoparticles.
   c. Compare the S layer of *C. jejuni* to that of *C. fetus*.
   d. Compare the S layer of *C. fetus* to that of *E. coli*.

Problem 3

Area: Microbial Growth
Microorganism: *Listeria monocytogenes*
Reference: Ko, R., Smith, L. T., and Smith, G. M. 1994. Glycine betaine confers enhanced osmotolerance and cryotolerance on *Listeria monocytogenes*. Journal of Bacteriology 176: 426-431.

Listeria monocytogenes is an opportunistic Gram-positive pathogen that causes listeriosis, a disease characterized by fever, diarrhea, and sore throat. This disease is fatal as much as 25% of the time and is normally transmitted through foods such as cheese, milk, and coleslaw. One factor that appears to contribute to outbreaks of listeriosis is the ability of *L. monocytogenes* to grow vigorously at refrigerator temperatures and to tolerate high salt concentrations. Ko et al. (J. Bacteriol. 176: 426-431, 1994) recently examined the accumulation of glycine betaine as an osmotically compatible solute by these bacteria.

1. Based on its vigorous growth at refrigerator temperatures (about 4^{o}C), one would classify *L. monocytogenes* as a:

   a. psychrophile
   b. mesophile
   c. thermophile
   d. refrigophile

Ko et al. first measured the intracellular concentrations of several different compounds after growth of *L. monocytogenes* in a rich complex medium under different conditions. The results are shown in the following table.

	intracellular concentration (mM) after growth in			
solute	0 NaCl/30^{o}C	8% NaCl/30^{o}C	0 NaCl/4^{o}C	8% NaCl/4^{o}C
glycine betaine	65	1300	310	1800
glutamate	200	640	350	430
carnitine	120	<100	430	<75

2. From these data, one can conclude that:

   a. the concentrations of the three compounds are not affected by growth conditions
   b. the concentrations of all three compounds increase in response to both increased salt concentration and reduced temperature.
   c. the concentrations of glycine betaine and carnitine increase in response to both increased salt concentration and reduced temperature.
   d. the concentration of glycine betaine increases in response to both increased salt concentration and reduced temperature.

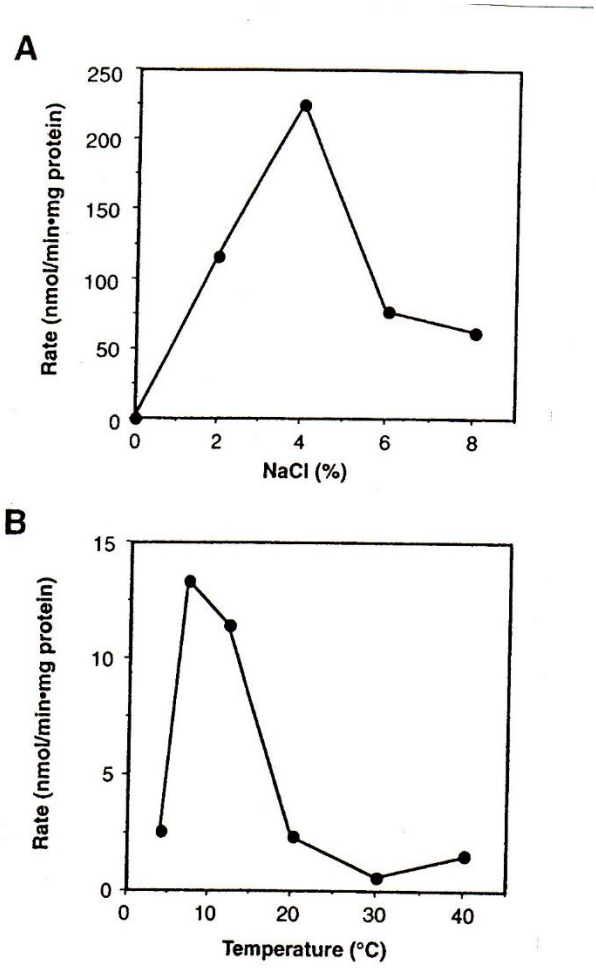
To determine if these bacteria can accumulate glycine betaine from the growth medium, Ko et al. compared the growth of *L. monocytogenes* on agar plates containing a chemically defined medium to which either NaCl or glycine betaine was added. The results are shown below.

		specific growth rate (gen/hr)	
temperature	% added NaCl	0 betaine	130 µM betaine
30	0	0.35	0.35
	2	0.22	0.26
	4	0.13	0.17
	8	0.008	0.09
7	0	0.027	0.033
	2	0.020	0.030
	4	no growth	0.014

3. These data indicate that:

a. addition of glycine betaine has no effect on growth rate.
b. addition of glycine betaine enhances growth only in unstressed bacteria.
c. addition of glycine betaine enhances growth only in severely salt-stressed bacteria.
d addition of glycine betaine enhances growth in both unstressed and severely salt-stressed bacteria.

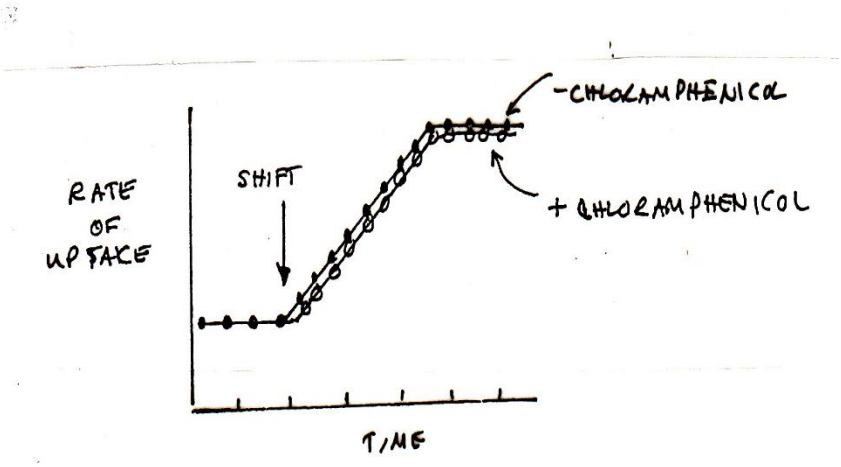
To study the uptake of glycine betaine by *L. monocytogenes*, Ko et al. grew bacteria in different concentrations of NaCl or at different temperatures. They then incubated the bacteria under the same conditions in the presence of radioactive [^{14}C]glycine betaine and measured the rates of uptake. The following figures show the results.



4. These results indicate that

   a. glycine betaine uptake is not affected by growth conditions.
   b. glycine betaine uptake is stimulated by high salt concentrations but not by reduced temperature.
   c. glycine betaine uptake is stimulated by reduced temperature but not by high salt concentrations.
   d. glycine betaine uptake is stimulated by both high salt concentrations and reduced temperatures.

To determine if the uptake of glycine betaine depends on the formation of new transport proteins, Ko et al. performed a "temperature shift experiment" in which bacteria growing at 30^{o}C were transferred to 37^{o}C in the presence or absence of chloramphenicol, an antibiotic that inhibits protein synthesis. The results are shown in the following figure.



5. These data indicate that

   a. there was no increase in glycine betaine uptake following the temperature shift.
   b. there was an increase in glycine betaine uptake due to synthesis of a new transport system.
   c. there was an increase in glycine betaine uptake due to activation of a pre-existing transport system.
   d. there was an increase in glycine betaine uptake due both to synthesis of a new transport system and to activation of a pre-existing transport system.

Problem 4

Area: Microbial Growth
Microorganism: *Streptococcus thermophilus*
Reference: Wa, Y., Zhang C., Sun, G., Qu. H., Chen, D., Huang, Y., and Gu, R. 2022. Effect of amino acids on free exopolysaccharide biosynthesis by *Streptococcus thermophilus* 937 in chemically defined medium. Journal of Dairy Science 105:6460-6468.

The bacterium *Streptococcus thermophilus* is widely used by the dairy industry in the production of yogurt. It grows anaerobically by a process of fermentation and during its growth, it produces a mixture of extracellular polysaccharides that improve the viscosity, texture, and mouth-feel of the yogurt. These exopolysaccharides also act as probiotics and may be useful as antioxidants and anti-inflammatory agents. Wa et al. (J. Dairy Sci. 105: 6460-6468, 2022) recently studied the growth and extracellular polysaccharide formation by *S. thermophilus* in a chemically defined medium.

To study the growth of *Streptococcus thermophilus* under carefully controlled conditions, Wa et al. first developed a chemically defined medium containing a mixture of 42 different nutrients. The pH of the medium was adjusted to 6.7 and the solution sterilized by filtration. The composition of the medium is shown in the next table.

Table 1. Composition of the chemically defined medium

Constituent	Concentration
Lactose	20 g/L
NH_4-citrate	0.6 g/L
Urea	0.24 g/L
l-Alanine	1 m*M*
l-Arginine	1 m*M*
l-Asparagine	1 m*M*
l-Aspartic acid	1 m*M*
l-Cysteine	1 m*M*
l-glutamate	1 m*M*
l-Glutamine	1 m*M*
Glycine	1 m*M*
l-Histidine	1 m*M*
l-Leucine	1 m*M*
l-Isoleucine	1 m*M*
l-Lysine	1 m*M*
l-Methionine	1 m*M*
l-Phenylalanine	1 m*M*
l-Proline	1 m*M*

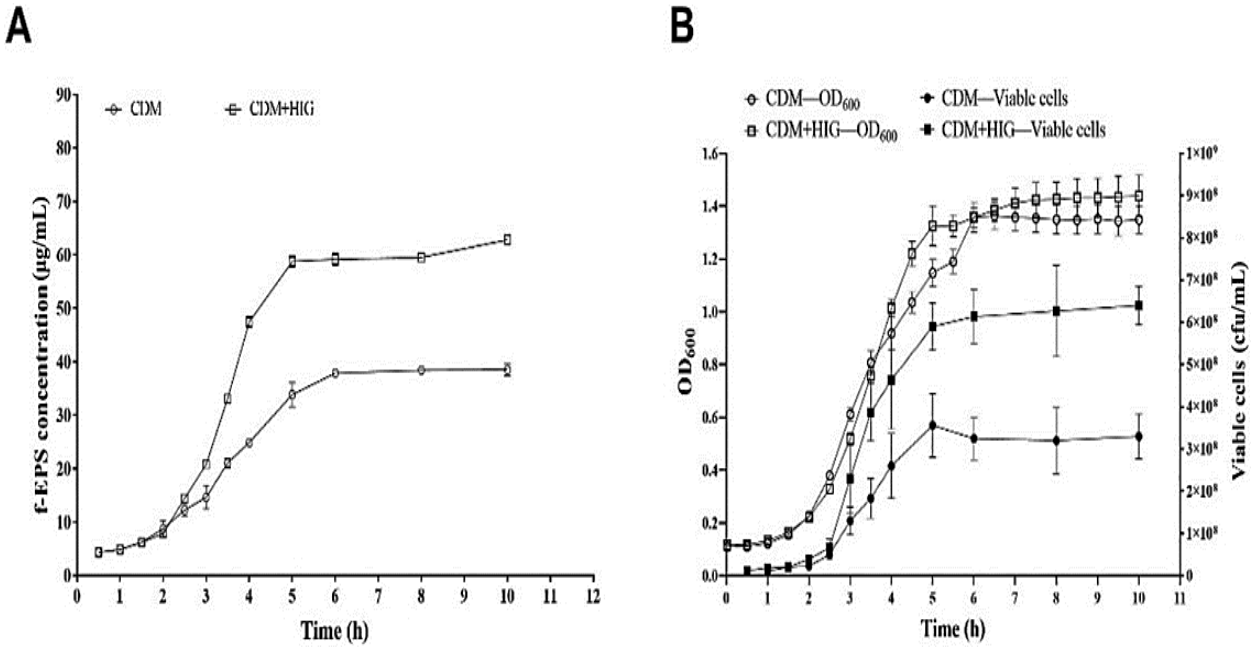
Constituent	Concentration
l-Serine	1 m*M*
l-Threonine	1 m*M*
l-tryptophan	1 m*M*
l-Tyrosine	1 m*M*
l-Valine	1 m*M*
Ascorbic acid	0.5 g/L
Biotin	0.01 g/L
Calcium pantothenate	0.001 g/L
Folic acid	0.001 g/L
Niacin	0.001 g/L
Pyridoxine hydrochloride	0.005 g/L
Riboflavin	0.001 g/L
Thiamine hydrochloride	0.001 g/L
Uracil	0.01 g/L
Adenine	0.01 g/L
Guanine	0.01 g/L
Thymine	0.01 g/L
KH_2PO_4	3 g/L
K_2HPO_4	3 g/L
$MgCl_2$	0.2 g/L
$CaCl_2$	0.05 g/L
NaH_2PO_4	30 m*M*
Na_2HPO_4	30 m*M*
Na-acetate	1 g/L

1. Based on what you know about milk and its nutrients, what is the primary carbon source for the bacteria in this medium?

   a. lactose
   b. urea
   c. ascorbic acid
   d. KH_2PO_4

To study the growth and formation of exopolysaccharides in this medium, Wa et al. grew cultures of *Streptococcus thermophilus* at 42°C, some in the standard medium (CDM) and some in a modified medium in which the concentrations of the amino acids histidine, isoleucine, and glutamate were increased to 15 mM (CDM+ HIG). They periodically measured the turbidity of the cultures at 600 nm and the number of viable cells/ml. They also removed 5 ml samples for free exopolysaccharide analysis. The bacteria were removed from the 5 ml samples by centrifugation and the proteins precipitated with trichloroacetic acid. The polysaccharides in the

supernatant fraction were precipitated with ethanol, washed, and quantified using a colorimetric assay for sugars. The results are shown in the next figure.



2. Both plots show curves typical of a bacterial growth curve. What are the major phases of this curve in the correct order?

   e. stationary phase, lag phase, exponential phase
   f. lag phase, stationary phase, exponential phase
   g. lag, phase, exponential phase, stationary phase
   h. exponential phase, lag phase, stationary phase.

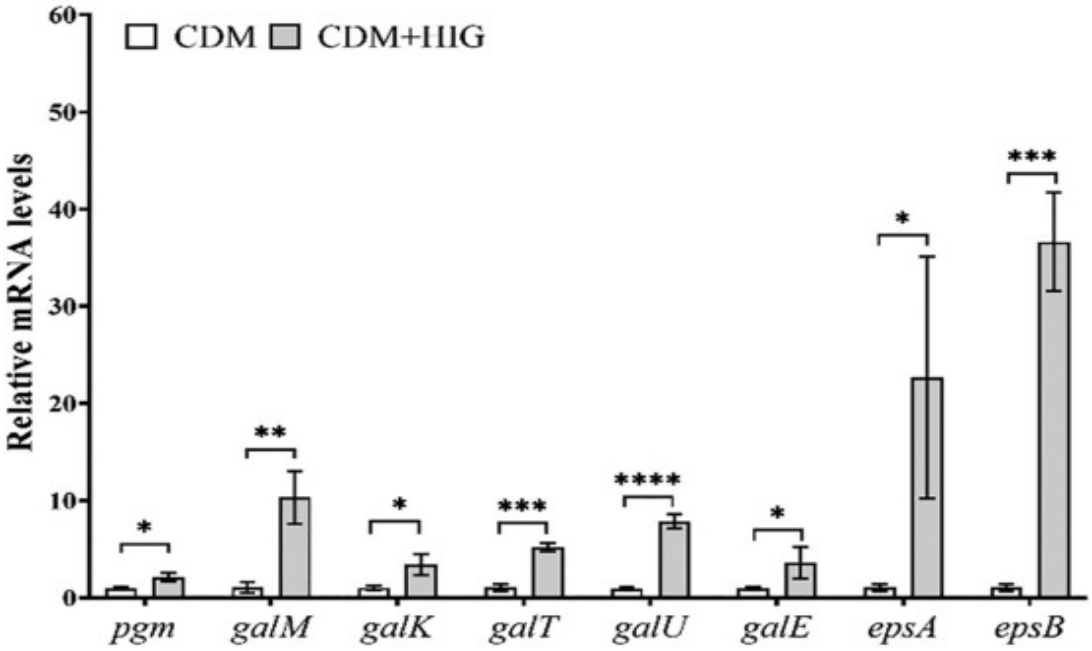
3. Look first at panel B. What can you conclude from these data?

   a. The addition of the extra amino acids had a small effect on both the number of viable cells/ml and the turbidity.
   b. The addition of the extra amino acids had a greater effect on the number of viable cells/ml than on the turbidity.
   c. The addition of the extra amino acids had a greater effect on turbidity than on the number of viable cells/ml.
   d. The addition of the extra amino acids greatly increased the growth rate during exponential phase

4. Now look at panel A. What can you conclude from these data?

   a. The addition of the extra amino acids increased the amount of exopolysaccharide that was formed.
   b. The addition of the extra amino acids had no effect on the amount of exopolysaccharide that was formed.
   c. The addition of the extra amino acids decreased the amount of exopolysaccharide that was formed.
   d. The addition of the extra amino acids shifted exopolysaccharide formation from exponential phase to stationary phase.

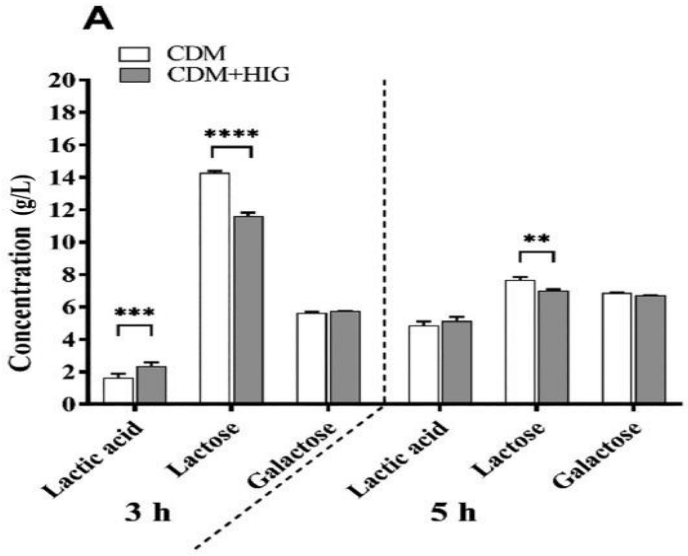
To study the basis of these results, Wa et al. measured the levels of transcription of several genes involved in exopolysaccharide formation. These genes *were pgm, galM, galK, galT, galU, galE, epsA, and epsB.* The next figure shows a comparison of the transcripts after growth for 3 hours in CDM and CDM + HIG.



5. What can you see in this image?

   e. The level of transcription of all of the genes was the same and did not change with the growth medium
   f. The level of transcription of the genes was variable but was consistently higher in bacteria growth in CDM + HIG.
   g. The level of transcription of the genes was variable but was consistently higher in CDM.
   h. The level of transcription of *epsA* and *epsB* was consistently lower than the other genes.

The metabolism of *Streptococcus thermophilus* like that of many lactic acid bacteria involves the uptake the lactose, the hydrolysis of lactose into D-glucose and D-galactose, and the formation of lactic acid as a product. Wa et al. measured concentration of lactic acid, lactose, and D-galactose in the medium of CDM and CDM + HIG cultures after 3 hours and 5 hours. The results are shown in the next figure.



6. What do these result indicate about the metabolism of the bacteria in the two media?

   d. After 3 hours, the bacteria in CDM have formed more lactic acid and degraded more lactose than those in CDM + HIG.
   b. After 3 hours, the bacteria in CDM + HIG have formed more lactic acid and degraded more lactose than those in CDM.
   c. After 5 hours, the differences in the concentrations of lactic acid and lactose persist and become more dramatic.
   d. The concentration of D-galactose is a good indicator of lactose metabolism.

7. In the original paper, Wa et al. states that they found in a previous study that the addition of histidine, isoleucine, and glutamate to the chemically defined medium led an increase in turbidity and to an increase in exopolysaccharide formation. Why might these three amino acids be specifically required?

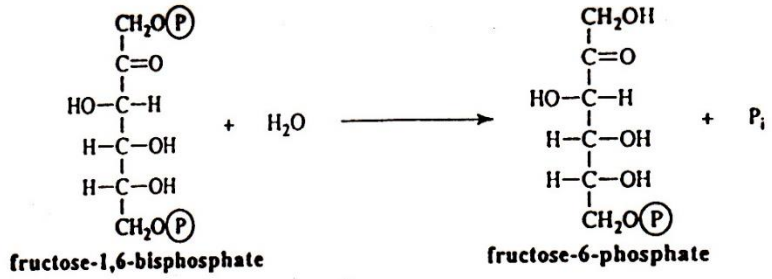
   a. These amino acids might be incorporated into the free exopolysaccharides.
   b. These amino acids might be converted to the sugars used in the free exopolysaccharides.
   c. These amino acids might be degraded as additional nitrogen sources by the bacteria.
   d. These amino acids might be taken up in small amounts by the bacteria and so this limits growth.

583

Problem 5

Area: Bacterial Metabolism
Microorganism: *Xanthobacter flavus*
Reference: van den Burgh, E. R. E., van der Kooij, T. A. W., Dijkhuizen, L., and Meijer, W. G. 1995. Fructosebisphosphatase isoenzymes of the chemoautotroph *Xanthobacter flavus*. Journal of Bacteriology 177: 5860-5864.

Xanthobacter flavus is a Gram-negative bacterium that can grow as either a chemoheterotroph or a chemoautotroph. In either case, it uses the enzyme fructose-1,6-bisphosphate phosphatase (FBPase) to catalyze the conversion of fructose-1,6-bisphosphate to fructose-6-phosphate. During chemoheterotrophic metabolism, this reaction is important in the formation of complex carbohydrates. During chemoautotrophic metabolism, this reaction is important in the overall process of carbon assimilation. The reaction is shown in the following figure.



In a series of experiments, van den Bergh et al. (Journal of Bacteriology 177: 5860-5864, 1995) showed that *X. flavus* synthesizes two different enzymes or isoenzymes catalyzing this reaction.

1. According to the basic system of enzyme classification, fructose-1,6-phosphate phosphatase is an example of a:

   a. oxidoreductase
   b. hydrolase
   c. lyase
   d. isomerase

2. The standard free energy values ($G^{o'}$) in kcal/mol for fructose-1,6-bisphosphate, water, fructose-6-phosphate, and phosphate are given as -622, -57, -420, and -262, respectively. Based on these values, the $\Delta G^{o'}$ for the reaction in the direction shown is:

   a. - 1361 kcal/mol
   b. + 3 kcal/mol
   c. - 3 kcal/mol
   d. - 723 kcal/mol

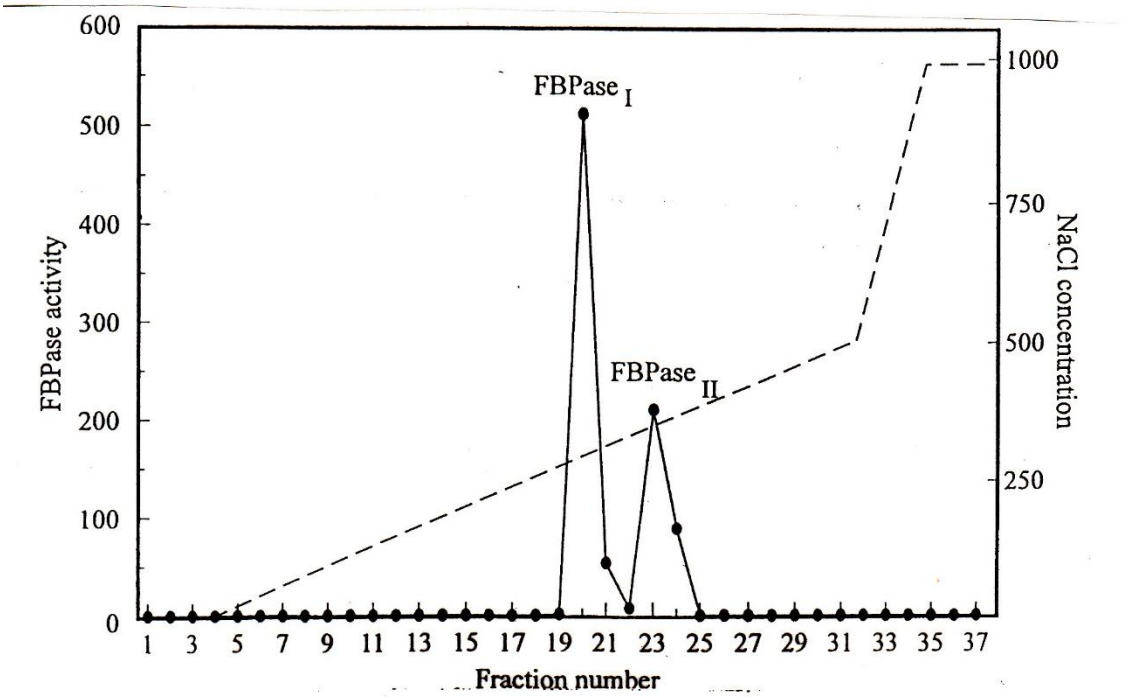
3. The role of fructose-1,6-bisphosphate phosphatase in the reaction is:

   a. to make $\Delta G^{o'}$ more positive
   b. to make $\Delta G^{o'}$ more negative
   c. to decrease the activation energy
   d. to increase the activation energy

4. Biochemically, fructose-1,6-bisphosphate phosphatase is a:

   a. nucleic acid
   b. protein
   c. carbohydrate
   d. lipid

To study this enzyme in more detail, van den Bergh et al. first grew *X. flavus* in a medium containing methanol as the sole carbon source. Methanol can be used by these bacteria as a substrate for chemoautotrophic metabolism. They then disrupted the cells and subjected the resulting extract to column chromatography. The material coming off of the column was collected as a series of fractions and then assayed for fructose-1,6-bisphosphate phosphatase activity. The results are shown in the following figure.

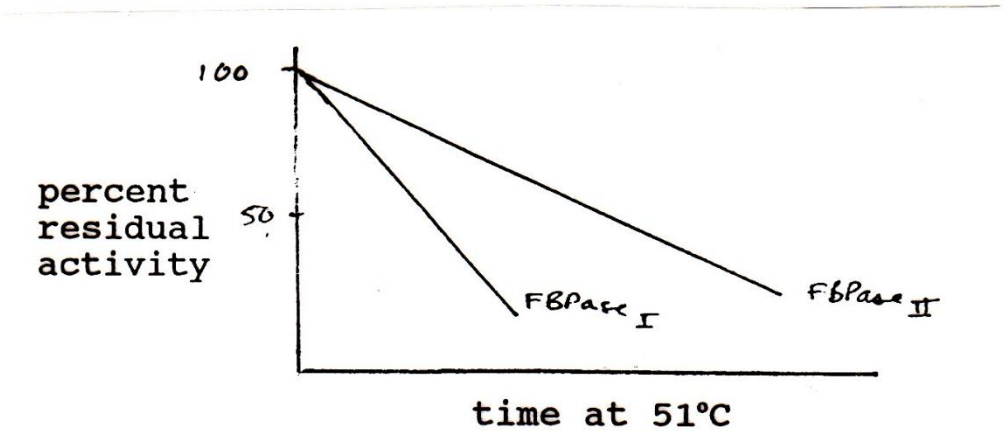


They then repeated this experiment with bacteria grown in a medium containing succinate as the sole carbon source. Succinate can only be used as a substrate for chemoheterotrophic metabolism. When the bacterial extract was analyzed by column chromatography, only $FBPase_I$ was present.

5. These results suggest that:

a. Both enzymes are involved in chemoautotrophic metabolism.
b. Both enzymes are involved in chemoheterotrophic metabolism.
c. $FBPase_I$ is specifically involved in chemoautotrophic metabolism.
d. $FBPase_{II}$ is specifically involved in chemoautotrophic metabolism.

van den Bergh then heated the two fractions containing fructose-1,6-bisphosphate phosphatase at 51°C. They removed samples periodically and determined the amount of activity that was still left. The results are shown in the following figure.



6. These results indicate that:

a. both fractions probably contain the same protein.
b. both enzymes are equally heat sensitive.
c. $FBPase_I$ is more heat sensitive than $FBPase_{II}$.
d. $FBPase_{II}$ is more heat sensitive than $FBPase_I$.

van den Bergh et al. then determined the ability of two fractions to use the structurally related compound sedoheptulose-1,7-bisphosphate as a substrate (this compound has 7 carbon atoms instead of 6). The ratio of the fructose-1,6-bisphosphate phosphatase activity to the sedoheptulose-1,7-bisphosphate phosphatase activity was 1.0 for $FBPase_I$ and 0.5 for $FBPase_{II}$.

7. These results indicate that:

a. Both fractions contain the same enzyme.
b. $FBPase_I$ prefers fructose-1,6-bisphosphate as a substrate.
c. $FBPase_{II}$ prefers fructose-1,6-bisphosphate as a substrate.
d. $FBPase_{II}$ prefers sedoheptulose-1,7-bisphosphate as a substrate.

Problem 6

Area: Microbial Metabolism
Microorganism: *Nitrosomonas europaea*
Reference: Deutch, C. E. 2013. L-Malate dehydrogenase activity in the reductive arm of the incomplete citric acid cycle of *Nitrosomonas europaea*. Antonie van Leeuwenhoek 104: 645-655.

The bacterium *Nitrosomonas europaea* is an example of the "nitrifying bacteria" and can obtain energy by the oxidation of ammonia to nitrite in the presence of oxygen as a terminal electron acceptor. It is an autotroph that can fix carbon through the Calvin-Benson-Bassham cycle and use it for all of its essential metabolites. When grown aerobically, *N. europaea* expresses all of the enzymes of the citric acid cycle except for 2-oxoglutarate (2-ketoglutarate or α-ketoglutarate) dehydrogenase. This includes the enzyme L-malate dehydrogenase, which catalyzes the reversible interconversion of L-malate and oxaloacetate using NAD^+/NADH or $NADP^+$/NADPH as the coenzyme electron carrier. This is shown in the following figure.

Deutch (Antonie van Leeuwenhoek 104: 645-655, 2013) studied the properties and metabolic functions of the MDH from *N. europaea*.

The next figure shows the process of ammonia oxidation.

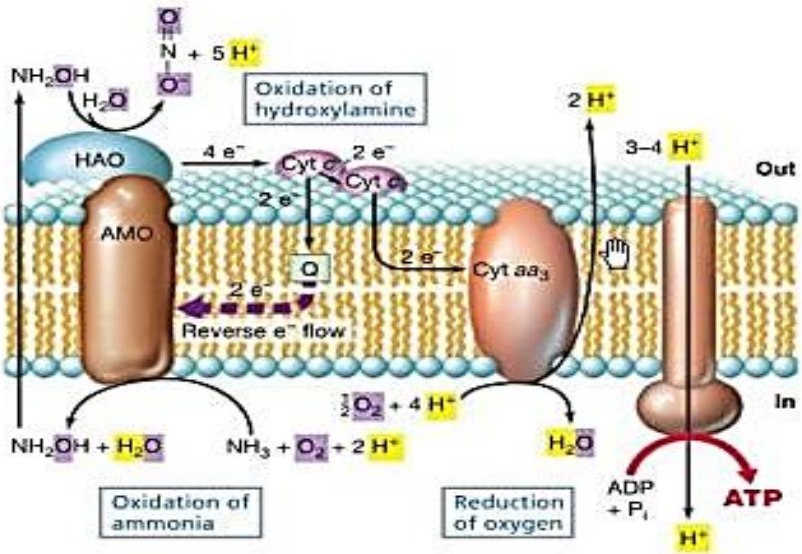


Figure 13.26 Oxidation of NH_3 and electron flow in ammonia-oxidizing bacteria. The reactants and the products of this reaction series are highlighted. The cytochrome *c* (Cyt *c*) in the periplasm is a different form of Cyt *c* than that in the membrane. AMO, ammonia monooxygenase; HAO, hydroxylamine oxidoreductase; Q, ubiquinone.

1. Based on this figure, what are the key steps in ammonia oxidation?

   a. ammonia to hydroxylamine to nitrate
   b. ammonia to nitrite to nitrate
   c. ammonia to hydroxylamine to nitrite
   d. ammonia to nitrate to nitrite

2. The mechanism of ATP synthesis in the system is called:

   a. photophosphorylation\
   b. substrate-level phosphorylation
   c. chemiosmotic or oxidative phosphorylation
   d. spontaneous phosphorylation

Deutch grew *N. europaea* in a chemically defined medium at 30°C, harvested the cells by centrifugation, disrupted the bacteria by three passages through a French pressure cell, and prepared an extract by centrifugation at 105,000 x g in an ultracentrifuge. L-malate dehydrogenase activity was measured at 340 nm in a double-beam UV-visible spectrophotometer. This was done both in the reductive direction (oxaloacetate to L-malate) and in the oxidative direction. The next figure shows the results when the coenzyme and the pH of the reactions was varied.

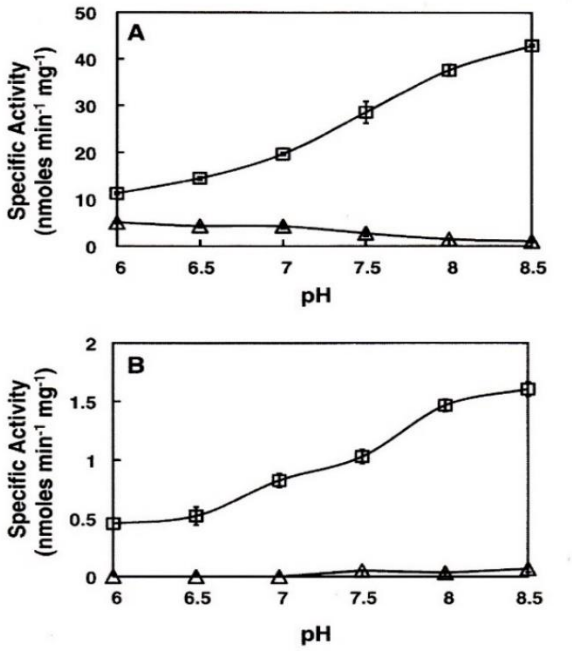


Fig. 2 Specific activities of L-malate dehydrogenase (MDH) from *N. europaea* as a function of buffer pH and coenzyme. **A** shows the specific activities in the reductive direction (oxaloacetate to L-malate) in the presence of NADH (*square*) or NADPH (*triangle*) in potassium phosphate buffers from pH 6.0 to 8.5. **B** shows the specific activities in the oxidative direction (L-malate to oxaloacetate) in the presence of NAD^+ (*square*) or $NADP^+$ (*triangle*) in potassium phosphate buffers from pH 6.0 to 8.5

3. What do these results indicate about the directionality and coenzyme preference of the *N. europeae* enzyme?

   a. The enzyme has greater activity in the oxidative direction and prefers NAD^+/NADH as the coenzyme.
   b. The enzyme has greater activity in the reductive direction and prefers NAD^+/NADH as the coenzyme.
   c. The enzyme has greater activity in the oxidative direction and prefers $NADP^+$/NADPH as the coenzyme.
   d. The enzyme has greater activity in the reductive direction and prefers $NADP^+$/NADPH as the coenzyme.

4. What is the optimal pH for the reaction?

   a. It is pH 6 for both the oxidative and reductive reactions.
   b. It is pH 8.5 for both the oxidative and reductive reactions.
   c. It is pH 8.5 in the reductive direction but 6.0 for the the oxidative direction.
   d. It is pH 6 in the reductive direction and pH 8.5 in the oxidative direction.

To determine if the reactions were sensitive to inhibition by the product, the reactions were done in the reductive direction (oxaloacetate to L-malate) in the presence of increasing concentrations of L-malate (panel A) or in the oxidative direction in the presence of increasing amounts of oxaloacetate (panel B).

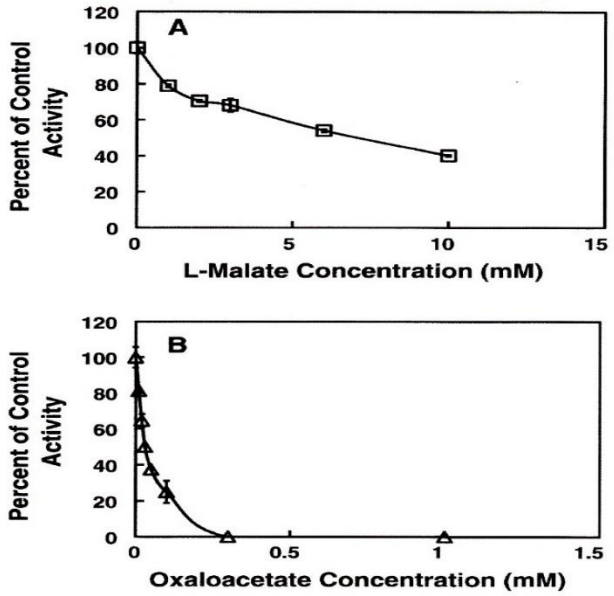


Fig. 3 Product inhibition of L-malate dehydrogenase (MDH) from *N. europaea*. **A** shows the percent of the control activity in the reductive direction (oxaloacetate to L-malate) in the presence of increasing concentrations of L-malate. The specific activity of the uninhibited reaction was 16.08 nmoles min^{-1} mg^{-1}. **B** shows the percent of the control activity in the oxidative direction (L-malate to oxaloacetate) in the presence of increasing concentrations of oxaloacetate. The specific activity of the uninhibited reaction was 0.81 nmoles min^{-1} mg^{-1}

5. What is indicated by these results?

a. Neither reaction is sensitive to product inhibition.
b. The reaction in the reductive direction is more sensitive to product inhibition than the reaction in the oxidative direction.
c. Both reactions are equally sensitive to product inhibition.
d. The reaction in the oxidative direction is more sensitive to product inhibition than the reaction in the oxidative direction.

In similar experiments, Deutch tested the effects of temperature and salt on the MDH reactions. The results are shown in the next figure.

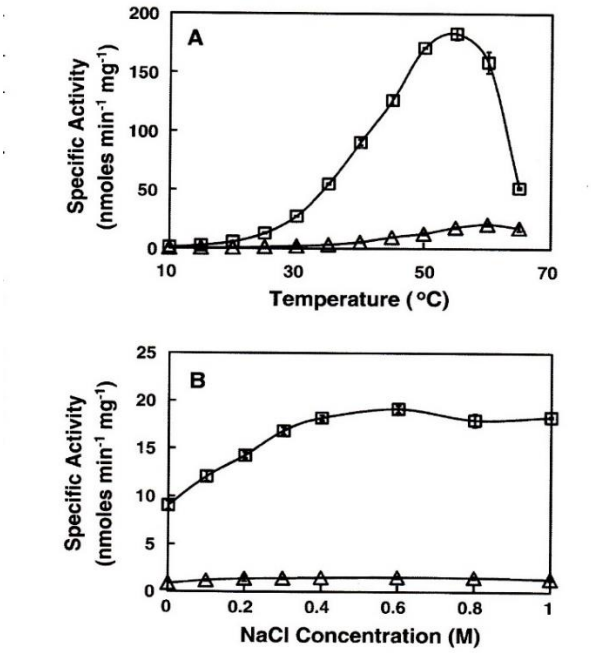


Fig. 4 Effects of temperature and salt concentration on the L-malate dehydrogenase (MDH) from *N. europaea*. **A** The specific activities in the reductive direction (oxaloacetate to L-malate, *square*) or in the oxidative direction (L-malate to oxaloacetate, *triangle*) at temperatures from 10 °C to 65 °C. **B** The specific activities in the reductive direction (oxaloacetate to L-malate, *square*) or in the oxidative direction (L-malate to oxaloacetate, *triangle*) at NaCl concentrations from 0 to 1.0 M

6. What can you conclude from these data?

a. The optimum temperature for both reactions is 50-55^{o}C but only the reaction in. the oxidative direction increases significantly.
b. The reaction in the oxidative direction is stimulated by NaCl but the one in the reductive direction is not.
c. The reaction in the reductive direction is inhibited by NaCl but the one in the oxidative direction is not.
d. The optimum temperature for both reactions is 50-55^{o}C but only the reaction in. the reductive direction increases significantly.

To determine if the MDH reaction was affected by other metabolites including those of the citric acid cycle, the reactions were tested in the presence of 1 mM or 10 mM concentrations of various compounds. The results are shown in the next table.

Table 1 Effect of metabolites on the activities of the L-malate dehydrogenase from *N. europaea*

Metabolite	Percent of control activity[a]		
	Conc. (mM)	OAA→ L-malate	L-malate→ OAA
Acetyl-CoA	10	86.9 ± 4.4	nd[b]
	1	104 ± 3.2	111 ± 0
L-Aspartate	10	89.2 ± 1.2	92.9 ± 6.7
	1	102 ± 3.8	103 ± 5.0
Citrate	10	102 ± 7.4	111 ± 0
	1	97.6 ± 11.9	103 ± 4.0
Fumarate	10	101 ± 1.6	104 ± 7.6
	1	98.9 ± 4.2	107 ± 2.5
Glyceraldehyde-3-P	10	60.8 ± 2.2	nd
	1	99.9 ± 4.8	91.6 ± 3.9
L-Lactate	10	115 ± 7.4	98.5 ± 2.5
	1	107 ± 0.9	nd
2-Oxoglutarate	10	69.8 ± 6.4	86.1 ± 11.7
	1	105 ± 12.2	108 ± 0
Phosphoenolpyruvate	10	61.9 ± 2.9	75.0 ± 4.0
	1	88.2 ± 1.4	100 ± 0
3-Phosphoglycerate	10	101 ± 4.1	nd
	1	115 ± 2.3	106 ± 7.8
Pyruvate	10	90.4 ± 4.0	100 ± 11.2
	1	97.8 ± 4.3	100 ± 11.8
Succinate	10	90.8 ± 3.3	107 ± 3.2
	1	90.5 ± 4.4	103 ± 4.0

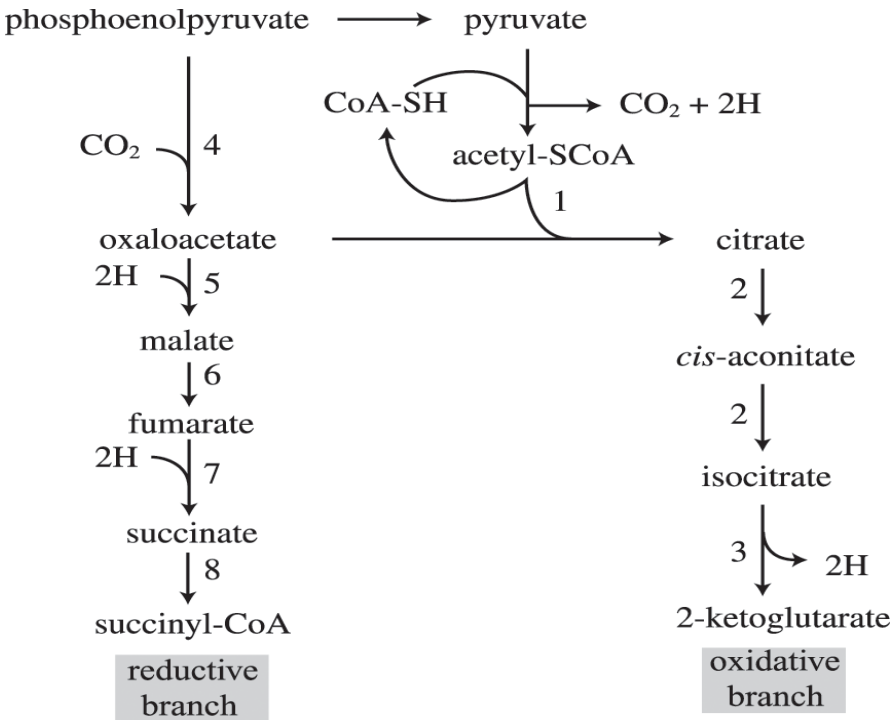
[a] Activities were expressed as mean percentages of control reactions±one standard deviation for replicate reactions with the same S_{105} preparation at 25 °C on the same day. The average specific activity for the control reactions in the reductive direction was 15.03 nmoles min^{-1} mg^{-1}. The average specific activity for the control reactions in the oxidative direction was 1.11 nmoles min^{-1} mg^{-1}

[b] A few potential effectors were not tested at every concentration because the compound was limited or because the particular S_{105} fraction was exhausted

7. What is the most obvious conclusion from these data?

a. There are major differences in the sensitivities of the reactions to all of the compounds.
b. There are no differences in the sensitivities of the reactions to all of the compounds.
c. There are small differences in the sensitivities of the reactions to all of the compounds.
d. There are some differences in the sensitivities of the reactions to 2-oxoglutarate and phosphoenolpyruvate.

The absence of absence of 2-oxoglutarate dehydrogenase activity in *N. europaea* means that the enzymes which normally work in a cyclic fashion in the TCA cycle function as two branches. This is shown in the next figure. Pyruvate formed during glycolysis can still be converted to acetyl-CoA and combined with oxaloacetate to form citrate. Citrate can go on to form isocitrate and 2-ketoglutarate (α-ketoglutarate or 2-oxoglutarate). This is a key intermediate in the formation of many amino acids. On the other hand, oxaloacetate can be converted by L-malate dehydrogenase to L-malate, which then be converted to fumarate, succinate, and succinyl-CoA. These also lead to key metabolites. The loss of 2-oxoglutarate dehydrogenase is not fatal to the cells.



8. Given that 2-oxoglutarate dehydrogenase is missing in *N. europaea*, in which direction would you expect the MDH reaction occur? Are the results consistent with your prediction?

   a. the reductive direction - yes
   b. the oxidative direction - yes
   c. both directions would be important - no
   d. the enzyme would no longer be necessary - no

Problem 7

Area: Microbial Genetics
Microorganism: *Vibrio vulnificus*
Reference: McDougald, D, Simpson, M. L., Oliver J. D., and Hudson M. C. 1994. Transformation of *Vibrio vulnificus* by electroporation. Current Microbiology 28: 289-291.

Vibrio vulnificus is a Gram-negative pathogenic bacterium found in the ocean. Most cases of *V. vulnificus* infection result from consumption of raw oysters or severe wounds that have been exposed to sea water. The bacteria multiply outside of the gastrointestinal tract and produce several extracellular protein toxins. *V. vulnificus* produces compounds that bind iron and can grow by fermentation of lactose. To study the genetics of this microorganism in more detail, McDougald et al. (Current Microbiol. 28: 289-291, 1994) attempted to introduce plasmid DNA into the bacteria by transformation.

1. Based on the information given, *V. vulnificus* exerts its primary pathogenic effects through the formation of:

   a. endotoxins.
   b. exotoxins.
   c. proteolytic enzymes.
   d. hypersensitivity reactions.

2. From the information given, it appears that *V. vulnificus* can grow:

   a. only anaerobically.
   b. only aerobically.
   c. both aerobically and anaerobically.
   d. photosynthetically.

To test for transformation, McDougald et al. first extracted plasmid DNAs carrying genes for resistance to tetracycline or kanamycin from several strains of *E. coli*. When the plasmid DNAs were mixed with *V. vulnificus* and the bacteria spread on agar plates containing tetracycline or kanamycin, no antibiotic resistant colonies were found. McDougald et al. then tried a technique called electroporation, in which the plasmid DNA/bacteria mixture was exposed to a series of high-voltage electrical shocks before plating. In this case, antibiotic-resistant colonies were recovered upon plating.

3. These results indicate that *V. vulnificus* is susceptible to:

   a. conjugation
   b. both natural transformation and artificial
   c. only natural transformation
   d. only artificial transformation

McDougald et al. then determined the number of antibiotic- resistant transformants as a function of DNA concentration for two strains of *V. vulnificus*. The results are shown below.

Table 3. Effect of plasmid DNA concentration on transformation efficiency utilizing pRT291

Strain	DNA concentration (μg/ml)	Transformants
MO6	2	1.7
	10	3.3
	50	25.0
	100	28.3
LC2	2	1.7
	10	3.3
	50	43.3
	100	75.0

4. These results suggest that:

   a. the efficiency of transformation is not affected by the DNA concentration.
   b. the efficiency of transformation decreases with DNA concentration.
   c. the efficiency of transformation increases equally with DNA concentration for both strains.
   d. the efficiency of transformation increases with DNA concentration for both strains but reaches saturation sooner with MO6.

To determine if the efficiency of transformation is affected by the composition of the growth medium, McDougald et al. compared the efficiency of transformation after growth in a rich medium (HI) or in a minimal medium (BSM) supplemented with glucose and glycine betaine, an osmotically compatible solute. The results are shown in the following table.

Table 4. Effect of growth medium on transformation efficiency of *Vibrio vulnificus* MO6 with pRT291

Medium[a]	Transformants/μg
HI	none
1% BSM + 0.2% glucose	1.8
1% BSM + 0.4% glucose	66.0
1% BSM + 0.4% glucose + 5 mM glycine betaine	240.0

[a] All cultures were grown overnight at 37°C with aeration.

5. These results suggest that transformation is most efficient in the presence of:

   a. BSM medium.
   b. BSM medium + glucose.
   c. BSM medium + glucose + glycine betaine
   d. BSM medium + glycine betaine

McDougald et al. found that *V. vulnificus* has two distinct colony types depending on the amount of extracellular polysaccharide formed: opaque colonies, which have a lot of polysaccharides, and translucent colonies, which have little polysaccharide. They then made two observations: 1) addition of glucose to the BSM medium caused the bacteria to form translucent colonies, and 2) translucent colonies were transformed much more efficiently than opaque colonies.

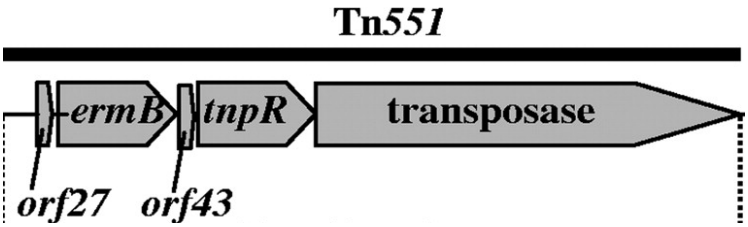
6. These observations suggest that:

   a. extracellular capsules promote DNA uptake and transformation.
   b. extracellular capsules have no effect on DNA uptake and transformation.
   c. extracellular capsules inhibit DNA uptake and transformation.
   d. extracellular capsules make the bacteria resistant to antibiotics.

Problem 8

Area: Microbial Genetics
Microorganism: *Staphylococcus aureus*
Reference: Wang, H., Ma, K., Shen, J., Fang, M., Pei, H., Li, Y., Zhu, C., Shu, F., Li, B., and Xue, T. 2023. Genes associated with desiccation stress in foodborne *Staphylococcus aureus* as revealed by transposon insertion mutagenesis. Food Research International 163: 112271. doi: 10.1016/j.foodres.2022.112271

The Gram-positive bacterium *Staphylococcus aureus* is an important human pathogen that causes a variety of opportunistic infections. Many of the sources of infection are sites in the food supply chain, and infections due to strains of *S. aureus* that are methicillin- or vancomycin-resistant can be particularly severe. One important aspect of food preservation is drying because the removal of water can limit bacterial growth. However, *S. aureus* has the ability to survive desiccation, and while some of the genes involved in desiccation tolerance have been identified, many have not. To search for additional genes that might contribute to the virulence of *S. aureus* in this way, Wang et al. used transposon mutagenesis to inactivate random genes in this microorganism. Transposons are mobile genetic elements that can move between DNA molecules and often carry genes for antibiotic resistance. They used a fragment of Tn551 carrying a gene for erythromycin resistance. This is shown in the next figure. Insertion of this DNA sequence into an existing gene would disrupt it and make the host organism resistant to this antibiotic.



1. Why is water so essential to the survival of a living organism?

   a. Water is the only source of the elements hydrogen and oxygen.
   b. Water commonly is used as an electron donor for metabolic processes.
   c. Cells are about 70% water and cellular biochemicals and structures are dissolved in water.
   d. Cells can actively transport water across the cell membrane.

To carry out transposon mutagenesis, Wang et al. introduced a plasmid called pBTn into a wild type strain of *S. aureus* called RMSA24 by electroporation. This plasmid carried a copy of Tn 551 and had additional gene causing resistance to the antibiotic chloramphenicol. The bacteria were grown at 30^{o}C into stationary phase in a rich medium called tryptic soy broth containing both erythromycin and chloramphenicol. They were then diluted into fresh medium containing only erythromycin and incubated at 42^{o}C to cause loss of the plasmid. Colonies were recovered on agar plates containing erythromycin and stored as glycerol stocks at -80^{o}C.

2. What is the simplest definition of a plasmid?

a. It is a small circular DNA that can be maintained in the cytoplasm of a host cell and contribute to its phenotype.
b. It is a long linear DNA that can be maintained in the cytoplasm of a host cell by recombination with the main chromosome.
c. It is a small circular RNA that can be maintained in the cytoplasm of a host cell and contribute to its phenotype.
d. It is a large protein that can contribute to the phenotype of a host cell.

To recover strains that might be more sensitive to desiccation, Wang et al. subjected the erythromycin-resistant isolates to three rounds of treatment. Overnight cultures were diluted into 3 ml of fresh broth to give an OD_{600} of about 0.05 and incubated at 37°C until the OD_{600} reached 1.0. 50 µl samples were then transferred to 48 well plastic tissue culture plates and dried at 37°C for four days. The viable cell count was then determined and those mutants with lower viable counts than the parent strain were identified. These mutants were then subjected to two more rounds of desiccation treatment. The bacteria with significantly lower rates of survival were then characterized further.

3. What would you expect to see in the wells of the plastic tissue culture plates after 4 days?

a. a thick layer of bacterial culture
b. a thin film of medium containing live bacteria
c. a thin film of medium containing both live and dead bacteria
d. a concentrated mass of dead bacteria

In this experiment, Wang et al. initially found 3154 mutants that were erythromycin resistant. After the first round of desiccation screening, this number was reduced to 232 and after the second and third rounds, the number was reduced to 18. Of these, 8 mutants with insertions at different sites were identified by PCR analysis and sequencing. All of these mutants showed a greater sensitivity to desiccation than the parent strain. The genes in these are listed in the following table.

Table 3
Identified transposon insertion sites.

Mutant strains	Gene name/ ID	Length of gene (bp)	Predicted protein function
M 1334	*NLG45_01240*	924	U32 family peptidase
M 2377	*NLG45_11885*	804	CHAP domain-containing protein
M 2233	*NLG45_11580*	969	YdcF family protein
M 2302	*NLG45_06760*	570	RNA polymerase sigma factor
M 2040	*NLG45_12715*	1374	EVE domain-containing protein
M 1640	*NLG45_10515*	1812	acetyltransferase
M 1870	*fmtB*	7422	LPXTG-anchored DUF1542 repeat protein FmtB
M 2130	*NLG45_04065*	522	CvpA family protein

4. What conclusion can you draw from these data?

   a. A very large number of genes is involved in desiccation sensitivity.
   b. A small number of genes with similar functions is involved in desiccation sensitivity.
   c. A small number of genes with different functions is involved in desiccation sensitivity.
   d. There is no genetic basis to desiccation sensitivity or tolerance.

To characterize these mutants, the parent strain and the mutants were subject to desiccation treatment. The results are shown in the next figure.

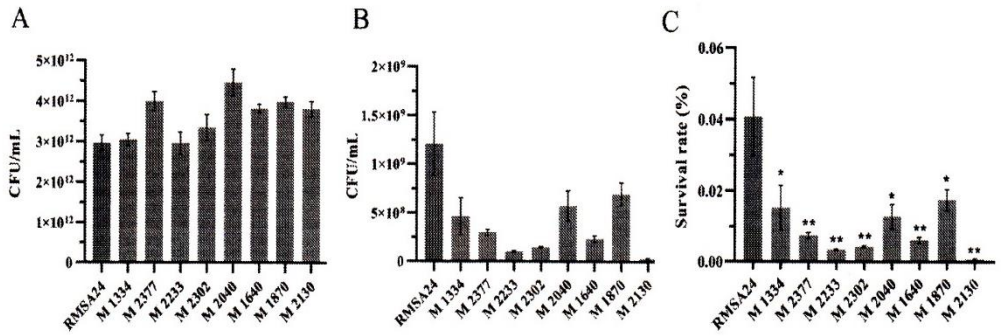


Fig. 1. Effects of desiccation stress for 4 days on the survival rate of *S. aureus* RMSA24 and its mutants. (A) Colony-forming unit statistics of *S. aureus* RMSA24 and its mutants before desiccation treatment. (B) Colony-forming unit statistics of *S. aureus* RMSA24 and its mutants after desiccation treatment. (C) Survival rate of *S. aureus* RMSA24 and its mutants before and after desiccation treatment. * represents $P < 0.05$; ** represents $P < 0.01$.

5. What do these results indicate?

   a. The survival of the mutants before the treatment was significantly lower than the parent strain.
   b. The survival of the mutants after the treatment was significantly lower than the parent strain.
   c. The survival of the mutants after the treatment was significantly higher than the parent strain.
   d. The survival of all of the mutants after the treatment was the same.

The genome of *S. aureus* has been completely sequenced. The following figure shows the positions of the new mutants in this genome.

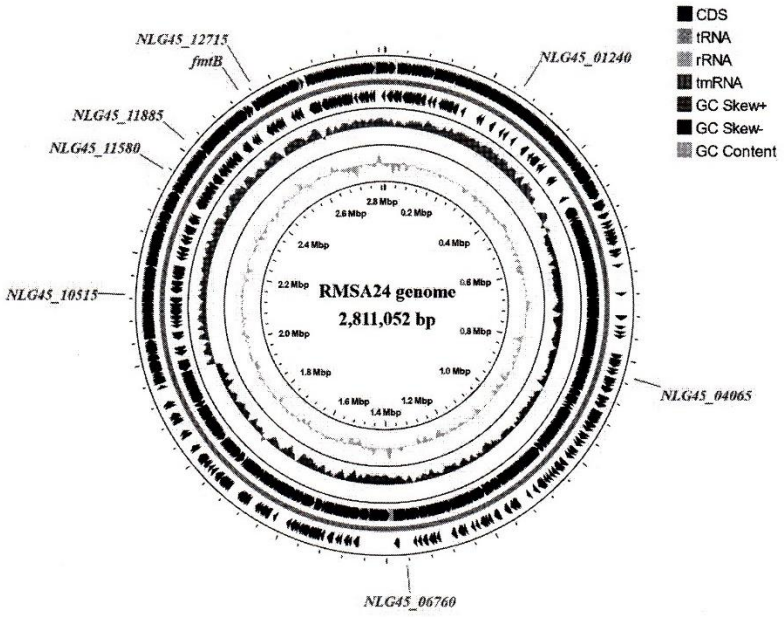
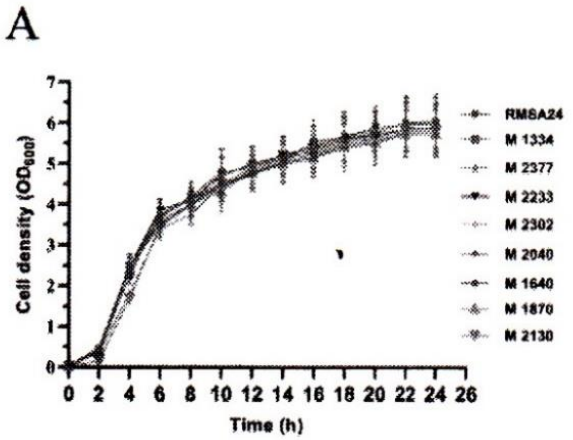


Fig. 2. The identified transposon insertion sites on the RMSA24 genome (the sequence moves clockwise).

6. These results indicate that:

   a. the genes for desiccation sensitivity lie near one another but do not form an operon.
   b. the genes for desiccation sensitivity lie near one another and form an operon.
   c. the genes for desiccation sensitivity are distributed around the genome but adjacent to known genes.
   d. the genes for desiccation sensitivity are distributed randomly in the genome.

To determine if the transposon mutants affects the growth of the bacteria, the parent strain and the mutants were grown in tryptic soy broth at 37°C for 24 hours. The results are shown in the next figure.



7. These results show that:

   a. the mutants all grow slower than the parent strain.
   b. the mutants vary but all grow slower than the parent strain.
   c. the mutants vary but all grow faster than the parent strain.
   d. the mutants do not differ from the parent strain.

Desiccation tolerance has sometimes been found to be related to the ability of the bacteria to form biofilms. Wang et al. tested for biofilm formation inoculating the wells of microtiter plates with the bacteria, incubating them for 24 hours, and then staining the adherent bacteria with crystal violet. The results are shown in the next figure.

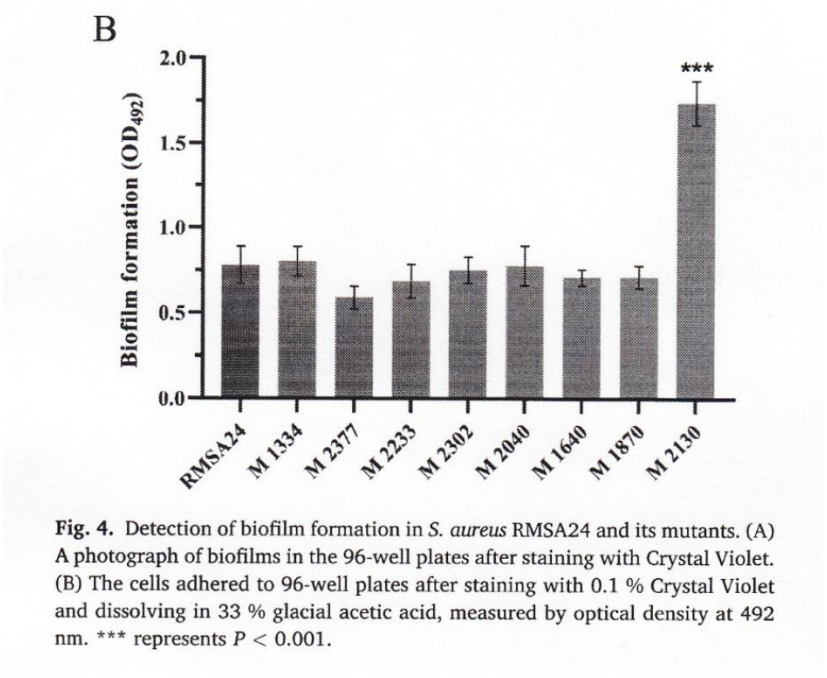


Fig. 4. Detection of biofilm formation in *S. aureus* RMSA24 and its mutants. (A) A photograph of biofilms in the 96-well plates after staining with Crystal Violet. (B) The cells adhered to 96-well plates after staining with 0.1 % Crystal Violet and dissolving in 33 % glacial acetic acid, measured by optical density at 492 nm. *** represents $P < 0.001$.

8. These results indicated that:

   a. the mutants all formed more biofilm than the parent strain.
   b. the mutants all formed less biofilm than the parent strain.
   c. only mutant M2130 formed more biofilm than the parent strain.
   d. only mutant M2130 formed less biofilm than the parent strain.

Finally, Wang et al. measured the levels of transcription of the genes in the mutant strains when the bacteria were not stress by desiccation and when they were stressed. The results are shown in the next figure.

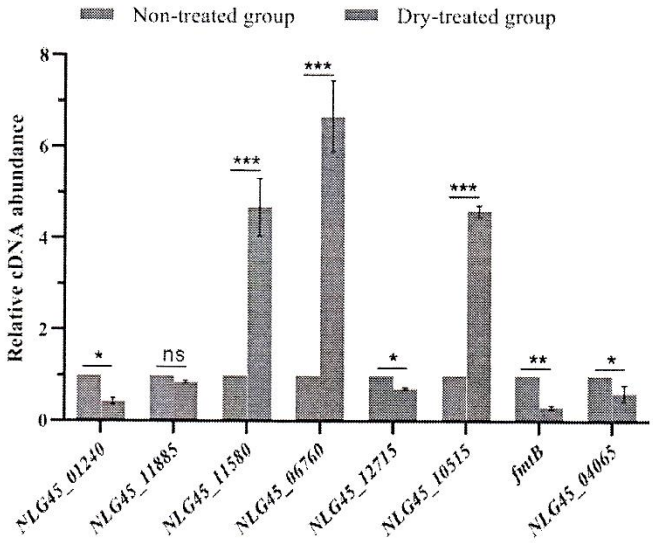


Fig. 6. Comparative measurement of the transcriptional level of relevant mutant genes of RMSA24 with or without desiccation stress. * represents $P < 0.05$, ** represents $P < 0.01$; *** represents $P < 0.001$; ns (no significant difference).

These results indicate that:

a. all of the genes increase in expression during desiccation.
b. all of the genes decrease in expression during desiccation.
c. the genes NLG45_11580, NLG45_06760, and NLG45_10515 all increase in expression during desiccation.
d. the genes NLG45_01240, NLG45_12715, and NLG45_104065 all increase in expression.

Problem 9

Area: Microbial Ecology
Microorganism: *Rhizobium meliloti*
Reference: Hornez, J.-P., Timinouni M., Defives, C., and Derieux, J.-C. 1994. Unaffected nodulation and nitrogen fixation in carbohydrate pleotropic mutants of *Rhizobium meliloti*. Current Microbiology 28:225-229.

Rhizobium meliloti is a Gram-negative bacterium that forms nodules in the roots of alfalfa plants. Fixation of nitrogen in these nodules provides ammonium as a nitrogen source for both the bacteria and the plants. To identify the compounds used as carbon sources by the bacteria in the plants, Hornez et al. isolated a mutant of *R. meliloti* that could not take up and degrade monosaccharides. They then tested this mutant for its ability to cause nodulation and nitrogen fixation in alfalfa.

1. Nitrogen fixation in root nodules is an example of:

    a. commensalism
    b. mutualism
    c. competition
    d. predation

2. The initial stage of infection of alfalfa by *Rhizobium* involves:

    a. penetration of the stem by insects.
    b. entry of the bacteria through the leaves.
    c. formation of infection threads in root hairs.
    d. penetration of the root by insects.

3. The enzyme that carries out the nitrogen fixation reaction usually contains:

    a. nitrogenase
    b. nitrogenase + nitrogenase reductase
    c. nitrogenase + nitrogenase reductase + ferredoxin
    d. nitrogenase reductase + ferredoxin

Hornez et al. began with a wild-type strain of *R. meliloti* designated M5N1 and isolated a mutant called 2-10 by mutagenesis in the laboratory. When they compared the ability of the parental strain and the mutant to grow in a minimal medium with various compounds as carbon sources, they obtained the results shown in the following table.

Table 2. Growth of parental M5N1 and mutant strains on several carbon sources

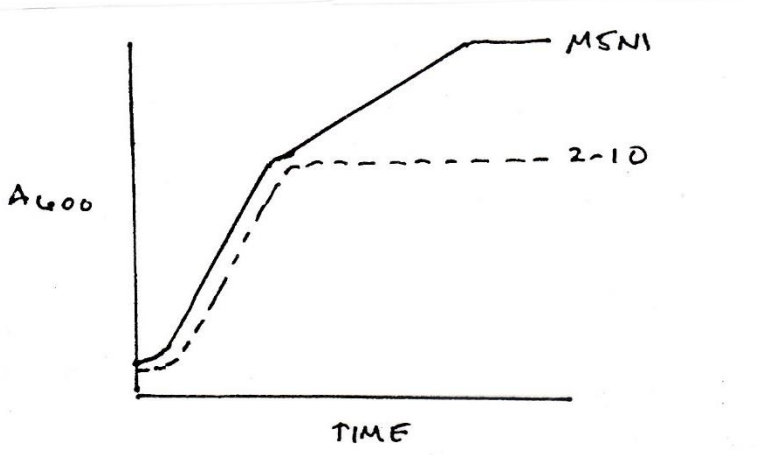
	Growth[a]	
Carbon sources	M5N1	2.10
glucose	+ (4)	−
fructose	+ (4)	−
galactose	+ (5)	−
mannitol	+ (4.5)	−
sorbitol	+ (4.5)	−
xylose	+ nd	−
ribose	+ nd	−
arabinose	+ nd	−
succinate	+ (4)	+ (4)
fumarate	+ (3)	+ (3)
malate	+ (4)	+ (3.5)
lactate	+ (2.5)	+ (2)
glutamate	+ nd	+ nd

[a] Cells were grown in minimal medium with 0.1% carbon sources. Generation times (indicated in parentheses) were determined by measurement of absorbance at 600 nm. Data are mean values of two experiments. nd, not determined; +, growth; −, no growth.

4. Which statement is best supported by these data?

a. The parental strain uses only monosaccharides as carbon sources.
b. The parental strain uses only organic acids as carbon sources.
c. The mutant strain uses only monosaccharides as carbon sources.
d. The mutant strain uses only organic acids as carbon sources.

Hornez et al. then compared the growth the strains M5N1 and 2-10 in a minimal medium containing both glucose and succinate. They obtained the results shown in the following graph.



5. These results indicate that:

   a. the growth rate of the parental strain is the same with either glucose or succinate.
   b. the growth rate of the parental strain with succinate as the carbon source is faster than that of the mutant.
   c. the parental strain first uses glucose as the carbon source and then uses succinate as the carbon source.
   d. the parental strain first uses succinate as the carbon source and then uses glucose as the carbon source.

Hornez then tested the ability of the parental strain and the mutant to form nodules within alfalfa roots and fix nitrogen. The results are shown in the following table.

Table 3. Nitrogen fixation activity of alfalfa nodules after inoculation with mutant or wild-type of *R. meliloti*

Strain	Nitrogenase activity (nmol ethylene produced/h per plant)	Mean plant dry weight (mg)
M5N1	28.3	27.6
2.10	20.8	21.3

6. These data indicate that:

   a. nitrogen fixation does not occur in nodules formed by the mutant.
   b. nitrogen fixation does occurs at the same rate in nodules formed by both the mutant and the parent.
   c. nitrogen fixation in nodules formed by the mutant occurs at about 2/3 the rate of the parent.
   d. nitrogen fixation and plant dry weight show no correlation.

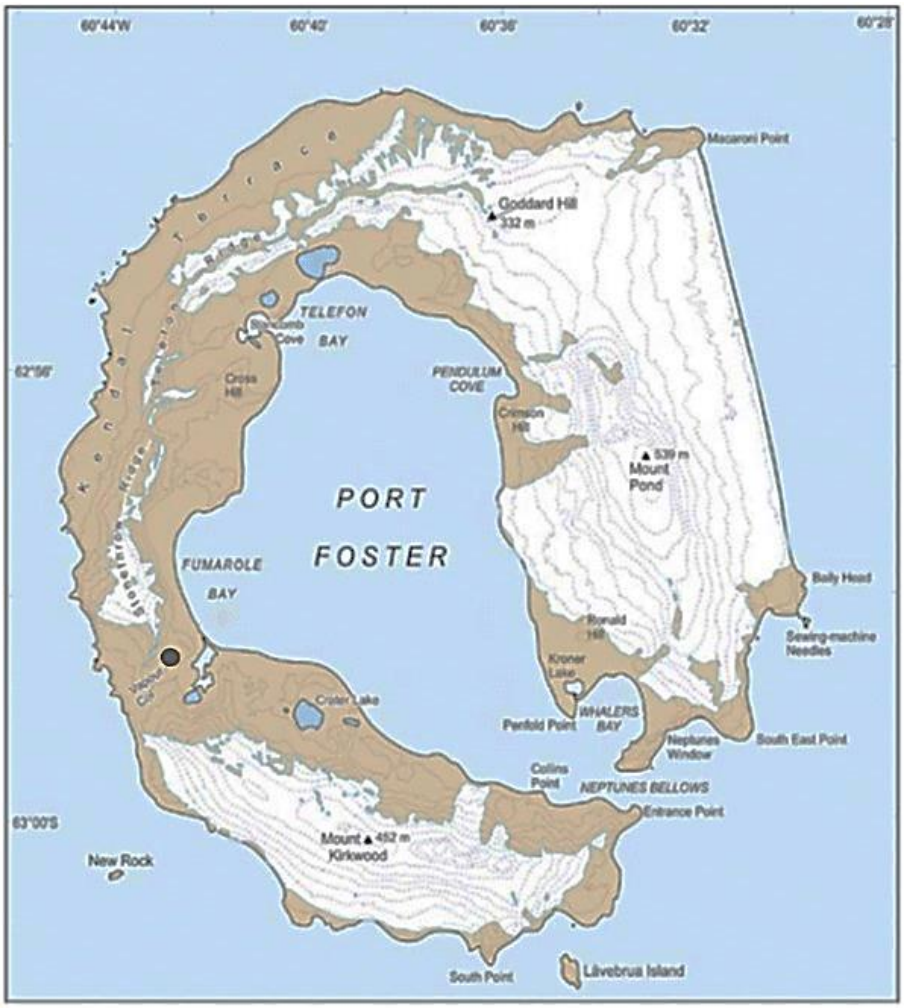
7. Based on these results, it can be concluded that:

   a. organic acids can serve as sole carbon sources for nitrogen fixation.
   b. only monosaccharides can serve as carbon sources for nitrogen fixation.
   c. monosaccharides are required as carbon sources for nitrogen fixation.
   d. only organic acids can serve as carbon sources for nitrogen fixation.

Problem 10

Area: Microbial Ecology
Microorganism: *Bacillus gelatini*
Reference: Flores, P. A. M., Correa-Llantén, and Blaney, J. M. 2018. A thermophilic microorganism from Deception Island, Antarctica with a thermostable glutamate dehydrogenase activity. Biological Research 51(1):55. doi: 10.1186/s40659-018-0206-3.

Environmental conditions at the poles of the planet Earth are more extreme than in more temperate regions. The Antarctic region at the southern pole is mainly cold, but it is far from uniform and contains areas that are hot due to volcanic activity. Deception Island is one of seven islands that constitute the South Shetland archipelago. The island is horseshoe in shape due to the collapse of the central part of a volcano. This shown in the following figure.

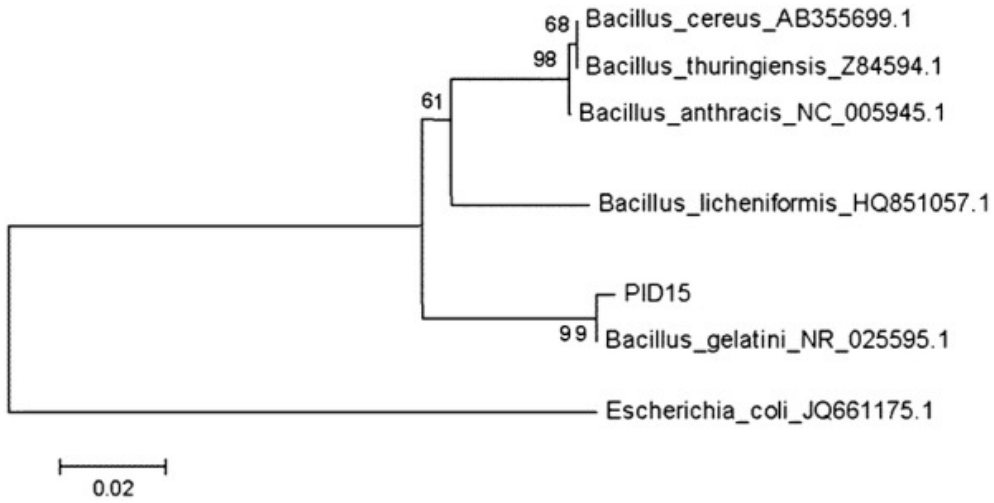


Flores et al. ((Biol. Res. 51: 55, 2018) recently describe the isolation and characterization of a novel thermophilic bacterium from this island. They collected soil samples during a Chilean research expedition from the geothermal site "Cerro Caliente." The temperature and pH at this site were 75 to 95^{o}C and pH 5.5, respectively.

1. Thermophilic microorganisms are usually defined as those that grow:

   a. at 0 to 20°C with an optimum at 15°C.
   b. at 10 to 45°C with an optimum at 37°C.
   c. at 35 to 80°C with an optimum at 65°C.
   d. at 65 to 110°C with an optimum at 95°C.

Flores et al. added 2 g samples of soil from this site to liquid tryptic soy broth and incubated them at 50°C and 70°C. The samples were serially diluted and plated on solid medium. Individual colonies were isolated and purified. The organism of most interest was labelled PID15. DNA from this microbe was isolated and the gene for the 16S ribosomal RNA amplified by PCR and sequenced. The following figure shows the phylogenetic position of this bacterium to several other organisms.



2. Why are 16S rRNA sequences commonly used to identify bacteria and define their phylogenetic relationships?

   a. They are found in all living organisms.
   b. They are found in all prokaryotic organisms.
   c. They are found only in all Gram-positive organisms.
   d. They are found in all eukaryotic organisms.

3. Based on this result, which bacterium is most closely related to PID15?

   a. *Bacillus cereus*
   b. *Bacillus licheniformis*
   c. *Bacillus gelatini*
   d. *Escherichia coli*

To further characterize the new isolated, Flores et al. subjected it to a series of standard test and compared it to *Bacillus gelatini*. The results are shown in the next table.

Table 1

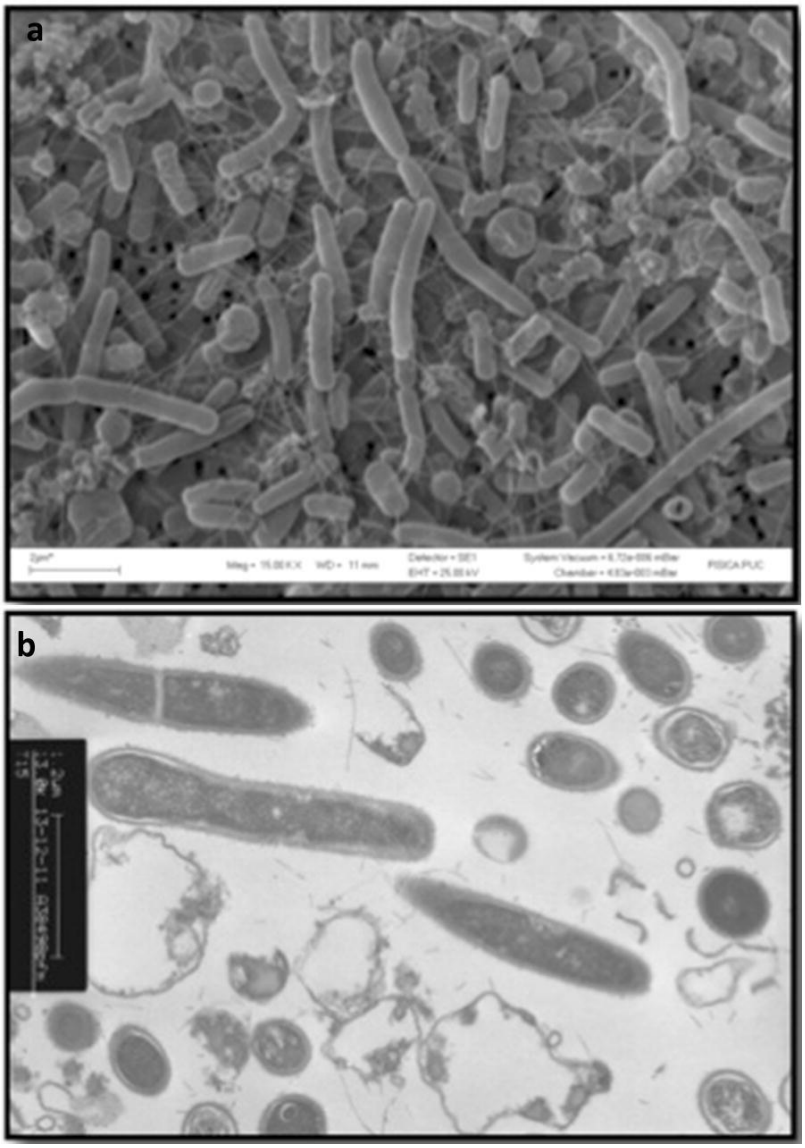
Biochemical characterization of by API20 E kit (bioMérieux, Inc.) and confirmed by tube assay

Biochemical test	**PID15**	***B. gelatini* (DSM 15865)**
Characteristics		
Gram	+	+
Temperature of growth (°C)	45–65	40–60
pH growth range	6.0–11.0	4.0–10.0
Motility	+	+
Amino acid degradation		
l-Arginine dehydrolase	(−)	(−)
l-Lysine decarboxylase	+	(−)
l-Ornithine decarboxylase	+	(−)
Tryptophan deaminase	(−)	(−)
Indole	(−)	(−)
Metabolism		
Hugh-Leifson (oxidation)	+	+
Hugh-Leifson (fermentation)	(−)	(−)
Methyl red	(−)	(−)
Catalase	+	+
Oxidase	(−)	(−)
Voges Proskauer	(−)	(−)
Hydrolysis of		
Gelatinase	+	+
Starch	(−)	(−)
β-galactosidase	(−)	(−)
Carbon source		
Citrate	(−)	(−)
d-Glucose	+	V
d-Mannitol	+	V
Galactose	+	(−)
Ribose	+	V
d-Sucrose	+	(−)
Lactose	+	(−)
Others		
H_2S production	(−)	(−)
Nitrate reduction	(−)	(−)
Urease	(−)	(−)

4. Based on these data, which is the most obvious difference between PID15 and *Bacillus gelatini*?

a. PID15 is Gram-positive and *Bacillus gelatini* is Gram-negative?
b. PID15 is motile and *Bacillus gelatini* is nonmotile?
c. PID15 can utilize more sugars as carbon sources than *Bacillus gelatini*.
d. PID15 grows by fermentation and *Bacillus gelatini* grows by respiration.

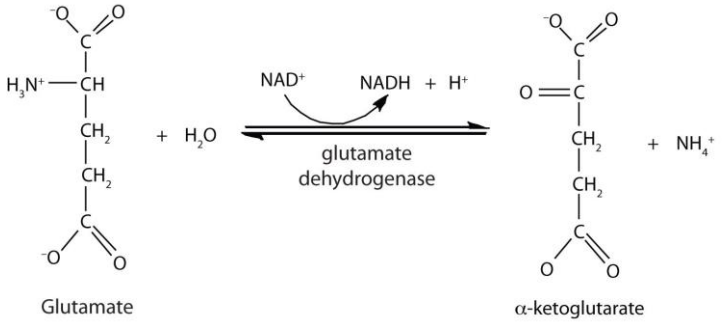
To characterize the bacterium microscopically, Flores examined the cells by both scanning and transmission electron microscopy. The results are shown in the next figures.



5. Based on these results, the organism is best described as a(n):

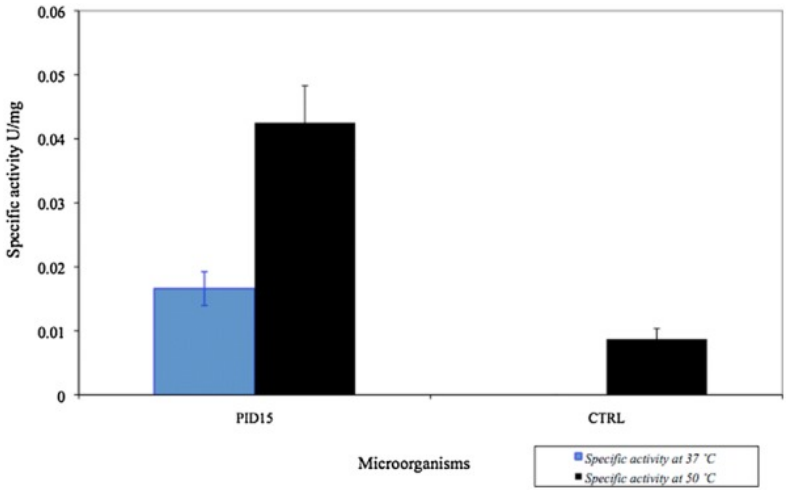
a. coccus
b. bacillus
c. spirillum
d actinomycete

One of the reasons Flores et al. were interested in isolating bacteria from this unusual site is that extremophiles often have promising applications in biotechnology. They looked specifically at the enzyme L-glutamate dehydrogenase, which catalyzes the following reaction called oxidative deamination:



\

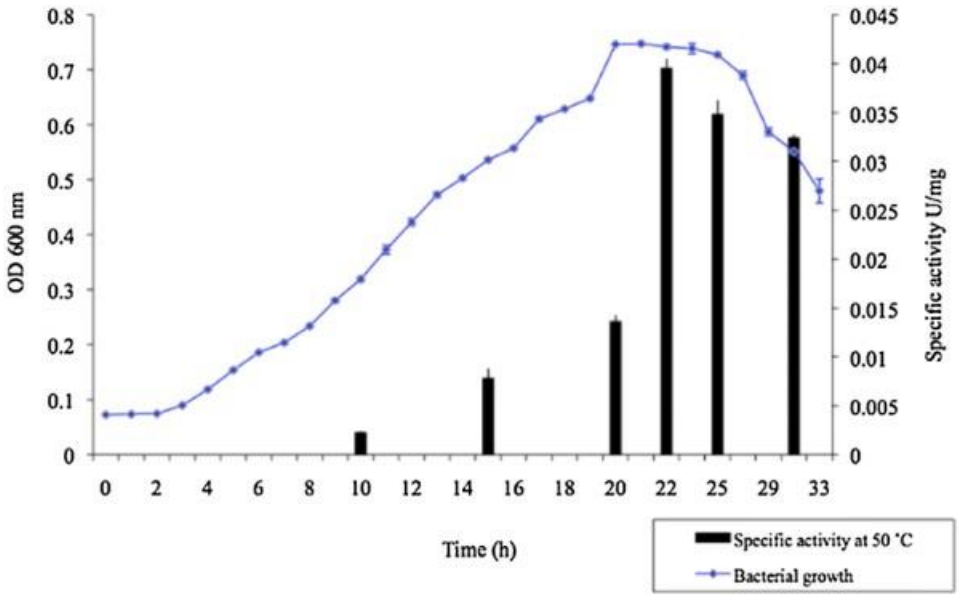
They measured enzyme activity at both 37°C and 50°C and obtained the results shown in the next figure. They compared PID15 to a control organism called GWE1.



6. What do these results indicate?

a. The enzyme from both microorganisms shows activity at 37°C and 50°C.
b. The enzyme PID15 shows activity at 37°C and 50°C but that from GWE1 only has activity at 37°C.
c. The enzyme GWE1 shows activity at 37°C and 50°C but that from PID15 only has activity at 37°C.
d. The enzyme GWE1 shows activity at 37°C and 50°C but that from PID15 only has activity at 50°C.

To study the formation of L-glutamate dehydrogenase by PID15, Flores et al. grew a culture at 50°C and periodically measured the optical density of the culture (OD_{600}) and the specific activity of the enzyme. The results are shown in the next figure.



7. What can you conclude from these data?

   a. The bacteria show a normal growth pattern and enzyme activity is highest during exponential phase.
   b. The bacteria show a normal growth pattern and enzyme activity is highest during stationary phase.
   c. The bacteria show an abnormal growth pattern and enzyme activity is highest during exponential phase.
   d. The bacteria show an abnormal growth pattern and enzyme activity is highest during stationary phase.

Problem 11

Area: Microbial Pathogenesis
Microorganism: *Vibrio cholerae*
Reference: Sengupta, T. K., Sengupta, D. K., and Ghose, A. C. 1993. A 20-kDa pilus protein with haemagglutination and intestinal adherence properties expressed by a clinical isolate of non-01 *Vibrio cholerae*. FEMS Microbiology Letters 112: 237-242.

The gastrointestinal disease cholera is caused by the Gram-negative bacterium *Vibrio cholerae*. This bacterium colonizes the mucosa of the small intestine and produces a toxin that alters membrane permeability. Sengupta et al. (FEMS Microbiol. Lett. 112: 237-241, 1993) recently described an unusual clinical isolate of *V. cholerae* and studied its ability to attach to host tissues.

1. Which of the following structures in the Gram-negative envelope is least likely to be involved in cell adhesion.

   a. cell membrane
   b. outer membrane
   c. S-layer
   d. pili

Sengupta et al. first compared the ability of the bacteria to cause hemagglutination (the aggregation of red blood cells) and to bind to isolated rabbit intestinal slices after growth in two different media: tryptic soy broth (TSB) and tris-buffered salts medium (T medium). The results are shown in the following table.

growth medium	hemagglutinating activity	adhesion index
TSB	128	12.9
T medium	4	2.0

2. These results indicate that:

a. the bacteria cannot cause hemagglutination.
b. the bacteria cannot adhere to intestinal cells.
c. hemagglutination and adhesion are greater after growth in TSB.
d. hemagglutination and adhesion are greater after growth in T medium.

When Sengupta et al. analyzed the lipopolysaccharide profiles of the bacteria after growth in TSB or T medium, they found no significant differences. However, when they analyzed the protein profiles of the cell envelopes after growth in each medium, they obtained the following results (a - indicates a protein of a certain mass was absent, a + indicates a protein of a certain mass was present).

protein	molecular mass	TSB	T medium
1	66,000	-	+
2	55,000	-	+
3	47,000	+	+
4	39,000	+	+
5	28,000	+	+
6	20,000	+	-

3. These results indicate that:

a. the protein composition of the cell envelope is the same under all growth conditions.
b. the protein composition of the cell envelope is completely different in the two media.
c. there are more different proteins in the cell envelope after growth in TSB.
d. there are more different proteins in the cell envelope after growth in T medium.

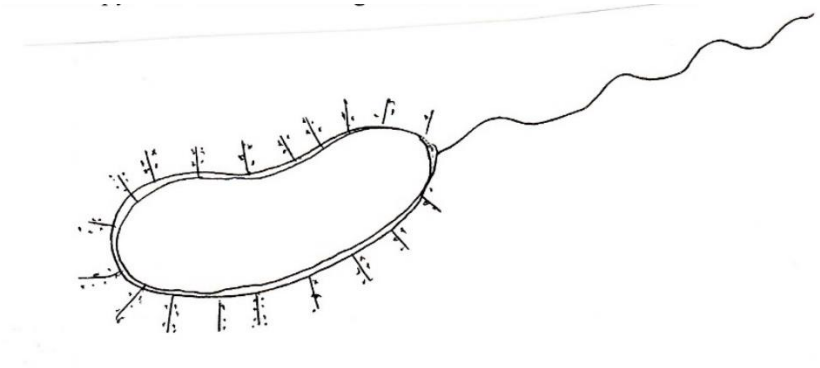
Sengupta et al. then tried to determine which of these proteins was important in adhesion of *V. cholerae* to intestinal cells. They isolated protein 1 (66,000 daltons), protein 4 (39,000 daltons), and protein 6 (20,000 daltons) and used each preparation to make antibodies that would bind to that particular protein. They then tested the effects of these antibodies on the ability of *V. cholerae* to adhere to rabbit intestinal slices. The results are shown below.

additions	adhesion index
V. cholerae + buffer	12.9
V. cholerae + antibodies to protein 1	12.8
V. cholerae + antibodies to protein 4	12.9
V. cholerae + antibodies to protein 6	1.2

4. From this, one can conclude that:

a. protein 1 (66,000) is important in adhesion.
b. protein 4 (39,000) is important in adhesion.
c. protein 6 (20,000) is important in adhesion.
d. none of the proteins is important in adhesion.

To identify the portion of the cell envelope with which protein 6 (20,000) was associated, they linked the antibodies specific to protein 6 to gold particles and used these particles to "stain" the bacteria for electron microscopy. The results are diagrammed below, where the dots represent the gold particles:



From this, one can concluded that protein 6 is:

a. a pilin
b. a flagellin
c. an integral outer membrane protein
d. a peripheral

Problem 12

Area: Microbial Pathogenesis
Microorganism: *Aeromonas trota*
Reference: Takahashi, E., Ozaki, H, Fujii, Y., Kobayashi, H., Yamanaka, H., Arimoto, S., Negishi T., and Okamoto, K. 2014. Properties of hemolysin and protease produced by *Aeromonas trota*. PloS One 9(3):e91149. doi: 10.1371/journal.pone.0091149.

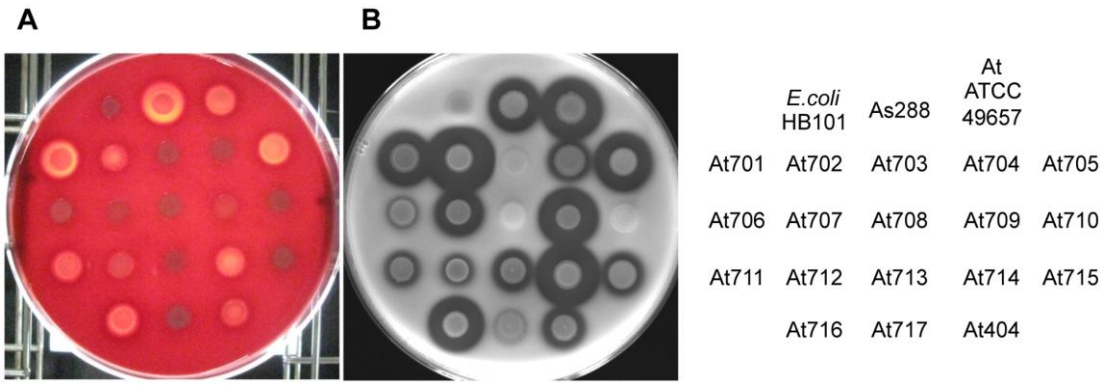
Bacteria of the genus *Aeromonas* are commonly found in aquatic habitats including fresh water, brackish water, and seawater. Many species can cause wound infections or sporadic diarrhea. *A. trota*, which has been previously called *A. enteropelogenes*, is a mesophilic species that is ampicillin sensitive but difficult to treat because is forms an enzyme called a β-lactamase that can degrade this antibiotic. *A. trota* was previously shown to cause diarrhea in an animal model. Takahashi et al. (PloS One 9(3):e91149, 2014) described the properties of a hemolytic protein (hemolysin) and a protease from this microorganism.

Several strains of *A. trota* were isolated from patients with diarrhea or from environmental water and soil. A type strain (ATCC 49657) was also obtained from the American Type Culture Collection. The bacteria were grown in nutrient broth medium at 37ºC with aeration.

1. What are the characteristics of diarrhea as a clinical disease of humans?

   a. the appearance of small red pustules on the surface of the skin.
   b. the occurrence of loose, watery, and more frequent bowel movements.
   c. the sudden onset of memory loss or mental confusion.
   d. the presence of muscle tremors.

To test for the ability of *A. trota* to form proteins that might lyse red blood cells or breakdown proteins, various strains were grown in liquid nutrient broth for 20 hours and 2 µl portions spotted onto nutrient agar plates supplemented with either 5% sheep erythrocytes or 1% skim milk. The plates were incubated at 37oC for 24 hours the presence or absence of areas of clearing observed around the bacterial spots. *E. coli* HB101 and *A. sobria* 288 were used as controls. The results are shown in the next figure.



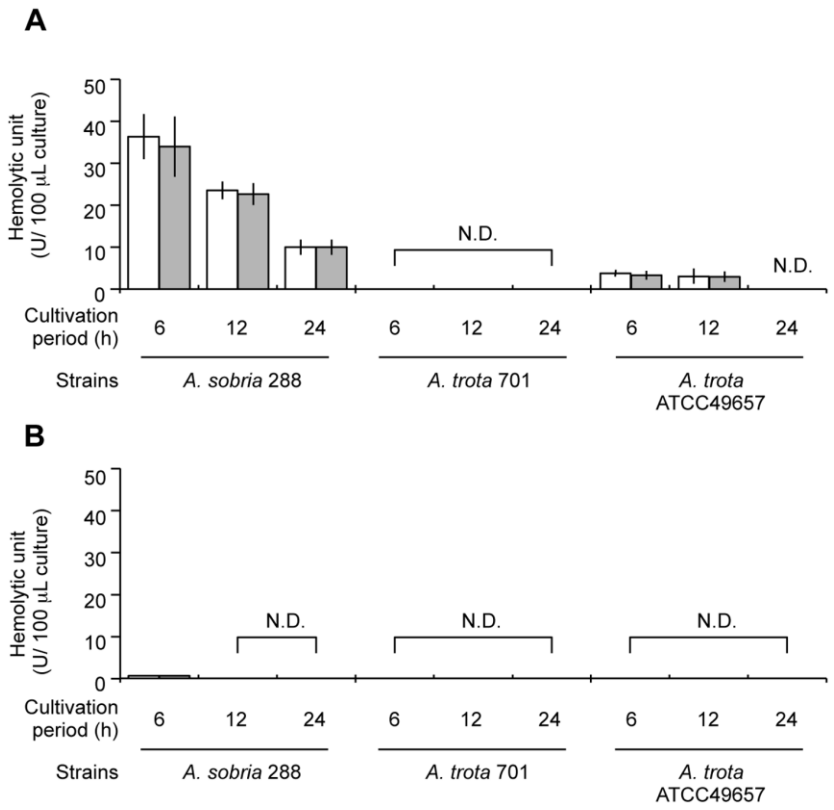
2. Look first at the three controls at the top of the plates. What can you see?

   a. HB101, As288, and At49657 all formed distinct zones of clearing on both types of medium.
   b. As288 and At49657 both formed distinct zones of clearing on both types of medium.
   c. As288 formed distinct zones of clearing on both types of medium but At49657 only showed clearing on the plate with red blood cells.
   d. As288 formed distinct zones of clearing on both types of medium but At49657 only showed distinct clearing on the plate with skim milk.

3. Now look at the look the spots for the various *A. trota* isolates. What can you conclude from these data?

   a. All of the isolates formed distinct zones of clearing on both types of medium.
   b. None of the isolates formed distinct zones of clearing on both types of medium.
   c. At701 formed distinct zones of clearing on both types of medium but At708 showed no zones of clearing.
   d. At707 formed a distinct zones of clearing on the blood agar plate but no clearing on the skim milk plate.

To determine if the proteins causing hemolysis could be formed and released when the bacteria were grown in liquid, medium, *A. trota* 701, *A. trota* 49657, and *A. sobria* 288 were cultivated in nutrient broth at 37oC. Samples were removed at 6, 12, and 24 hours and centrifuged at 15,000 x *g* for 5 min. The supernatant (culture fluid) was decanted and saved. The cell pellet was suspended in buffer and the bacteria disrupted by sonication. Each sample was then tested for hemolytic activity with the results shown in the next figure.



4. What can you conclude from these data?

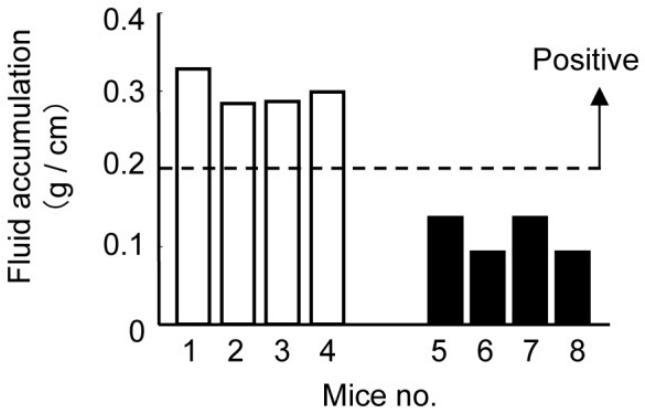
   a. *A. sobria* 288 forms a hemolysin that is released into the medium early during growth but it is not retained in the cells.
   b. *A. trota* 49657 forms a hemolysin that is released into the medium early during growth but it is not retained in the cells.
   c. *A. trota* 49657 and *A. trota* 701 form more hemolysins when it is grown in liquid medium than when they are grown on agar plates.
   d. *A. trota* 701 forms more hemolysis than *A. trota* 49657 when it is grown in liquid medium.

To compare the genes for the hemolysins, the *alh* genes from *A. sobria, A. trota* 49657, and *A. trota* 701 were amplified by PCR and sequenced. The genes from *A. trota* 49657 and *A. trota* 701 showed 61.9% sequence identity and 93% sequence similarity to that of *A. sobria*.

5. What does the observation contribute to this analysis?

   a. It does contribute at all.
   b. It suggests that amino acid replacements in the *A. trota* protein have led to a decrease in activity.
   c. It suggests that amino acid replacements in the A. trota protein have prevented its release from the cells.
   d. It suggests that amino acids are not important in determining the activity of the protein.

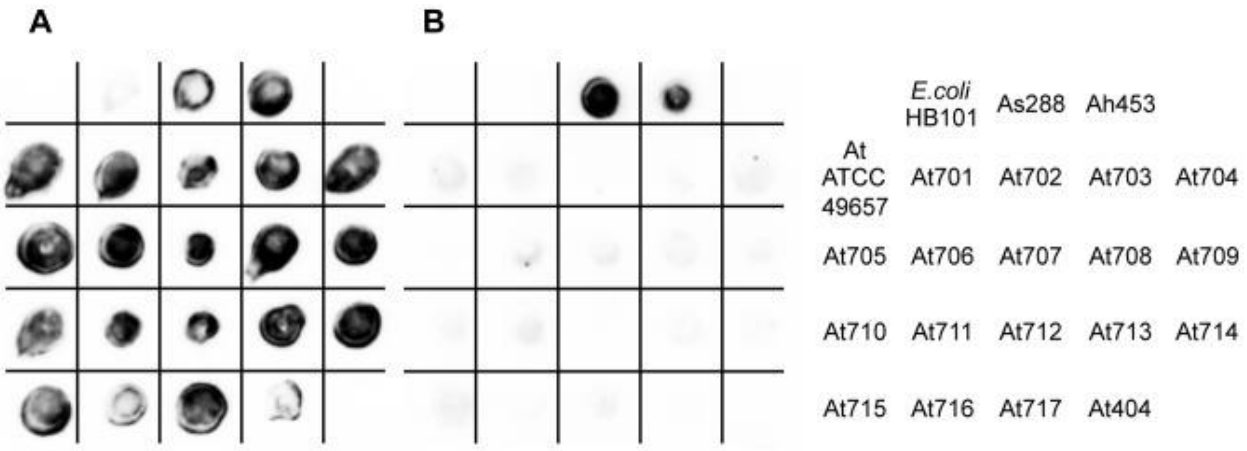
To determine if the hemolysin from *A. trota* 701 has toxin activity, suspensions of the bacteria containing a total of 3 x 10^7 cells mixed with either preimmune serum or serum containing hemolysin antibodies were injected into loops of mouse intestine. The mice were killed after 3 hours and the weights of the intestinal loops determined. The results are shown in the next figure. The open bars show samples treated with preimmune serum and the filled bar show samples treated with hemolysin antibodies.



6. What can you conclude from these data?

   a. The protein from *A. trota* 701 in an enterotoxin.
   b. The protein from *A. trota* 701 not an enterotoxin but the antibodies against it are.
   c. The protein from *A. trota* 701 is an enterotoxin but only when combined with the antibodies against it
   d. The mouse model is not a valid test for a toxin.

Takahashi et al. then followed up on the experiment with skim milk agar by testing for the presence of a gene coding for a serine protease. The various *A. trota* isolates, the control strains used before, and another control called *A. hydrophila* 453 were grown on nutrient agar plates at 37°C for 24 hours. The bacteria were then transferred to a Hybond-N nylon membrane, lysed, and the DNA denatured. The spots were then process and tested using synthetic probes specific to serine protease and metalloprotease genes of *A. sobria*. Hybridization was observed by a chemical assay. The results are shown in the next figure.



7. What does these data indicate about the presence of the serine protease and metalloprotease genes in these bacteria?

   a. *E. coli* and *A. sobria* contain both genes.
   b. None of the *Aeromonas* strains contain both genes.
   c. All of the *A. trota* isolates contain both genes.
   d. 17 of the 19 *A. trota* isolates and the ATCC 49657 strain contain a serine protease gene but not a metalloprotease gene.

When Takahashi et al. attempted to find the serine protease activity in the liquid cultures of *A. trota* using antibodies against the protein from *A. sobria*, they could not find it either in the cultures supernatants or the cell lysates. They also could not detect a protease activity use azocasein (a colored polymer) as a substrate.

8. Since the many of the *A. trota* strains did show good zones of clearing on skim milk agar plates, what might you want to study next?

   a. the genomes of the bacteria in liquid cultures and solid media.
   b. the transcription of the hemolysis and protease genes in liquid cultures and solid media.
   c. the synthesis of the hemolysis and protease activities in liquid cultures and solid media.
   d. the stability of the hemolysis and protease activities in liquid cultures and solid media.