

## Chapter 2

# Genetic Control of Cell Chemistry Using *Serratia marcescens*

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## Contents

Introduction.....	22
Materials .....	23
Student Outline .....	23
Introduction.....	23
Study of Biochemical Pathways .....	24
Branched Biochemical Pathways.....	27
Prodigiosin Synthesis in <i>Serratia marcescens</i> .....	28
Procedure .....	29
Student Worksheet.....	31
Notes for the Instructor .....	32
Acknowledgements.....	32
Further Reading .....	33

## Introduction

This exercise enables students to carry out and visualize results of cross-feeding experiments involving *Serratia marcescens*. Because of the color expression which results from either a complete pathway or successful cross-feeding, results are easily apparent. The biochemical pathway under study is the one involved in synthesizing *prodigiosin*, a red pigment apparent in the wild-type bacterium. Five mutants are provided to the students, each mutant is a different color. When the mutant strains are plated in pair-wise combinations, cross-feeding occurs in which strains that are mutant for a step further along the pathway can feed those which are lacking a step at an earlier place in the pathway. The result of cross-feeding is a wild-type color appearing in the strain which is “fed” and this is very apparent in most pair-wise comparisons because of its strong red color.

This exercise can be used as an easily “readable” cross-feeding experiment, to elucidate genetic control of cell chemistry, to teach sterile technique, and it can be simplified for more basic classes (see Notes for the Instructor). It is best if the instructor performs the experiment to observe results before presenting it to the class because color expression is not always easily readable; if the instructor is familiar with potential problems, he/she can aid students in interpreting the results. It is also essential for the instructor to be able to visualize the pathway as students interpret their results and diagram the pathway. The exercise, as written here, is for students who have had some introductory biology, it was used by us in the genetics lab and provided a challenge to the students. There are some ideas in the Notes for the Instructor section for simplifying the exercise.

Color expression in *Serratia marcescens* is best observed by growing cultures on peptone-glycerol (PG) plates that have been prepared a week ahead (see Materials section). Mutant strains should be plated several days before the lab so that each group of students will have access to cultures to plate their cross-wise comparisons. During the lab, each group of students will plate all strains in pair-wise combinations; at the same time, several plates of each strain alone should be plated as a reference for normal color development in the mutant bacteria. *Note:* Make sure students store plates right-side-up as some volatile substance are formed.

## Materials

Each group of students, three or four per group, will need the following:

Stock plates with the *Serratia marcescens* auxotroph strains, labelled A–E (one set of stock plates per two groups of students can be used if desired) (5)

Stock plate of the *Serratia* wild-type strain (1)

Peptone-glycerol agar plates, sterile (10)

Inoculating loop (1)

Bunsen burner (1)

EtOH, 95% (for sterilizing work space)

Marking pen

Parafilm

To make 1 liter of peptone-glycerol agar plates add:

Bacto-peptone	5 g
Glycerol	10 ml (about 12 g)
Bacto-agar	15 g
Water	1 liter

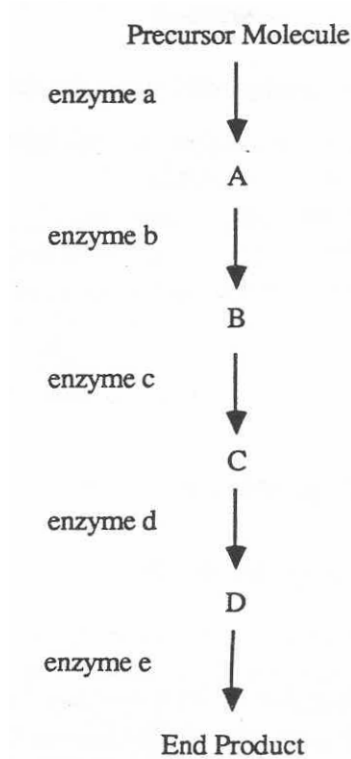
Place the above ingredients in a 1.5–2 liter flask and autoclave for approximately 20 minutes. Cool until the flask is still quite warm but you are able to handle it. Pour plates and store for 1–4 weeks before use. Three liters of media will make about 100 plates but 1 liter is about all you can pour conveniently by hand.

## Student Outline

### Introduction

An organism's physical attributes or phenotype is a manifestation of its genetic make-up or genotype. The phenotype of an organism is the sum of the physical expression of the many different genes contained within its genome which control this through cell chemistry. Different alleles for specific genes are expressed by different organisms and can sometimes be interpreted from physical, identifiable characteristics. If mutations are incorporated into the genome of a cell or organism, these will often be apparent through the resulting phenotypic differences between cells or organisms. Information about underlying genetic differences can thus often be obtained by careful study of phenotypic expression.

It is often possible for biologists to relate many of an organism's structural or functional features (phenotype) to chemical reactions that take place within its cells. A *biochemical pathway* combines or alters a number of intermediate products through chemical reactions to produce a final product. Typically, a biochemical pathway begins with certain *precursor* molecules, these are the raw materials which will be modified by the various chemical reactions of the pathway to produce a *final product*. Precursor molecules can be obtained from the environment or some other pathway. Often there are a number of intermediate steps which are catalyzed by different enzymes and which produce intermediate compounds (Figure 2.1), before the final product is formed.



**Figure 2.1.** Hypothetical biochemical pathway showing substrates, genes, and enzymes.

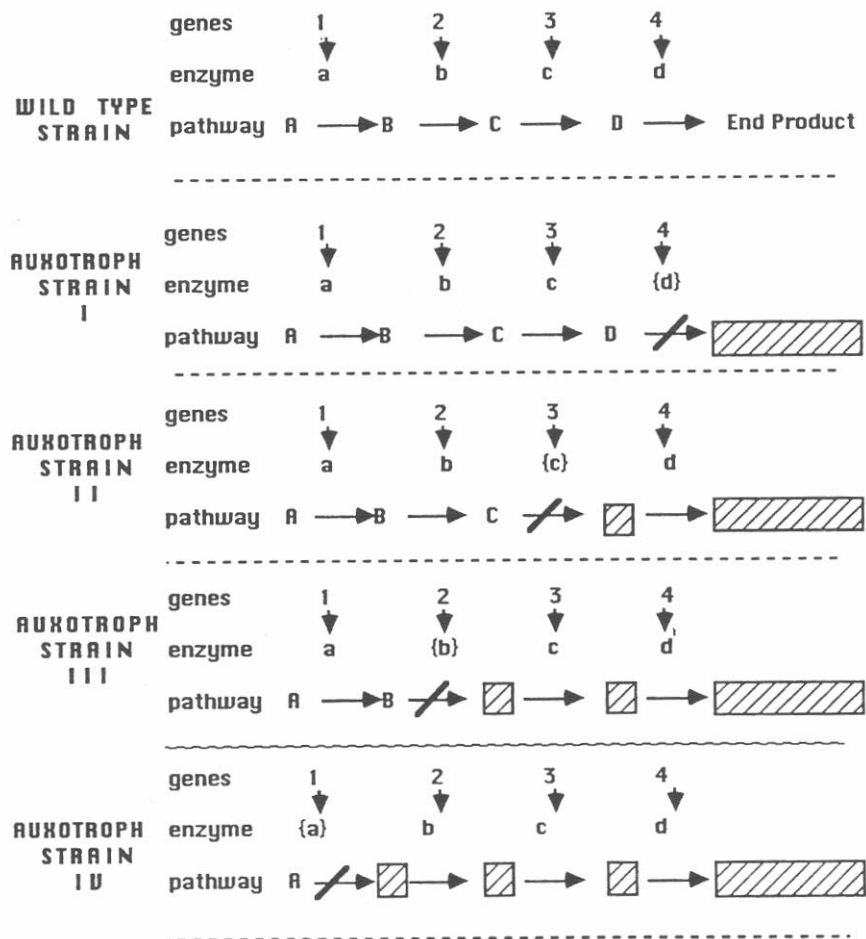
It is through genetic control of the biochemical pathways on a cellular level that an organism's phenotype is determined. Enzymes which catalyze each step of the biochemical pathway are coded for by specific genes, genes are a result of the specific nucleotide sequence of a particular portion of the DNA molecule. Within the nucleus, the DNA nucleotide sequence or *allele* for the gene produces a complementary RNA nucleotide sequence (the messenger RNA or mRNA) which couples with ribosomes in the cytoplasm and directs the synthesis of the enzyme. The mRNA nucleotide sequence determines the amino acid sequence of the enzyme. The presence of the appropriate enzyme allows a specific step of the pathway to be completed. All of the genes coding for all of the enzymes involved in the pathway must be functioning normally (must be producing normal, functional enzymes) if the pathway is to be complete and product is to be formed.

### Study of Biochemical Pathways

The method for studying the genetic control of biochemical pathways is quite simple once the underlying principles are understood. The wild-type strain (*prototroph*) of the organism is able to produce the end product of the biochemical pathway being studied. The pathway is functional in this organism because the genetic information is complete and contains wild-type alleles for each gene controlling each step of the pathway. *Auxotroph* strains are those strains which are unable to complete the pathway due to a mutation in one or more of the genes coding for enzymes which carry out steps in the pathway. These can be produced by irradiating cells with x-rays or ultraviolet light; sometimes mutations arise naturally in populations but this is very infrequent.

Figure 2.2 shows an example of a biochemical pathway with four steps: precursor A is changed to the end product through four enzyme-catalyzed reactions which produce intermediates B, C, and D. Each of these enzymes is coded for by a specific allele. An auxotroph is produced by a mutation in one of these alleles; this produces a non-functional enzyme which blocks the conversion from one intermediate to the next. Each of the four auxotrophs (I–IV) has a block at a different step in the pathway because of a mutant allele for one of the four genes controlling the pathway (the other genes produce normal enzymes). In auxotroph Strain I the mutagen changed the nucleotide sequence in gene 4, converting the wild-type allele to a mutant allele. This causes the mutant allele to code for altered enzyme {d} instead of normal enzyme d. Because enzyme {d} has been changed, it cannot catalyze the change from intermediate product D to the end product and auxotroph I is blocked in this step of the pathway.

□ Examine the other three strains in Figure 2.2 to determine where they are blocked in the pathway. Notice that the phenotypic result is the same, none of the auxotrophic strains can product final end product.



**Figure 2.2.** Four auxotrophic strains (I–IV) resulting from blocks in a simple linear biochemical pathway. Each block results from a mutation in one of the four genes controlling the pathway.

Scientists often utilize auxotrophs to study synthesis or degradation of a particular compound because auxotrophs are unable to grow or grow poorly unless the compound in question is supplied in its growth medium. A few auxotroph strains are produced after mutagenic treatment and can be separated from the more numerous prototrophs by the use of an antibiotic such as penicillin. Penicillin is only harmful to growing cells and has no effect on those cells which are not growing (auxotrophic strains). Auxotrophic strains will not be killed by the antibiotic while prototrophs will, thus the investigator can select for only auxotrophic strains. After the incubation mixture is washed free of penicillin, the auxotrophic strains can be grown on medium which supplies the compound necessary for growth. If one wants to study the biochemical pathway for synthesis of tryptophan, one can isolate a number of auxotrophs produced by independent events and can put them into several groups. If one then supplements the growth medium for these strains with the compounds suspected to be involved in the synthesis of tryptophan, the biochemical differences between the strains becomes apparent. Some strains will grow on indole, some will grow on anthranilic acid but not indole, some will grow on indole and tryptophan but not anthranilic acid, others on anthranilic acid and tryptophan but not indole and some will grow on all three. The fact that some organisms which cannot grow on tryptophan alone will grow when supplied with one of the two alternative chemicals listed above indicates that these compounds may play a direct role in the biosynthesis of tryptophan and can be inferred to be intermediate compounds.

An investigator who is interested in the formation of end product, for example tryptophan, might ask three questions:

1. How many reaction steps are in the pathway?
2. What is the shape of the pathway?
3. Which genes control which steps in the pathway?

The first step is to sequence the auxotrophic strains in the same order as they are blocked in the pathway. There is one very important difference between auxotrophic and wild-type strains that allows us to do this. Wild-type organisms produce an end product utilized by the cell and have incorporated into the genome a control to “turn on” production of the end product by activating the pathway as it is needed and to “turn off” production as soon as sufficient end product accumulates in the cell. Because auxotrophic strains cannot produce end product their pathway operates continuously but cannot continue to completion. As a result, large quantities of the intermediate product that is the substrate for the blocked reaction step are produced and are secreted by the cell into the nutrient medium.

□ Examine Figure 2.2 again and determine which substance would be secreted for each auxotrophic strain, for example Strain I would secrete substance D.

Notice that Strains II, III, and IV are normal for enzyme d and would be able to convert precursor D to the end product if it were supplied. Because of this fact, if Strain II were grown on the same medium as Strain I, it would be able to utilize secreted precursor D from Strain I to produce end product and thus grow normally (the same is true for Strains III and IV). This principle is the reason for one of the general rules for elucidation of a biochemical pathway:

1. If an auxotroph strain has its block in the last reaction step of the pathway, then it will *feed all other* strains but be fed by none.

Examination of Figure 2.2 will also reveal the Strain IV cannot feed Strain I or any other strain because although it secretes precursor A the other strains are blocked subsequent to that step and will still be unable to continue to the end product. For example, even when grown with Strain IV,

Strain I will still be blocked at step 4 and will not be able to produce end product. This gives us Rule 2.

2. If an auxotrophic strain has its block in the first step of the biochemical pathway, it will be *fed by all other strains* and will feed none.

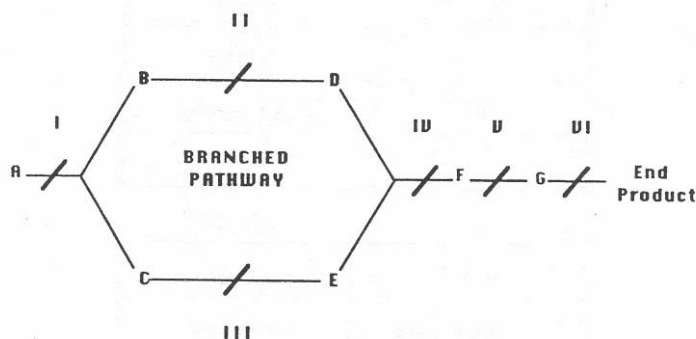
Rule 3 describes a general rule which will be apparent from examination of the pathways in Figure 2.2.

3. For strains with blocks in steps between the first and last step, the strain with a block close to the beginning of the pathway will be fed by any strains blocked later in the pathway and strains with blocks late in the biochemical pathway will be able to feed all strains with blocks earlier in the pathway.

### Branched Biochemical Pathways

Figure 2.3 shows a pathway that is branched, there are six reaction steps each of which is catalyzed by a different enzyme. This means that there are potentially six auxotrophic strains which can be produced by mutation of the gene that produces the enzyme for that step. The principles are the same as a simple pathway except for one difference. Remember that an auxotrophic strain is blocked in only one step and secretes the substrate produced before the block. A branched pathway begins with either one or two compounds; Figure 2.3 illustrates a single precursor. In this figure, compound A is split by an enzyme to produce B and C which are both converted by separate enzymes to D and E. Intermediates D and E are joined by an enzyme to produce intermediate F. F is converted to G and G is changed into the end product. In Figure 2.3 the strains are labelled I–VI and are each blocked at a single step. Strain II cannot convert compound B to D but all other enzymes in the pathway are fully functional. This means that compound B accumulates in the medium because it cannot be converted. Notice that E must be combined with D to form intermediate F, this step cannot happen because Strain II cannot produce D. Because of this, intermediate E also builds up and is secreted into the medium. Strain III is just the reverse, it can convert B to D but is blocked in the C to E step. Strain III therefore secretes both C and D. A little examination of the branched pathway will show why Rule 4 is true.

4. If a pathway is branched, “mutual feeding” will occur between all strains with blocks on opposite branches of the pathway. “Mutual feeding” means that one auxotroph feeds another strain and is in turn fed by that same strain. Mutual feeding always occurs between strains blocked on opposite arms of the pathway.



**Figure 2.3.** A representation of a simple branched biochemical pathway. Each auxotrophic strain is a result of a single mutation causing a malfunctional enzyme at a single step, all other steps are functional. Pathway substances are represented by the letters A–G.

□ Make certain that you understand how the information in Table 2.1 relates to the diagram in Figure 2.3. This is a good time to test whether you understand the principles behind the four rules of the explanatory system.

**Table 2.1.** Important features of the six auxotrophic strains in the pathway shown in Figure 2.3.

Auxotroph strain	Substance(s) secreted due to block	Recipient strain	Donor strains
I	A	None	II, III, IV, V, VI
II	B, E	I, III	III, IV, V, VI
III	C, D	I, II	II, IV, V, VI
IV	D, E	I, II, III	V, VI
V	F	I, II, III, IV	VI
VI	G	I, II, III, IV, V	None

### Prodigiosin Synthesis in *Serratia marcescens*

In this laboratory exercise you will use the explanatory system to study the genetic control of a biochemical pathway that produces an end product called *prodigiosin*. This is a deep red pigment found within the bacterium *Serratia marcescens* and causes the wild-type bacteria to exhibit a deep red color. You will study five auxotroph strains of *Serratia*, each blocked at a different step within the pathway, and each a different color. The auxotroph strains were produced by ultraviolet light treatment of the wild-type. Strain designations and coloration are shown in Table 2.2.

**Table 2.2.** Strains of *Serratia marcescens* and their coloration.

Color	Strain
OF	orange
WF	light orange
C-11	light pink
XII-20	purple
9-3-3	pink
D1 (wild-type)	dark red



During this exercise you will perform pair-wise feeding trials to determine which secreted intermediates will allow completion of the prodigiosin pathway. Because of the nature of this pathway, the procedure is quite simple. Auxotrophic strains are able to grow and produce colonies that differ from wild-type only in coloration. If “feeding” occurs, the auxotrophic strain being fed will develop the wild-type color. Careful examination of each pair-wise trial and comparison with coloration of the strains as they grow alone will enable you to elucidate this pathway.

### Procedure

This lab provides good experience in utilizing sterile procedure in order to produce plates that are not contaminated with other bacteria or fungi. *Serratia* can cause skin and eye irritations so use care in handling and wash your hands when finished.

Work in groups of three or four for this study. Each group will need the following materials:

Stock plates with the *Serratia marcescens* auxotroph strains (labelled A–E) (5)

Stock plate of the *Serratia* wild-type strain (1)

Peptone-glycerol agar plates, sterile (10)

Inoculating loop (1)

Bunsen burner (1)

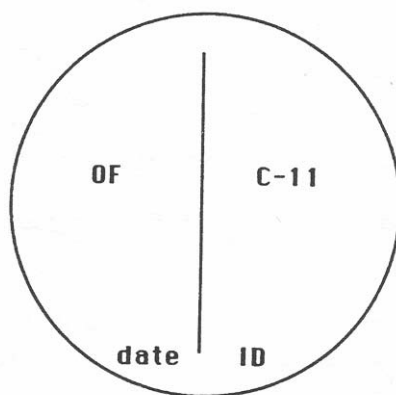
EtOH, 95% (for sterilizing work space)

Marking pen

Parafilm

Disinfect your work area by wiping down the surface with a paper towel soaked in 95% EtOH. *Do not ignite the bunsen burner until all the alcohol has evaporated.*

Each of the five auxotrophic strains will be plated next to each other in pair-wise combinations. This means that you will need 10 feeding trial plates. Label them on the bottom to indicate which strains will be plated, one on each side. Write the date in the center bottom edge of the plate and indicate your group using the initials of the group members (see Figure 2.4).



**Figure 2.4.** Before plating, make sure the bottom of your plate is carefully labelled with strains, date, and group identification.

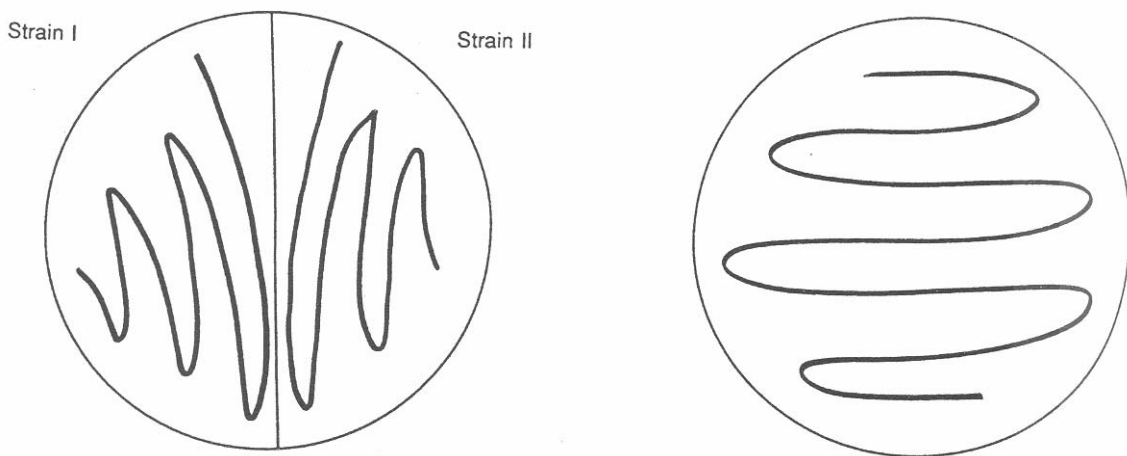
□ The auxotrophs will be plated by themselves during the lab to serve as a reference so that you can observe color development of these strains as they grow by themselves. Because color changes over time, it is important to compare your plates with the reference plates.

□ All group members should have experience in setting up feeding trial plates. To do this:

1. Draw a line bisecting the center of each plate and perpendicular to the date at the bottom. The narrow end of the streak marks should be at the bottom above the date. See Figure 2.5 for an example.
2. Have the two stock plates you will be using nearby.
3. Flame the inoculating loop in the bunsen burner flame.
4. Lift the lid of the stock plate and cool the loop by pressing it against the sterile lid for a few seconds. Be sure to just tilt the lid up and keep it over the plate.
5. Scrape the loop over the surface of the bacteria to gather some up, only a very small amount is needed.
6. Quickly transfer the inoculum to the feeding trial plate, you can use Figure 2.5 as a template.
7. Lift the lid only enough to insert the loop and carefully move the loop according to the template to form one-half of the “V” pattern on the trial plate.
8. Re-flame the loop and transfer the other auxotroph strain to form the other half of the “V” streak pattern following the above steps, *be certain you don't overlap the bottom of the “V” — it should be close but not touching*.

*Note:* You will not be able to see the inoculum you have plated. When plating bacteria, a little goes a long way.

9. Continue with the procedure until all 10 feeding trial plates have been streaked.



**Figure 2.5.** Streak patterns for pair-wise “feeding” trials (*left*) and the color reference plates (*right*).

### Student Worksheet

Name:

Group ID:

- Complete Table 2.3 using the results from your cross-feeding experiment. Use a plus sign (+) to indicate prodigiosin was formed and a minus sign (-) to indicate it was not.

**Table 2.3.** Results from cross-feeding.

Donor	Recipient				
	A	B	C	D	E
A	—				
B		—			
C			—		
D				—	
E					—

- In Table 2.4 fill in the important features of the five auxotroph strains in the prodigiosin pathway of *Serratia marcescens*.

**Table 2.4.** Features of the five auxotrophic strains.

Auxotroph strain	Substances secreted due to block	Recipients	Donors
A			
B			
C			
D			
E			

- Use the results from Table 2.4 and the rules of the explanatory system to construct a pathway for prodigiosin synthesis. Provide a diagram of the pathway.

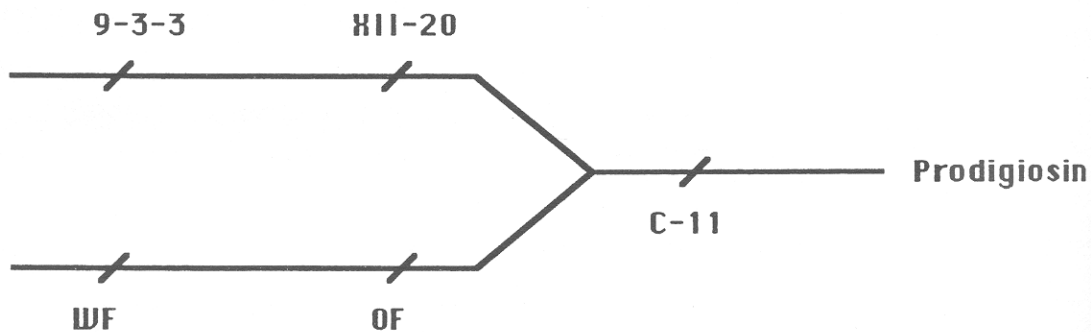
### Notes for the Instructor

Students will invariably have trouble interpreting results, especially with pair-wise combinations involving the OF strain because of its orange color and slow development. This is why I strongly suggest running this for yourself before presenting it to your students, pay attention to timing of color development, strength of color, and interpreting of the results relative to the actual pathway. You may notice that some pair-wise combinations involve color formation only along the bottom of the “V”, this is a result of intermediate products that are soluble in the medium, such as those secreted by strains WF and OF. When color appears all along the leading edge of the bacteria, this is a result of secretion of volatile intermediate products, such as those secreted by XII-20 and 9-3-3. This is why it is important for students to store plates right-side-up.

This exercise can be simplified by giving students strains mutant for enzymes on only one branch of the pathway, for example, 9-3-3, XII-20, and C-11 (Figure 2.6). The results indicate a straight, simple path and are easily interpreted by students in beginning biology classes.

Remember, the mutant strains are deficient for *enzymes* that control the pathway, this should be clear in the students' diagrams. Don't let students put letters indicating the strains in the pathway itself.

Prodigiosin is a secondary metabolite which is constructed from several amino acids that may accumulate in the cell as a result of primary metabolism. Proline is incorporated intact in the prodigiosin molecule, histidine is used indirectly, methionine contributes a methyl group, and alanine is entirely incorporated except for a carboxyl group. It is thought the formation of prodigiosin allows the cell to remove toxic accumulation of metabolites such as these amino acids.



**Figure 2.6.** The branched pathway for biosynthesis of prodigiosin showing the locations of each mutant strain.

### Acknowledgements

I would like to thank Bill Wischusen for introducing this lab to UNLV, Bob Geever (UNLV) for many hours of assistance in experimental work with mutant *Serratia marcescens*, and Jon C. Glase (Cornell University) for a copy of his lab on *S. marcescens*.

### Further Reading

- Allen, M. K. 1980. The elucidation of a biochemical pathway. Pages 145–153, *in* Tested studies for laboratory teaching, Volume 1 (J. C. Glase, Editor). Proceedings of the First Workshop/Conference of the Association for Biology Laboratory Education (ABLE), Kendall/Hunt Publishing Co., Dubuque, Iowa, 272 pages.
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