Conjugation Lab Report

Introduction

Bacteria are unicellular, haploid organisms that are suited for experiments because they reproduce rapidly by binary fission, produce genetically identical progeny, and are genetically simple. Normal bacteria can grow on minimal media, media containing only water, a carbon source, and inorganic salts, but some mutant auxotrophic bacteria have specific nutritional requirements. These requirements can be used to select for or against specific strains. Some bacteria can transfer a copy of accessory DNA to another by conjugation. During conjugation, cells containing a sex factor, F^+, unidirectionally transfer a copy of this sex factor to cells lacking the sex factor, F^-, through a conjugation canal. Because the conjugation canal is easily broken, the entire F factor rarely transfers, and so genes closer to the origin of the F factor (the first point to enter the F^- cells) will appear with greater frequency among the resulting progeny. Once the F factor has been transferred the new genes will be expressed if they are dominant, and recombination can occur between the new genes and the DNA of the recipient cell. This phenomenon can be used to map bacterial genes. The frequency with which a particular gene appears in exconjugant progeny depends on its distance from the origin, and the number of recombinant progeny can be used to determine relative map distance between genes. In this experiment we conjugated F^+ and F^- cells with different genotypes and plated the resulting exconjugants onto different selective media plates. Our purpose was to determine the order of the genes involved with the selected traits relative to the point of origin, and to determine relative map distances between genes if possible.

Results

From the media 1 replica plates, we counted a total of 144 colonies, with the most on media 1, followed by media 5, media 2, media 3, and media 4 (with zero colonies). From the media 2 replica plates, we counted a total of 137 colonies, with the most on media 2, followed by media 5, media 3, media 1, and media 4 (with zero colonies). The tabulated results are in Tables 1 and 2.

Discussion

Plate 1 lacked threonine-leucine and selected for TL^+, plate 2 lacked proline and selected for pro^+, plate 3 lacked glucose and contained lactose and selected for lac^+, plate 4 lacked glucose and contained galactose and selected for gal^+, and plate lacked methionine and selected for met^+. Plate 3 selected for lac^+ and plate 4 selected for gal^+ because each of those plates selected for bacteria that could utilize lactose and galactose as the sole carbon source, respectively. Among the media 1 replica plates, colonies 1, 2, 4, 10, 20, 23, and 36 did not grow on the media 1 plate, and therefore could not be taken into account for any of the other plates since we could not tell if these colonies on other plates did not grow because of the specific media they were on or because they were plated improperly (Table 3). Among the media 2 replica plates, colonies 1, 6, 34, and 35 did not grow on the media 2 plate, and therefore could not be taken into account for the other plates (Table 4). With these adjustments, the total number of colonies on media 2 replicate plates was 134 and the total number of colonies on media 1 replicate plates was 130. More colonies grew overall on media 2 replica plates than media 1 replica plates, indicating that more colonies received the pro^+ gene to survive on the pro^- media than received the TL^+ gene to

survive on the TL^- media, and that the pro gene must then come before the TL gene on the F factor. To determine the order of the other genes with respect to each of these markers, for each set we ranked the plates in order of most colonies grown due to received DNA. For plates 1, 2, 3, and 4, this was the number of colonies grown, but for plate 5 this was actually the number of colonies that did not grow since the original F^- bacteria were met^+ (Tables 3 and 4). So, F^- bacteria grew on plate 5, but exconjugant bacteria that received the met^- gene did not. For the media 1 replica plates, the colonies grew best on media 1, followed by media 2, media 3, media 5, and media 4. Based on these results, the gene map is: TL, pro, lac, met, and gal (Fig. 1). For the media 2 replica plates, the colonies grew best on media 2, followed by media 3, media 1, media 5, and media 4. Based on these results, the gene map is: pro, lac, TL, met, and gal (Fig. 2). The map based on media 2 plates is better because we know from above that the pro gene is closer to the origin than the TL gene. Since the two maps are inconsistent, we have to modify map 2 to take into account map 1. Based on the example in lecture notes, the modified map is lac, pro, TL, met, and gal (Fig. 3). On this map, the pro and lac genes are closer to the origin than the TL gene, so we can use the TL^+ data to determine the distances between pro-TL and TL-lac (but not with gal or met since you can only determine map distances between markers and genes that enter the F^- cell before them). Based on the TL^+ data, pro and TL are 37.2 map units apart and lac and TL are 60.5 map units apart. From this we infer that lac and pro are 23.3 map units apart. However, looking at colonies selected for lac^+ out of the pro^+ colonies, the calculated map distance is 23.9 map units. Since calculating small distances is more accurate, we conclude that the map distance between lac and TL is 61.1 map units (Fig. 3). Once again, we cannot calculate any distances involving met or gal since they are farther from the origin than both of the markers we selected for. The slight discrepancy in the results could be due to scoring

error, plating error, or other random error we have no control over (such as bacteria that does not grow as well as it should or bacteria that spontaneously mutate to grow when it should not). To compensate for the scoring error, we could dilute our bacteria by another factor of 10, but this would probably make the resulting data pool too small. A better alternative would be to repeat the experiment several times and then calculate the genetic distances using a large pool of data.

Conclusion

We studied conjugation in bacteria and the gradient of transmission method to map five genes on an F factor. We determined that with respect to the point of origin, the genes are in the order: lac, pro, TL, met, and gal, and that the relative map distance between lac and pro is 23.9 map units, between lac and TL is 61.1 map units, and between pro and TL is 37.2 map units.

Appendix A – Figures

Figure 1 – Gene Map 1 (based on media 1 replicate plates)

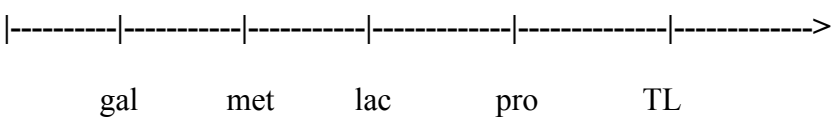


Figure 2 – Gene Map 2 (based on media 2 replicate plates)

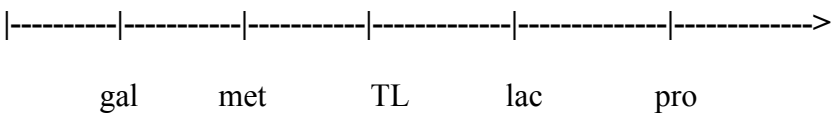
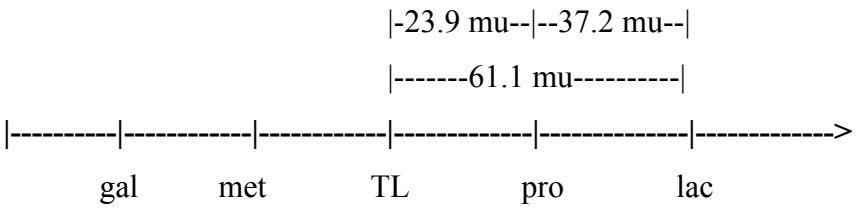


Figure 3 – Composite Gene Map



Appendix B – Tables

Table 1 – Compiled Media 1 Plates Raw Data

	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Pro$^+$	x	x	x		x		x	x	x		x		x	x	x	x		x			x		x	x	
lac$^+$		x	x	x	x			x	x	x	x		x	x	x	x					x	x	x	x	
gal$^+$																									
met$^+$		x	x	x	x		x	x			x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
TL$^+$			x		x	x	x	x	x		x	x	x	x	x	x	x	x	x		x	x		x	x

	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50
pro$^+$		x	x	x	x			x	x			x	x			x		x	x	x	x		x		
lac$^+$		x		x		x	x	x	x			x	x						x	x	x	x	x		
gal$^+$																									
met$^+$	x	x	x	x	x	x	x	x		x	x	x			x	x	x		x			x	x	x	x
TL$^+$	x	x	x	x	x	x	x	x	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x

Table 2 – Compiled Media 2 Plates Raw Data

	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
TL^+				x					x		x		x			x								x	
lac^+		x	x	x			x	x	x	x		x	x	x	x	x			x	x	x	x	x	x	
gal^+																									
met^+		X	x	x	x		x	x			x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
pro^+		x	x	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x

	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50
TL^+	x				x	x		x	x	x			x	x				x			x				
lac^+		x	x	x	x	x	x			x	x	x	x	x	x		x	x	x		x				x
gal^+																									
met^+	x	x	x	x	x	x	x	x		x	x	x			x	x	x		x			x	x	x	x
pro^+	x	x	x	x	x	x	x	x		x	x	x	x	x	x	x	x	x	x		x	x	x	x	x

Table 3 – Adjusted Media 1 Plates Data (unbolded, italicized columns were not used in calculations)

	01	*02*	**03**	*04*	**05**	**06**	**07**	**08**	**09**	*10*	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	*20*	**21**	**22**	*23*	**24**	**25**
pro$^+$	*x*	*x*	x		x		x	x	x		x		x	x	x	x		x			x		*x*	x	
lac$^+$		*x*	x	*x*	x			x	x	*x*	x		x	x	x	x					x	x	*x*	x	
gal$^+$																									
met$^-$						x			x																
TL$^+$			x		x	x	x	x	x		x	x	x	x	x	x	x	x	x		x	x		x	x

	26	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**	*36*	**37**	**38**	**39**	**40**	**41**	**42**	**43**	**44**	**45**	**46**	**47**	**48**	**49**	**50**
pro$^+$		x	x	x	x			x	x			x	x			x		x	x	x	x		x		
lac$^+$		x		x		x	x	x	x			x	x						x	x	x	x	x		
gal$^+$																									
met$^-$									x				x	x				x		x	x				
TL$^+$	x	x	x	x	x	x	x	x	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x

Table 4 – Adjusted Media 2 Plates Data (unbolded, italicized columns were not used in calculations)

	01	**02**	**03**	**04**	**05**	*06*	**07**	**08**	**09**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**
TL$^+$				x					x		x		x			x								x	
lac$^+$		x	x	x			x	x	x	x		x	x	x	x	x			x	x	x	x	x	x	
gal$^+$																									
met$^-$	*x*					*x*			x	x															
pro$^+$		x	x	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x

	26	**27**	**28**	**29**	**30**	**31**	**32**	**33**	*34*	**35**	**36**	**37**	**38**	**39**	**40**	**41**	**42**	**43**	**44**	*45*	**46**	**47**	**48**	**49**	**50**
TL$^+$	x				x	x		x	*x*	x			x	x				x			x				
lac$^+$		x	x	x	x	x	x			x	x	x	x	x	x		x	x	x		x				x
gal$^+$																									
met$^-$									*x*				x	x				x		*x*	x				
pro$^+$	x	x	x	x	x	x	x	x		x	x	x	x	x	x	x	x	x	x		x	x	x	x	x