Protein Profiles of Bacteria Under Short Term and Long Term Exposure To Environmental Stress

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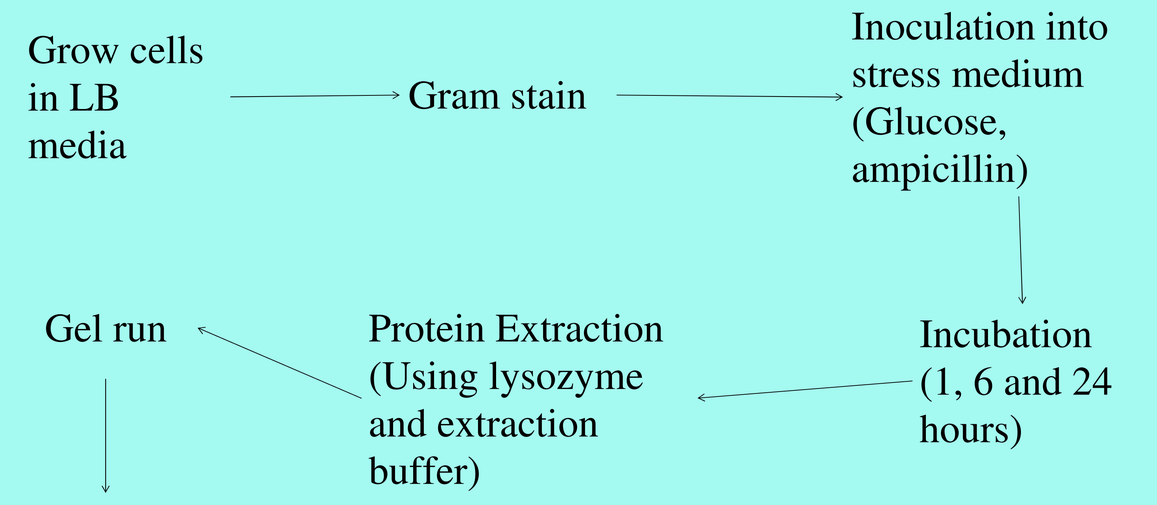
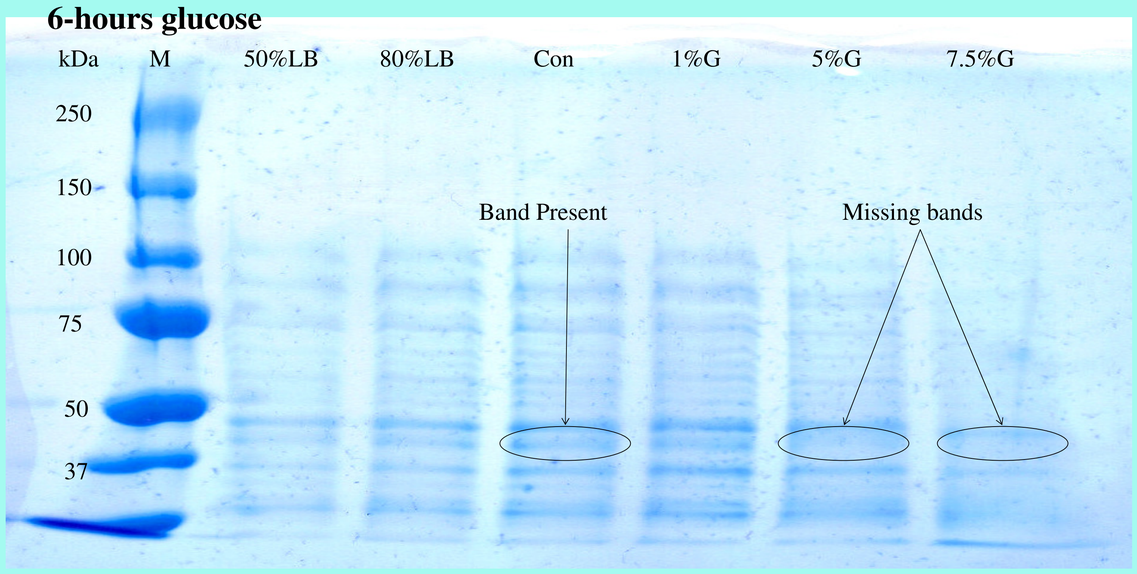
Abstract: Bacteria regulate their protein expression differently when exposed to environmental stress. The main objective of this project is to study the environmental stress (glucose and ampiciillin) on *Serratia marcescens* by observing their protein expression with the use of common detection methods like Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE). Cells were cultivated on Luria-broth agar plates and transferred to nutrient broth solution and stressed for 1-hour, 6-hours and 24-hours. The profiles of the proteins were obtained using SDS-PAGE. Restriction and excess of glucose on *S.marcescens* cells were studied and SDS-PAGE showed missing bands of sizes between 50kDa to75kDa when *S.marcescens* were stressed at different incubation times. Cell growth increased when the cells were incubated with higher concentrations of glucose. There was also a significant increased in protein concentration with increased glucose concentration and longer incubation time. When exposed to ampicillin at 0.1mg/mL, a pink pigment was observed. Exposure to high glucose concentration (as high as 75mg/mL) showed some missing protein bands in SDS-PAGE. The missing protein bands were of sizes 42kDa to 60kDa. We hypothesized a few possible identities of these missing proteins by searching *Escherichia coli* protein database.

Flow Diagram Illustrating the Main Process of the Experiment

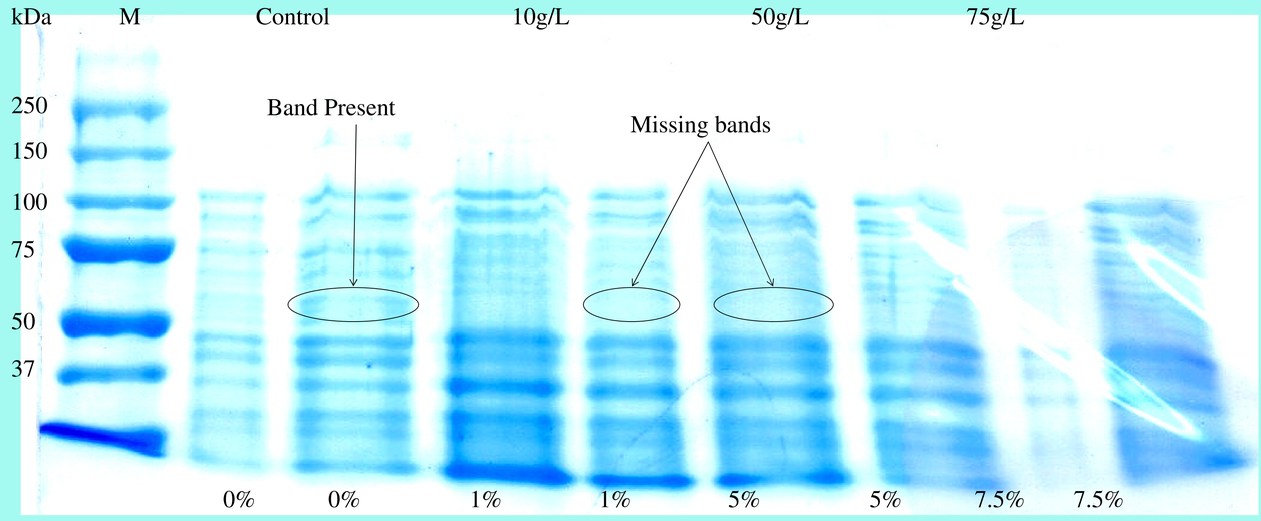


6-hours glucose



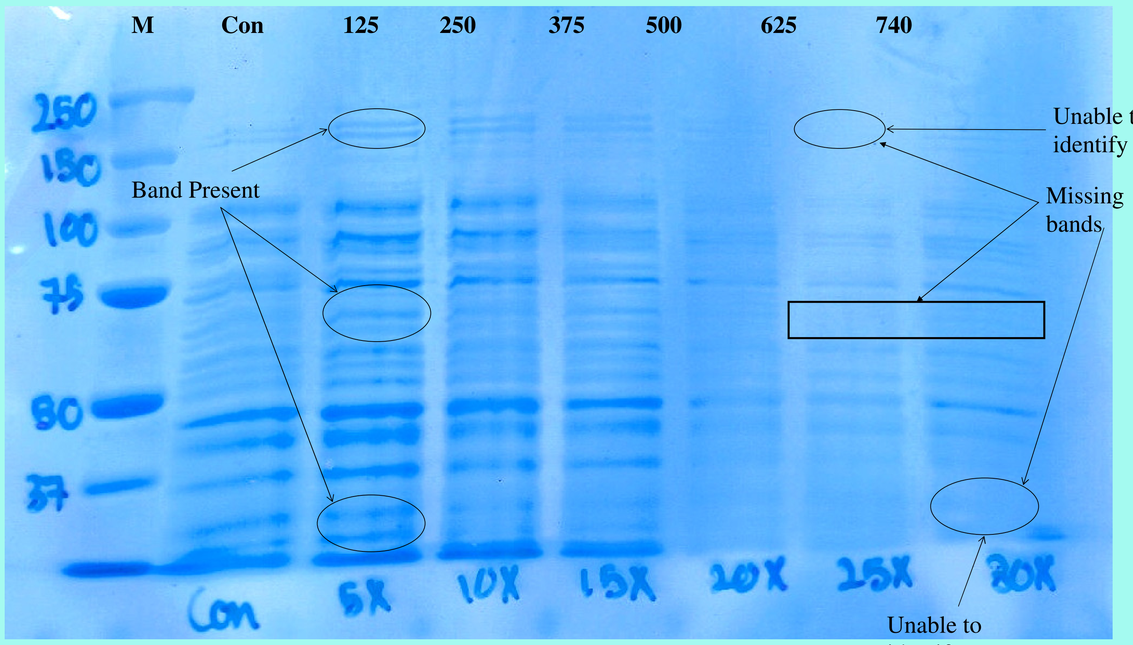
Protein bands are absent at high glucose concentration (5% glucose, 7.5% glucose)
Possible identities of the 2 proteins based on *Escherichia coli* protein profile are MalE (43kDa), AceA (42kDa) and Ydcs (42kDa)
MalE (Maltose binding periplasmic protein), AceA (Isocitrate Lyase and Ydcs (Putative ABC transporter periplasmic binding protein)

24-hours glucose



Proteins bands are absent at high concentrations (1% glucose, 5% glucose)
Possible identities of the 2 proteins based on *Escherichia coli* protein profile are AtpA (55 kDa) and DppA (60 kDa)
AtpA (ATP synthase Alpha Chain) and DppA (Periplasmic Dipeptide Transport Protein)

24-hour ampicllin stress



Protein bands are absent in high ampicillin concentrations (625µ/mL and 740µg/mL)
Identity of protein band missing near 60kDa region was hypothesized to be DppA (60kDa)
Identity of protein bands near 150kDa and 20kDa region could not be determined from *E. coli* protein database

Table of cell concentration for glucose stress

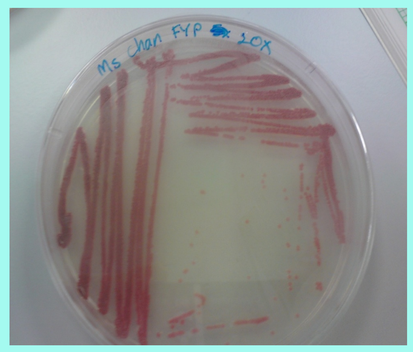
	Cell Concentration (x 10^8cells/mL)	
Stress conditions	**6 hours stress**	**24 hours stress**
50% LB	2.46	5.13
80% LB	3.35	6.77
Control	3.82 (C)	6.98 (C)
1% glucose	**6.53 (1.7C)**	**13.9 (2C)**
5% glucose	3.85	13.5
7.5% glucose	3.94	5.86

1% glucose is ideal for *S.marcescens* growth

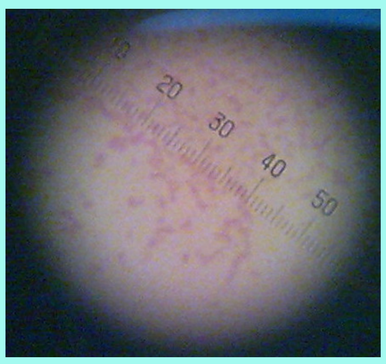
Table of cell concentration for ampicillin and glucose stress

	Cell Concentration (x 10^8 cells/mL)	
Stress Conditions	6 hour	24 hour
Control	3.99 (C)	11.86 (C)
5X	4.27 (1.07C)	6.54 (0.55C)
10X	4.69 (1.17C)	6.76 (0.57C)
15X	3.96 (0.99C)	6.23 (0.53C)
20X	3.83 (0.95C)	6.21 (0.52C)
25X	4.02 (1.00C)	6.89 (0.58C)
30X	4.06 (1.01C)	6.96 (0.58C)

Addition of 7.5% glucose to ampicillin stress reduced the effect of ampicillin on *S.marcescens*



S. marcescens grown on LB agar



S. marcescens under microscopic view

Conclusion

1) Ampicillin is detrimental to *S. marcescens* growth.
2) Under stressful conditions, *S. marcescens* has been found to down regulate many genes.
3) Virulence of *Serratia* might be linked to the glucose level of different individuals such as respiratory and urinary tract infection.
4) *S. marcescens* is able to grow well in high glucose concentration (10mg/mL).

Future Work

1) Use high and low concentration gels to get a complete profile of *S. marcescens.*
2) Use another β-lactam antibiotics like amoxicillin.
3) Carry out N-terminal sequencing for cell membrane proteins on *S. marcescens.*
4) Use silver staining and mass spectrometry to identify missing protein bands.
5) Use Isoelectric Focusing (IEF) for more accurate separation of proeins.

References

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