Journal of Experimental Microbiology and Immunology (JEMI) Vol. 11:54-59

Effects of streptomycin and kanamycin on the production of capsular polysaccharides in *Escherichia coli* B23 cells

STEPHANIE GANAL, CATHERINE GAUDIN, KERSTIN ROENSCH, MARINA TRAN
Department of Microbiology and Immunology, UBC

Aminoglycosides are a class of antibiotics that inhibit protein synthesis in bacteria by binding to the 30S ribosomal subunit. However, it has been reported that certain aminoglycosides have secondary effects on the cell's overall carbohydrate concentration. Previous studies have also reported an increase in capsule size. This study attempted to investigate the effects of kanamycin and streptomycin on carbohydrate production, particularly the sugars found in the capsular polysaccharides. *Escherichia coli* B23 was incubated in the presence of kanamycin and streptomycin. It was found that the concentration of glucuronic acid in the capsule increased in both the kanamycin and streptomycin treated cells, which correlated with the increase in the concentration of carbohydrate in the capsule and in the cell. However, a decrease in the amount of capsular 6-deoxyhexose was observed in streptomycin treated cells, correlating with a decrease in the overall concentration of 6-deoxyhexose.

Capsular polysaccharides are found in all members of the Enterobacteriaceae family of bacteria, and have important roles in interactions between *Escherichia coli* strains and their environments. Most wild-type *E. coli* strains synthesize extensive capsules composed of lipopolysaccharide-associated O antigens and capsular K antigens that encapsulate the entire cell and are firmly attached to the outer membrane; such capsules are well-known virulence factors and in enteropathogenic strains they can mediate adhesion to the host gastrointestinal tract cells (12). However, in the laboratory strain K12, O and K antigens are no longer produced; instead, it synthesizes a capsule composed mainly of colanic acid (4). Colanic acid has repeating units of glucose, galactose, fucose and glucuronic acid, in a molar ratio of 1:2:2:1, respectively (10). It has also been shown in many previous studies that the amount of colanic acid, as well as its composition, can be influenced by external cues from the environment, such as osmotic stress and the presence of beta-lactam antibiotics (8, 9).

Streptomycin and kanamycin are antibiotic drugs of the aminoglycoside family, which are known to inhibit protein synthesis by blocking the translocation of peptidyl-tRNAs during translation. Previous studies have confirmed this effect and have shown a decrease in the overall protein concentration after treatment of *E. coli* B23 cells (a subtype of K12) with aminoglycosides (2). In contrast, carbohydrate concentrations in whole cell lysates were observed to be higher post-treatment (2). It was determined that primarily hexose sugars, a prominent component of the capsular polysaccharides, were increasing in these cells (2). Therefore, the hypothesis that treatment with aminoglycosides causes an increase in the *E. coli* K12 capsule was put forward. Investigations were carried out by isolating the capsular polysaccharides and conducting assays for the four sugars that were expected to be found in colanic acid: glucose, galactose, fucose and glucuronic acid.

MATERIALS AND METHODS

Conditions setup. *Escherichia coli B23* was obtained from the MICB 421 laboratory stock in the Microbiology and Immunology Department of the University of British Columbia. An overnight culture of *E. coli* B23 was prepared by inoculating *E. coli* B23 in 51 ml of M9 minimal medium (8.4 mM NaCl, 48 mM Na_2HPO_4, 22 mM KH_2PO_4, 18 mM NH_4Cl, 0.80 mM $MgSO_4 \cdot 7H_2O$, and 0.4% (w/v) glycerol) and placed in a shaking water bath at 37°C. Twenty millilitres of the overnight culture was used to inoculate 478.8 ml of M9 minimal medium. The newly inoculated culture was grown to 0.8 OD_{460} (5h) in a shaking water bath at 37°C. Kanamycin monosulfate (Sigma S6501 – 50G) and streptomycin sulfate (Sigma K-4000) were each mixed with distilled water to give a final concentration of 20 mg/ml and then filter-sterilized. The five-hour culture was split such that three 130 ml samples of the incubated culture were placed in three separate flasks. One hundred thirty microlitres of the kanamycin solution was added to one flask to create a 20 μg/ml kanamycin condition. One hundred thirty microlitres of the streptomycin solution was added to another flask to create a 20 μg/ml streptomycin condition. The third flask acted as a control. The three flasks were incubated for 1 h in a shaking water bath at 37°C in order to see the effects of the antibiotics during exponential growth. Turbidity measurements were taken at the initial inoculation of the culture, before the 5 h incubation and after the 5 h incubation using a spectrophotometer (Spectronic 20) at OD_{460}.

Preparation of cell lysate for the modified Park-Hancock protocol. Thirty millilitres of each of the flasks were placed in three separate centrifuge tubes and centrifuged for 10 min at 7000 rpm at 15°C. The supernatants were discarded and the pellets were resuspended in 2 ml of distilled water. The samples were again centrifuged for 10 min at 7000 rpm. The supernatants were discarded and the pellets were resuspended in 2.5 ml of chilled 25% (w/v) trichloroacetic acid (TCA). The suspensions were allowed to sit in an ice bath for 10 min and then centrifuged at 7000 rpm for 10 min at 0°C. The pellets were resuspended in 2.5 ml of 75% (v/v) ethanol

and allowed to sit at room temperature for 10 min. They were frozen for a day before the modified Park-Hancock protocol (7) was performed on them.

Modified Park-Hancock protocol. This procedure was followed as described (7) to fractionate the three samples into proteins and carbohydrates. The lysates were thawed and centrifuged at 7000 rpm at 15°C for 10 min. The supernatants were removed and the pellets were resuspended in 2.5 ml of 5% (w/v) TCA solution. The suspensions were placed in a boiling water bath for 5 min and then cooled to room temperature. They were then centrifuged for 10 min at 7000 rpm at 15°C. The supernatants were discarded and the pellets were resuspended in one milliliter of distilled water.

Capsule Isolation. Isolation of capsular polysaccharides was carried out according to a protocol by Ghiorse et al. (5). The culture samples were centrifuged at 17,000 x g for 20 min. The resulting supernatant was discarded and the pellet was resuspended in PBS to ¼ of its original volume, then blended in a Waring blender for 5min at maximum speed. Exopolymers were precipitated by adding two volumes of ice-cold acetone. The suspension was centrifuged at 6000 x g for 10 min. The pellet was resuspended in 10 ml of distilled deionized water and dialysed against 1L of distilled deionized water at 4°C for 48h. Spectra/Por^R Molecularporous membrane dialysis tubes with a molecular weight cut-off of 6,000-8,000 kDa were used and the litre of dialysis water was changed twice a day. The exopolymers were dried overnight using a lyophilizer. The crude exopolymers were dissolved in 20 ml of sterile deionized autoclaved water containing 10 mM $MgCl_2$. Deoxyribonuclease I (DNaseI) was added to a final concentration of 5µg/ml and ribonuclease A (RNase A) was added to a final concentration of 0.1 mg/ml. After an incubation period of 5 h at 37°C, pronase was added to a final concentration of 0.1 mg/ml. The mixture was incubated at 37°C for 24 h. It was heated to 80°C for 30 min and centrifuged at 17000 x g for 20 min. The supernatant was dialysed and dried in a lyophilizer as before. The partially purified dry exopolysaccharides were dissolved in 0.05 M Tris base containing 0.1 M NaCl. Sodium deoxycholate was added to a final concentration equal to 0.75 critical micellar concentration (approximately 1.5 mM). The mixture was incubated at 65°C for 15 min and cooled on ice to room temperature, then acetic acid (20%) was added to a final concentration of 1%. Lipopolysaccharide (LPS) and deoxycholate were pelleted by centrifugation at 16,000 x g for 5 min. The supernatant containing the purified capsule was stored at 4°C until usage.

Anthrone Carbohydrate Assay (13). The samples were dissolved in distilled H_2O up to a final volume of 0.5 ml then were put into glass test tubes. One millilitre of 4% (w/v) phenol was added to the samples. Two and one-half millilitres of concentrated sulfuric acid was quickly put on top of the liquid surface. The tubes were mixed immediately. After a 30 min incubation time, the absorbance was scanned from 460 nm to 520 nm in a spectrophotometer. The control sample and kanamycin-treated sample had extremely similar absorption spectra, with a peak at 490 nm; however, the streptomycin-treated sample had a slightly different absorption spectrum, with a peak at 485 nm. It was decided that 490 nm was the optimal wavelength for analysis. Glucose standards ranging from 0 µg/ml to 100 µg/ml were treated in the same way and the absorbance was measured at 490 nm in order to generate a standard curve to determine sample concentrations at 490 nm.

Carbazole Glucuronic Acid Assay (1). The sodium tetraborahydrate solution was made by dissolving 573 mg of sodium tetraborahydrate in 60 ml of concentrated sulfuric acid. A 0.125% (w/w) carbazole solution was made by dissolving 0.125 g of carbazole in 10 g of ethanol. Three millilitres of sodium tetraborahydrate solution was placed in each test tube and tubes were cooled to 4°C. Half a millilitre of the prepared glucuronic acid standard solutions (0, 10, 20, 40, 60, 80, 100 µg/ml) was layered over the sodium tetraborahydrate. The closed tubes were shaken gently at first and then vigorously shaken with constant cooling. Tubes were heated for 10 min in a boiling water bath then cooled to room temperature. One-tenth of a millilitre of the carbazole solution was then added to the tubes and the tubes were shaken again. The test tubes were heated in a boiling water bath once more for 15 min then cooled down to room temperature. Absorption of the samples was measured at 530 nm against blanks of distilled water.

Preparation of glucose standards and diluent/blank. A solution of 12 mg/ml glucose (the glucose standard) in 0.05 M Tris + 0.1 M NaCl (to mimic the final conditions of capsule isolation samples) was made as well as the buffer solution without carbohydrate (the diluent/blank). Sodium deoxycholate was added to each solution to a final concentration of 0.75 CMC (1.5 mM) and the mixtures were incubated at 65°C for 15 min, then cooled on ice. Solutions were then centrifuged at 16,000 x g for 5 min and the supernatant (normally containing the capsule) was removed to a new tube. After acid hydrolysis a series of glucose standards (0, 25, 50, 100, 200 and 300 ug/ml) were created by diluting the 12 mg/ml glucose solution with the diluent/blank.

Acid hydrolysis. Samples (including diluent/blank and glucose standard) were treated with concentrated 18 M H_2SO_4 to a final concentration of 1 M and heated in a 100°C water bath for 2 h. Samples were then cooled to room temperature and treated with 2.2 M NaOH to neutralize the H_2SO_4 then distilled water was added to give a final volume of 10 ml. The diluted samples were then used for glucose determination.

Glucose assay (6). Concentration of glucose in samples was determined enzymatically using the GAHK20 Glucose (HK) assay kit (Sigma-Aldrich). Ten microlitres of each sample and 100 ul of Assay Reagent were combined and allowed to sit at room temperature for 15 min, then the A_{340} was read using a Beckman-Coulter DU530 spectrophotometer zeroed with the diluent/blank.

6-deoxyhexose assay. This procedure was followed as described (3) and was used to measure the fucose concentration in the different samples of the cell lysate and the isolated capsule. Standards were made from 0, 1, 2, 4, 6, 8, 10 µl of 2 µg/ml fucose solution. Two hundred microlitres of each of the standards and samples were cooled in an ice bath. Nine hundred microlitres of reagent A (4 ml dH_2O and 24 ml concentrated H_2SO_4) was added to each of the samples and allowed to warm to room temperature. The samples were boiled in a hot water bath for 5 min and then cooled to room temperature in a cold water bath. Twenty microlitres of freshly made reagent B (0.3 g of cysteine HCl in 10 ml dH_2O) were added to each of the samples. They were shaken and then allowed to stand for three hours in the dark at room temperature. The samples were assayed at 396 nm and 427 nm using a spectrophotometer. The difference between the two absorbance readings was correlated to the difference in the standards to estimate the 6-deoxyhexose concentration.

RESULTS

Carbohydrate Assay. The total carbohydrate concentration increased in antibiotic treated cells. Total carbohydrate measured in the anthrone assay was about 10% higher in kanamycin treated cells and increased by about 41% in streptomycin treated cells compared to the control.

This increase in carbohydrates was also seen in the isolated capsule of the *E. coli* B23 culture. The capsule of both the kanamycin- and streptomycin-treated bacteria had a higher concentration of carbohydrates than the capsule of the control. The control sample contained approximately 0.75 µg/ml of carbohydrates, whereas the carbohydrate concentration in the antibiotic-treated samples lay around 1.0 µg/ml. The increase was similar under both treatment conditions,

with 30 to 40% more total carbohydrate in antibiotic-treated cultures. Thus, the percentage increase in the total carbohydrates and in the carbohydrates in the capsule lay in the same range (~40%).

Glucuronic Acid Assay. The concentration of glucuronic acid in the total carbohydrate isolates increased 2.6-fold from 0.8 µg/ml in the control to 2.0 µg/ml in the kanamycin-treated sample; however, a significant decrease in glucuronic acid concentration was measured in the cells incubated in the presence of streptomycin.

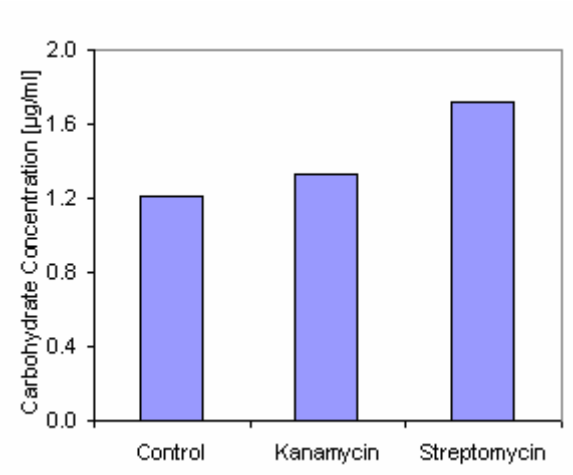

Fig. 1: Total Carbohydrate Concentration in *E. coli* B23 cells. Among the different samples, "Control" refers to the absence of treatment with aminoglycosides. "Kanamycin" and "Streptomycin" samples were grown in the presence of 20 µg/ml of the corresponding aminoglycoside. Cells were grown to log phase in minimal M9 medium at 37°C, treated with corresponding antibiotic and incubated for another hour before the total carbohydrates were isolated in a cell fractionation. Carbohydrate concentrations were determined using the anthrone assay.

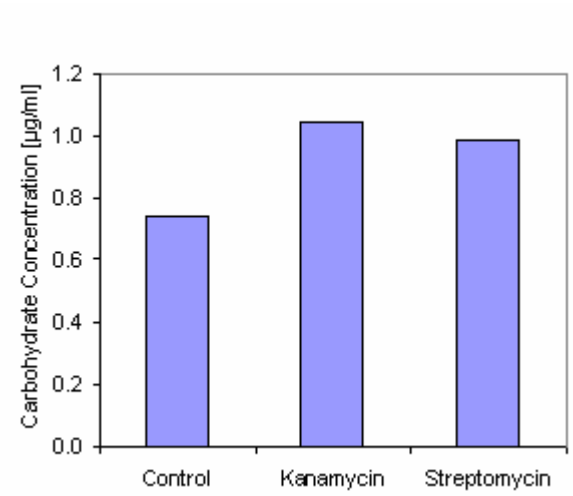

Fig. 2: Total Carbohydrate Concentration in *E. coli* B23 capsule. Among the different samples, "Control" refers to the absence of treatment with aminoglycosides. "Kanamycin" and "Streptomycin" samples were grown in the presence of 20 µg/ml of the corresponding aminoglycoside. Cells were grown to log phase in minimal M9 medium at 37°C, treated with corresponding antibiotic and incubated for another hour prior to the isolation of capsule polysaccharides. Carbohydrate concentrations were determined using the anthrone assay.

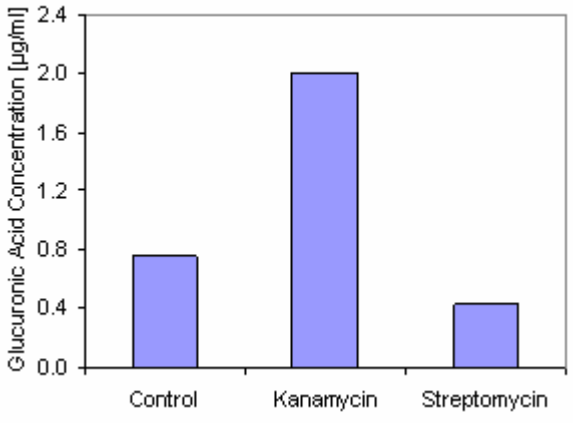

Fig. 3: Glucuronic Acid Concentration in *E. coli* B23 cells. Among the different samples, "Control" refers to the absence of treatment with aminoglycosides. "Kanamycin" and "Streptomycin" samples were grown in the presence of 20 µg/ml of the corresponding aminoglycoside. Cells were grown to log phase in minimal M9 medium at 37°C, treated with corresponding antibiotic and incubated for another hour before the total carbohydrates were isolated in a cell fractionation. Glucuronic acid concentrations were determined using the carbazole method.

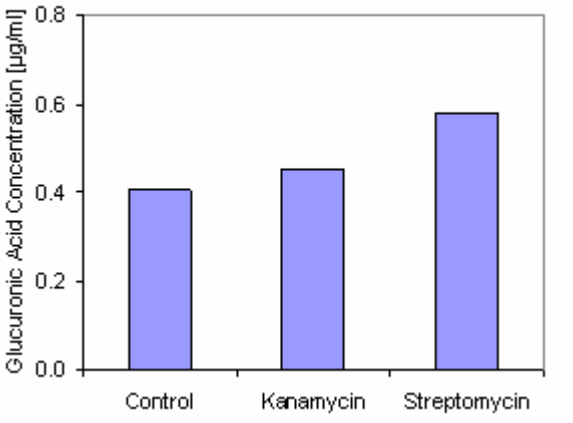

Fig. 4: Glucuronic Acid Concentration in *E. coli* B23 capsule. Among the different samples, "Control" refers to the absence of treatment with aminoglycosides. "Kanamycin" and "Streptomycin" samples were grown in the presence of 20 µg/ml of the corresponding aminoglycoside. Cells were grown to log phase in minimal M9 medium at 37°C, treated with corresponding antibiotic and incubated for another hour prior to the isolation of capsule polysaccharides. Glucuronic acid concentrations were determined using the carbazole method.

Within the isolated capsule a different pattern was observed. In total, an increase of glucuronic acid was detected in the two samples treated with the aminoglycosides. The kanamycin-treated sample revealed a slight increase in glucuronic acid (12% above control concentration). Incubation with streptomycin increased the amount of glucuronic acid by 42%, from 0.40 µg/ml to 0.57 µg/ml.

6-Deoxyhexose Assay. When total carbohydrate was isolated and the concentration of 6-deoxyhexose was measured, it was observed that the highest concentration was within the untreated control. The kanamycin-treated cells showed a decrease in 6-deoxyhexose concentration by 23%, whereas the streptomycin treatment reduced the concentration by 90%.

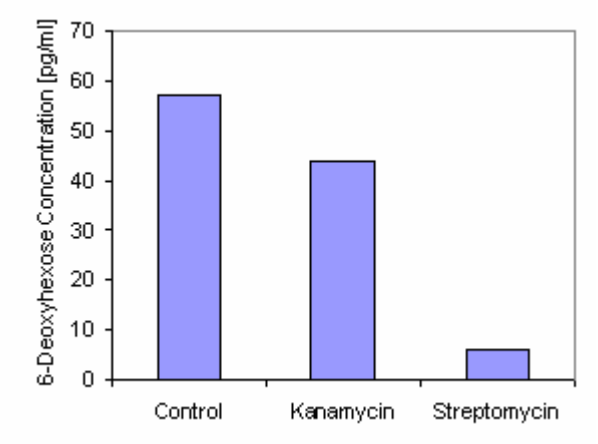

Fig. 5: 6-Deoxyhexose Concentration in *E. coli* B23 cells. Among the different samples, "Control" refers to the absence of treatment with aminoglycosides. "Kanamycin" and "Streptomycin" samples were grown in the presence of 20 µg/ml of the corresponding aminoglycoside. Cells were grown to log phase in minimal M9 medium at 37°C, treated with corresponding antibiotic and incubated for another hour before the total carbohydrates were isolated in a cell fractionation. 6-Deoxyhexose concentrations were determined using a photometric assay.

The 6-deoxyhexose concentrations in the capsule isolates were distinct from those observed in the total carbohydrate samples. The concentration of 6-deoxyhexose was unaltered in case of kanamycin treatment. Uniquely, the streptomycin sample showed a decrease of approximately 50% compared to the control sample.

Enzymatic Glucose Assay. Results from the glucose assay were unsatisfactory and may not be viewed as having any significance. Both the original isolated samples of total carbohydrates and capsule exopolymers as well as the samples treated with acid hydrolysis gave extremely low readings in the spectrophotometer although the standard glucose solutions gave optimal measurements; furthermore, a complete lack of glucose in the control samples as well as the treated samples was the conclusion of the enzyme assay. The sample readings lay outside the range of significant values of the spectrophotometer. Although the pH of the neutralized samples was not checked to ensure that it was in a suitable range for the enzymatic reaction (~pH 7), it can be assumed that an acidic pH was not the reason for the lack of results, since the glucose standards underwent the exact same procedure of acid hydrolysis and neutralization, and gave optimal results in the spectrophotometer assay.

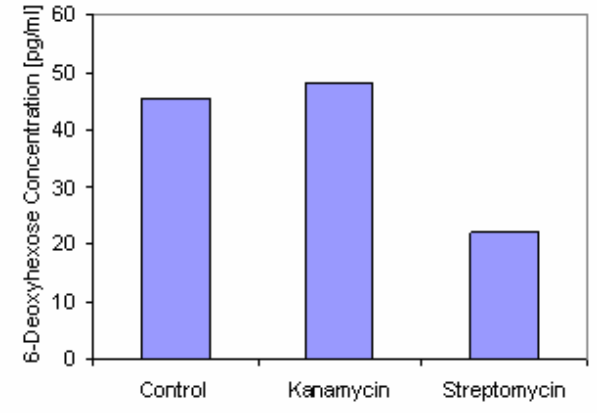

Fig. 6: 6-Deoxyhexose Concentration in *E. coli* B23 capsule. Among the different samples, "Control" refers to the absence of treatment with aminoglycosides. "Kanamycin" and "Streptomycin" samples were grown in the presence of 20 µg/ml of the corresponding aminoglycoside. Cells were grown to log phase in minimal M9 medium at 37°C, treated with corresponding antibiotic and incubated for another hour prior to the isolation of capsule polysaccharides. 6-Deoxyhexose concentrations were determined using a photometric assay.

DISCUSSION

The initial results (Fig.1) supported the observation that the production of carbohydrates increased in cells growing in the presence of aminoglycosides compared to cells growing in untreated minimal M9 medium (2). Additionally, we were able to isolate the bacterial capsule and measure the amount of carbohydrates present in this compartment (Fig.2). According to anthrone assay protocol, the absorbance of the samples was scanned from 460 nm to 520 nm, in order to compare their maxima of absorbance; slightly different maxima are an indication that different sugars dominate in the different samples. This appeared to be the case, since the absorption spectrum of the streptomycin-treated sample differed somewhat from the absorption

spectra of the control and kanamycin-treated samples. Despite this slight discrepancy, in the capsule a higher amount of total carbohydrates was observed after treatment with aminoglycosides. Thus, our main hypothesis that the production of colanic acid increased in presence of the antibiotics kanamycin and streptomycin is confirmed.

Since it is known that the bacterial capsule of *E. coli* K12 strain consists of the sugars fucose, glucose, galactose and glucuronic acid in the molar ratio 2:1:2:1 (11), further sugar assays for specific carbohydrates were performed. We used the carbazole method to test for glucuronic acid, the 6-deoxyhexose-assay to examine the amount of fucose and performed an enzymatic glucose test. No suitable test for galactose was available. However, the enzymatic glucose test did not lead to useful results. No glucose could be measured within the spectrophotometric analysis. The readings were too low, thus they lay out of the significant range of the spectrophotometer. It is most likely that the glucose concentration was diluted too highly during acid hydrolysis and therefore glucose was undetectable; if glucose is assumed to occur in a 1:1 ratio with glucuronic acid in the capsule, and the glucuronic acid concentration was observed to be 0.4-0.6 µg/ml in the capsule, then mathematically the glucose concentration of the samples subjected to spectrophotometry were far below the optimal range of 0.5-50 µg of total glucose stated as necessary for proper enzymatic analysis in the GAHK-20 Glucose Assay Kit product information. Another possibility is that the acid hydrolysis did not work out; therefore, the capsule sugars were still present as enzymatically non-detectable polymers instead of monomers. A complete absence of glucose is possible but unlikely as glucose is an important component of both the whole cell and the capsule.

With the carbazole method, we determined the amount of glucuronic acid in the cell as well as in the capsule. In general, an increase in the glucuronic acid concentration was observed in the cells growing in the presence of the investigated aminoglycosides (Fig.3 and Fig.4). However, the decrease in the concentration of glucuronic acid observed in the total carbohydrates in the sample treated with streptomycin does not support this hypothesis. As the concentration of glucuronic acid was higher in the capsule than in the total carbohydrates in the sample (0.58µg/ml compared to 0.43µg/ml), it is likely that some glucuronic acid may have been lost during preparation. The value for the streptomycin sample in the total carbohydrates was therefore considered inaccurate (Fig.3). Although there was an increase in the amount of glucuronic acid in the bacterial capsule, the increase in the capsule was lower than the increase in the total cell. This was inconsistent with our expectation that the production of exopolymers is the main reaction to antibiotic stress. However, it is possible that the cell accumulates glucuronic acid to high intracellular concentrations in the presence of aminoglycosides, then secretes it after a time to become part of the modified capsule. Looking for an increase in capsular glucuronic acid at later timepoints could prove or disprove this hypothesis. A longer incubation time would have been required to investigate this issue.

As already described in the literature (2), the addition of kanamycin and streptomycin lowered the concentration of fucose in the cell (Fig.5). We were able to confirm this observation in the bacterial capsule as well (Fig.6). As fucose is a very important component of colanic acid, these results did not support the hypothesis that production of capsular carbohydrates is the response to aminoglycoside treatment. However, it was reported that the composition of colanic acid varies depending on the environment (10). The presence of oxidizing agents, for example, results in an almost complete loss of fucose within the colanic acid (10). This observation shows that an alteration of the composition of bacterial capsule is, in principle, possible. Thus, we suggest that aminoglycosides cannot only increase the amount of exopolymers but also their composition. Aminoglycosides seem to be one of the stimuli that induce these changes. Similar alterations were observed as a response to dessication (12), alkali-treatment (10) or exposure to other antibiotics (8). This alteration seems to be regulated by a certain gene cluster, the so-called *cps* genes. Colanic acid is produced by enzymes encoded by the *cps* genes and the colanic acid biosynthesis genes are part of an extensive regulon responding to environmental changes (4).

Thus, our experiments showed that an increased or decreased expression of certain sugars in the capsule as a measure of cellular self-protection is a likely response of bacterial cells to aminoglycoside treatment. This observation serves to highlight the importance of proper antibiotic administration during the course of treatment, since it is clear from this study that sub-lethal doses of antibiotics actually improve *E. coli*'s ability to defend itself from these antibiotics.

FUTURE EXPERIMENTS

Galactose is so far the only sugar of the colanic acid capsule which was not investigated separately. Thus, it would be useful to perform a galactose assay.

As previous publications proved that the expression of exopolymers is controlled by a gene cluster called *cps* (4), it would be interesting to measure the level of gene expression after treatment with antibiotics

compared to the level of gene expression in the control sample. To complete this experiment, not only the amount of mRNA should be measured (a Northern blot would be sufficient to compare levels of *cps* gene cluster mRNA present under different conditions), but also the half-life of the mRNA in the cells should be investigated, for example by isolating the RNA at regular time intervals from cells treated with a transcriptional inhibitor such as actinomycin D.

ACKNOWLEDGEMENTS

We would like to thank Dr. Bill Ramey for donating generous amounts of his time, support and guidance in the course of this experiment, as well as Jennifer Sibley for her warm bench-side manner and patience, without which we might have succumbed to panic and stress. Lastly, we wish to humbly thank Lando Robillo for his assistance with the lyophilizer, and all the staff of the media room for their tireless efforts to provide us with the necessary materials.

REFERENCES

1. **Bitter, T. and M. Muir**. 1962. A modified uronic acid carbazole reaction. Anal. Biochem, **4**: 330-334.
2. **Chung, C., G. Hung, C. Lam, and L. Madera.** 2006. Secondary effects of streptomycin and kanamycin on macromolecular composition of *Escherichia coli* B23 cell. J. Exp. Microbiol. Immunol. **9**: 11-15.
3. **Ferro-Luzzi, G. A.** 1997, posting date. Carbohydrate assay. University of California, Berkley. [Online.] http://www.bio.com/protocolstools/protocol.jhtml.
4. **Gottesman, S., and V. Stout.** 1991. Regulation of capsular polysaccharide synthesis in *Escherichia coli* K12. Mol. Microbiol. **5**: 1599-1606.
5. **Kachlany, S. C., S. B. Levery, J. S. Kim, B. L. Reuhs, L. W. Lion and W. C. Ghiorse.** 2001. Structure and carbohydrate analysis of the exopolysaccharide capsule of Pseudomonas putida G7. J. Environ. Microbiol. **3**: 774-784.
6. **Kunst, A., Draeger, B. and Ziegenhorn, J.** 1984. Methods of Enzymatic Analysis. 3rd ed. 163-172.
7. **Park, J. T., and R. Hancock.** 1960. A fractionation procedure for studies of the synthesis of cell-wall mucopeptide and of other polymers in cells of *Staphylococcus aureus.* J. Gen. Microbiol. **22**: 249-258.
8. **Sailer, F. C., B. M. Meberg, and K. D. Young.** 2003. ß-Lactam induction of colanic acid in gene expression in *Escherichia coli*. FEMS Microbiol. Lett. **226**: 245-249.
9. **Sledjeski, D.D., and S. Gottesman.** 1996. Osmotic shock induction of capsule synthesis in *Escherichia coli* K-12. J. Bacteriol. **178**:1204-1206.
10. **Sutherland, I. W.** 1969. Strucutural studies on colanic acid, the common exopolysaccharide found in Enterobacteriaceae, by partial acid hydrolysis. Oligosaccharides from colanic acid. Biochem. J. **115**: 935-945.
11. **Sutherland, I.W.** 1971. Enzymic hydrolysis of colonic acid. Eur. J. Biochem. **23**: 582-587.
12. **Whitfield, C.** 2006. Biosynthesis and assembly of capsular polysaccharides in Escherichia coli. Annu. Rev. Biochem. **75**: 39-68.
13. **Wright, B. G., and P. A. Rebers.** 1972. Procedure for determining heptose and hexose in lipopolysaccharides. Modification of the cysteine-sulfuric acid method. Anal Biochem. **49**: 307-319.