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Intra-strain Variation of Lipopolysaccharides from *Citrobacter freundii* E9750 by *in vitro* Passages

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The genus *Citrobacter* is closely related to *Salmonella* and *Escherichia coli*, and there are some serological cross-reactions existing among these species. These cross-reactivities are caused by O-specific polysaccharide antigens in the LPS, which contain similar epitopes. In some cross-reactive strains, O-specific polysaccharides exhibit structural homology. In order to determinate the frequency of intra-strain variation of lipopolysaccharides (LPS) from *Citrobacter freundii* E9750 by *in vitro* passages at two different growth temperatures, isolates obtained from *Citrobacter freundii* E9750 *in vitro* passages at 37 and 42°C were analyzed for carbohydrate concentration, reactions to serological tests, and heterogeneity of lipopolysaccharides using SDS-PAGE. The genetic relatedness of the isolates was investigated using the PCR-RFLP profile of *rfb* and Pulsed Field Gel Electrophoresis (PFGE) profiles. Variation in expression of LPS was observed as a change in the concentration of carbohydrates. In addition, low reactivity with *C. freundii* E9750 O-antiserum, serological cross reactivity with *S. senftenberg* O-antiserum, and heterogeneity in electrophoretic mobility, were found. The PFGE and RFLP patterns of all isolates were indistinguishable from each other, including those that presented a serological reaction with the O -antigen sera from *S. senftenberg*. *C. freundii* E9750 NCTC isolated strains showed a high frequency in LPS variability. Analysis of the results suggested that heterogeneity was due to changes in the structure of the LPS carbohydrates. Since lipopolysaccharides are important factors in virulence and determine the basis for serotyping, the high frequency of intra-strain O-antigen heterogeneity in *C. freundii* E9750 is important.

Key words: Lipopolysaccharide, *Citrobacter freundii,* intra-strain variation, serological cross-reaction, O-antigen, SDS-PAGE, RFLP.

INTRODUCTION

Citrobacter freundii is a member of the Enterobacteriaceae family, which is commonly found in the environment and in the intestinal tracts of animals, including humans. Although *C. freundii* is often considered a commensal bacteria of the human intestinal microbiota, this ubiquitous organism has been associated with noso-comial infections in the urinary, respiratory, and biliary tracts of compromised patients, and in patients suffering from diarrhea, gastritis, meningitis, brain abscesses, neonatal

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sepsis, and in some cases, although rare, in newborns with meningitis (Badger et al*.,* 1999; Gupta et al., 2003; Nada et al*.,* 2004).

Citrobacter infections were found to represent 0.8% of Gram -negative infections in a large surveillance study, while in hospital settings, *Citrobacter* spp. have accounted for 3–6% of all isolates of Entero-bacteriaceae. The mortality rate of hospitalized patients with *Citrobacter* infections has been observed to be 6.8%. This Figure increases to 18 – 56% for patients with *Citrobacter* bacteremia (Samonis et al., 2009).

In terms of culture and biochemical characteristics, certain strains of *Citrobacter* spp. resemble another enteric bacterium. The genus *Citrobacter* is closely related to *Salmonella* and *E. coli*, and serological cross-reactions exist between the species of these genera. Cross-reactivity is caused by O-specific polysaccharide antigens in the LPS, which contain similar epitopes; in some cross-reactive strains, O-specific polysaccharides exhibit structural homology (Nishiuchi et al., 2000; Samuel et al., 2004).

Lipopolysaccharide (LPS) is the major component of the outer membrane of Gram-negative bacteria and serological heterogeneity is defined by the diversity in the structure of LPS at the cell-surface. In "smooth-type" lipopolysaccharides, the LPS molecule is composed of two biosynthetic entities: the lipid A-core and the O-polysaccharide (O-antigen) with most of the heterogeneity in LPS molecules being found in the O-antigen (Lerouge and Vanderleyden, 2001).

Transition from smooth (S) form LPS to rough (R) form occurs occasionally. Rough isolates do not produce O-antigens as a result of mutations in one or more of the multiple genes controlling their syntheses and polymerization. Characteristically, genes coding enzymes involved in O -antigen synthesis are clustered in a region called the *rfb* cluster (Coimbra et al., 2000).

The O-chain, consisting of repetitive subunits of one to eight monosaccharides, is responsible for much of the immunospecificity of the bacterial cell (Samuel and Reeves, 2003; Caroff and Karibian, 2003). The chemical composition and structure of the O-antigen is strain-specific but can also vary within one bacterial strain (intrastrain LPS heterogeneity) (Lerouge and Vanderleyden, 2001). Moreover, the O-chains determine the specificity of each bacterial serotype, a kind of fingerprint for bacteria. Bacterial structures such as LPS that are selected for use in serotyping schemes must remain antigenically stable in order to provide reliable results and useful information.

Combination of monosaccharide diversity, the numerous possibilities of glycosidic linkage, substitution and configuration of sugars, and the genetic capacities of diverse organisms have all contributed to the uniqueness of the vast majority of O- chain structures. The resulting diversity of chain lengths on different LPS molecules in culture is responsible for the well-known "ladder-like pattern" of LPS molecules that are produced on SDS-electrophoretic gels (Palva et al., 1980). This procedure is especially useful for determining the number and distribution of O-antigen repeat units, which are displayed as a ladder of bands following silver staining. The *Salmonella typhimurium* DT104, provide a further example of the potential epidemiological application of the technique of LPS profiling (Lawson et al., 2002).

Understanding LPS structural variation is important because several studies have indicated that the composition or size of the O-antigen might be a reliable indicator of virulence potential, and that these important features often differ within the same bacterial strain (Lerouge and Vanderleyden, 2001). For instance, in *Salmonella enteritidis*, it was shown that virulent isolates obtained from chick spleens produce LPS with a larger O-chain: core ratios indicated a higher number of O-chain repeats in the LPS molecules, as compared with no-virulent isolates (Rahman et al., 1997).

In general, O- antigen modifications seem to play an important role at several stages of the infection process, including the colonization (adherence) step, and the ability to bypass or overcome host defense mechanisms (Lerouge and Vanderleyden, 2001).

While the structure of lipid A and core LPS are highly conserved, considerable heterogeneity may exist in the composition of the subunits forming long-chain LPS. This heterogeneity can be useful to discriminate between strains of bacteria within a given species. Within enterobacteriacae, this has been demonstrated amongst isolates of *Citrobacter freundii* and *Escherichia coli* strains belonging to serogroup O157 H7 (Chart et al., 1993a; Chart et al., 1993b).

The composition of most elements of the bacterial cell can be influenced by changes in environmental conditions during growth, for example, variations in the amount and composition of LPS (Korczynski and Wheat, 1970).

However, it is clear that the material isolated as LPS from Gram-negative bacteria varies markedly in composition depending upon the environmental conditions during growth and the treatment given to cells (example, heat) prior to LPS isolation. Therefore, it is becoming clear that changes in the growth environment can lead to variations in the composition of bacterial walls and isolated LPS (McDonald and Adams, 1971).

The aim of this study was to determine the frequency of intra- strain variation of lipopolysaccharides extracted from *Citrobacter freundii* E9750 by *in vitro* passages at two different growth temperatures.

MATERIALS AND METHODS

Strains and culture conditions

Citrobacter freundii NCTC E9750 was grown in tubes containing 10 ml of Luria broth (LB) for 24 h at 37ëC. Aliquots of 10 µl were transferred daily to a new tube under the same growth conditions. The test culture was grown under a temperatures of 37 and 42°C. The cultures were transferred every day for 558 day. The temperatures of 37 and 42°C were chosen for incubation in order to mimic

normal human body temperature and hyperpirexia respectively.

Serologic assays

The *Citrobacter freundii* E9750 antiserum was prepared by injecting in rabbits intravenously with boiled (100°C, 30 min) preparations of bacterial cells diluted in saline to McFarlands 4. Rabbits received 0.5 ml of antigen on day 1, 1.0 ml on days 5 and 10, and 2 ml on days 15 and 20. The rabbits were exanguinated 7 days later and the sera stored at -20°C. The antisera were used for agglutination testing. Titre determination was carried out after a serial 10-fold dilution, and plate agglutination. Agglutination tests were performed using isolated, viable *C*. *freundii*, S. *senftenberg* and *E*. *coli* 0157 H7.

Micro agglutination assay

Strains were checked for purity in blood and MacConkey agars following overnight incubation at 37°C. Smooth colonies were then taken from the blood agar culture, plated onto tryptone soy agar and incubated overnight at 37°C. The growth from each plate was harvested and diluted in 10 ml saline solution (0.15 M). The suspension was then incubated in a water bath at 100°C for 1 h. After this time, the suspension was adjusted with 0.6% formaldehyde in saline solution to a standard No. 3 of the MacFarland nephelometer scale to give a concentration of 9.0×10^8 bacteria/ml. For the agglutination assays, 50 µl of serial dilutions of the serum were dispensed into 96 well microtiter plates (Nunc, Denmark) containing 50 µl of the adjusted bacterial suspension and incubated overnight at 50°C.

Obtaining the LPS

Samples obtained from both cultures (100 µl) were streaked on Luria agar. After being left overnight, five single colonies from the culture at 37°C and five single colonies from the culture at 42°C were selected and grown separately in 10 ml LB for 4 to 6 h at 37 and 42°C respectively. The ten cultures were then streaked (100 µl) on Luria Agar (14 dishes per culture colony) and grown at their respective temperature for 18 -24 h. Bacterial growth from the 14 plates of each culture colony were collected with NaCl 0.85%, pooled together, and centrifuged at 5000 x g for 10 min.

The LPS of all cultured colonies were obtained using the Phenol-water method. (Westphal and Jann, 1965). Briefly, the phenol and aqueous layers were combined and dialyzed with distilled water for 48 h using a membrane with a cut-off at 6000 -8000 Da (Spectrum Laboratories). After dialysis, the product was freeze-dried and the LPS was resuspended in 5 ml of 25 mM Tris-HCl, pH 7.5, containing 20 µg/ml RNAse and 20 µg/ml DNAse (Sigma Chemical Co, ST Louis, Mo. USA). Following incubation at 37°C with gentle agitation for 3 h, proteinase K (Sigma) was added at a concentration of 20 µg/ml and incubation continued for a further 3 h (Siddon and Chapman, 1993). The product was dialyzed and freeze-dried once more and then stored at room temperature until further use. The concentration of carbohydrates was determined by the phenol-sulfuric acid method (Dubois et al., 1956).

SDS-PAGE of LPS

Due the tendency of LPS to aggregate, 0.5% SDS in polyacrylamide gel PAGE (Del Mar Tavío et al., 2000) was used to disperse the LPS aggregates, in order to obtain a better resolution of the LPS O-antigen in the high-molecular-weight bands.

The LPS were examined by electrophoresis according to Laemmli (1970) with some modifications. The SDS-gel was enhanced with a 4% (w/v) stacking gel and a 12% (w/v) separation gel with 4 M urea, and 0.5% SDS; 40 µg of each LPS was loaded in each lane. Electrophoresis was run at a constant 200 V for 1.5 h and low-Mr markers were used (Bio-Rad Laboratories, Richmond, CA). The band profiles were detected by silver staining (Tsai and Frasch, 1982) and counter-stained with Coomassie blue. LPS analysis based on SDS-PAGE was repeated three times for each isolate colony to ensure reproducibility of the results.

Obtaining high molecular weight DNA

DNA was obtained following a method originally developed by Schwartz and Cantor (1984) with modifications proposed by Coimbra et al. (1999, 2000). Briefly, 10 µl of the culture was transferred into 10 ml of LB broth and incubated for 4 – 6 h at 37 or 42°C depending on the origin. Cell concentration was estimated by measuring the optical density of the culture at a wavelength of 600 nm, approximately 2×10^9 bacteria. DNA was obtained and used as a template for the long PCR reaction.

Pulsed field gel electrophoresis profiles

Chromosomal DNA contained in agarose plugs was digested with 10 – 20 U of *Xba*I (Roche), and PFGE was performed using the CHEF DR II system (Bio-Rad) in 0.5 X Tris Borate EDTA buffer. DNA macrorestriction fragments *Xba*I digest isolate strains were resolved in 1% (w/v) agarose gels (Bio-Rad). Electrophoresis conditions were as follows: 5.4 V cm^{-1} for 44 h ramped at 6 to 72 s.

PCR-RFLP

The published oligonucleotides 482 and 412, complementary to JUMPstart and *gnd*, respectively, were used to amplify the O-antigen gene cluster of *C. freundii* E9750 in a long PCR reaction (Wang and Reeves, 1998). Long PCR was carried out with the Expand Long Template PCR system (Boerhinger Mannheim, Germany) using a 50 µl mixture reaction containing the following: 500 ng of DNA; 300 nM of each primer, 500 µM each of dNTPs, $MgCl_2$, 3 mM; and 2.6 U of Expand Long Template PCR System (Boerhinger, Mannheim, Germany). The PCR assay was performed in an iQ Cycler Q- Real Time PCR Detector System Bio Rad (Bio-Rad Laboratories, Hercules, CA, USA). The PCR program was repeated 10 times as follows: denaturation at 94°C for 10 s, annealing at 64°C for 30 s, and extension at 68°C for 15 min. For the next 20 cycles, the extension step was increased by 20 s per cycle. One initial denaturing step (94°C for 2 min) and one final elongation step (72°C for 7 min) were added. Amplification products were verified on 0.6% agarose gel in TBE buffer. PCR product sizes were estimated using the Lambda *HindIII* ladder, a commercial standard molecular weight marker (Promega, Madison, WI, USA).
Ten micro litres of PCR product were mixed with 10 U of *Mbo*II (Amersham Pharmacia Biotech, Buckinghamshire, UK) in a digestion buffer. After overnight incubation at 37°C, the tubes were heated at 72°C for 30 min (Giammanco et al., 1999). The RFLP were resolved in 6.5% polyacrylamide gels and 10% glycerol in TBE buffer [Standard molecular weight marker AmpliSize Molecular Ruler (Bio-Rad Laboratories, Hercules, CA, USA)]. Following electrophoresis, gels were stained with ethidium bromide. Gel images were electronically captured using a Windows Kodak Image Analysis software program version 3.5 (EDAS 290 system). *Hha*I, *Hind*II *and EcoR*I restriction patterns were obtained, and the RFLP were resolved according to the manufacturer's instructions.

Statistical analysis

For the analysis of LPS by electrophoretic profiles, "non-variation (A)" and "with variation (B, C, D, E y F)" dichotomous categories were established and the isolates proportion differences were determined with the findings of changes between two levels of temperatures (37 and 42°C) in ^{2} test. The concentration of LPS carbohydrates in each of the groups of the isolates and the control strain was compared by means of Kruskal-Wallis test, while the multiple comparisons were carried out using Bonferroni test.

All statistical analysis except Bonferroni test were carried out by JMP8 (SAS Institute, Inc.).

RESULTS

Over the duration of the study, 116 isolates were obtained from *Citrobacter freundii* E9750 from successive cultures grown *in vitro* at 37 and 42°C. In terms of growth rate, the proportion of growth was 1.28 times higher for the cultures at a temperature of 37°C than those at 42°C (Means of three assays).

The isolates were differentiated into six groups according to electrophoretic mobility of SDS-PAGE profile, and the presence of an immunoreactive O -chain: those that had an immunoreactive O-chain were classified as smooth isolates (A, B, C, D and E profiles); and those lacking an immunoreactive O-chain were determined to be rough isolates (F profile).

Serological reaction of LPS

Isolates from successive cultures were analyzed for serologic reaction with *Citrobacter freundii E9750*, *Salmonella* and *E. coli* antisera. The result of plate agglutination between isolates extracted from cultures grown at 37 and 42°C was statistically significant ($P < 0.05$).

The isolates showed a variation in agglutination reactions against the *C. freundii* E9750 O-antiserum, and approximately 34% (37°C) and 25% (42°C) of isolates showed no reaction.

Agglutination of boiled cultures of the isolates with C*. freundii* antiserum resulted in O titers between 1:20 to 1:6,400 that were significantly different from the C*. freundii* O reference strains, which showed agglutination titers up to 1:3,200.

Serological cross-reactivity was found in 8% of all isolates reacting with *Salmonella senftenberg* O-antiserum, which showed agglutinating titers between 1:100 and 1:3200. It is important to note that *C. freundii* reference strain did not react with *S. senftenberg* anti-O sera before the study, and none of the isolates reacted with *Escherichia coli* anti-O sera.

Determination of carbohydrate

There was a substantial variation in the carbohydrate content of the LPS's. The concentration of carbohydrate in the LPS of each isolate showed a decrease in the carbohydrates content (Kruskal Wallis rank sums test: $p = 0.0011$; Bonferroni's correction: $p < 0.05$) (Figure 5).

Intra-strain antigenic variations of *C. freundii* surface LPS

LPS of *C*. *freundii*, *E. coli* 0157 H7 and *S. senftenberg* 74210 were extracted using the hot phenol-water method. SDS-PAGE analysis of the LPS of these strains showed a characteristic ladder pattern.

The SDS-PAGE profile of the LPS from *C. freundii* E9750 was different from that obtained from *E. coli* 0157 H7 and *S. Senftenberg.*

The ladder-like pattern of the isolates produced one to six distinct LPS SDS-PAGE profiles designated as A (non-variation profile), B, C, D, E and F (variation profile). These profiles were generated according to similarities between the LPS profiles, with each profile showing a typical LPS "ladder" pattern. There were no profile variations between the three replicates of each isolate tested (Figure 1A and 1B).

Of the 116 isolates, 53 showed different electrophoretic mobility, with 34 of these being grown at 37°C and 19 at 42°C showed a statistically significant difference ($P = 0.0003$) (Table 1).

The genetic relatedness of the isolates

Restriction analysis for the *rfb* clusters from *C. freundii* E9750, *S. senftenberg* 74210 and *E. coli* O157 H7 showed differences at a genomic level (Figure 2). The PFGE patterns of all isolates were indistinguishable from each other (Figure 3). An amplified product in the *rfb* gene cluster of *C. freundii* of approximately 15 Kbp in size was obtained from all PCR assays.

Consistent RFLP profiles after *Mbo*II, *Hha*I*, Hind*III *and Eco*RI cleavage for all *C. freundii* E9750 isolates were obtained, even for those that presented a serological reaction with the anti-O serum from *S*. *senftenberg* (Figure 2).

DISCUSSION

The chemical and serological classifications of the genus *Citrobacter* are controversial (Brenner et al., 1999).

According to Knirel et al. (2002), immunochemical studies are needed to: (i) Elucidate the O-antigen structures in serogroups not yet studied, (ii) Reveal serological relationships at a molecular level between strains of different *Citrobacter* serogroups and different enterobacterial genera, and (iii) To find the position in the classification scheme of non-typed *Citrobacter* strains. Heterogeneity in the expression of LPS can provide a means for discrimination within a given species. Within

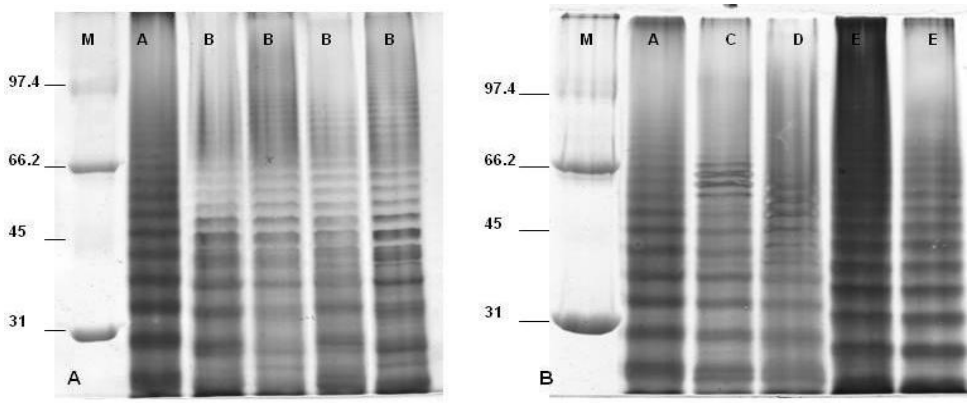

Figure 1. Silver-stained SDS-PAGE profiles of LPS: Profiles of individual colonies isolated from *C. freundii* E9750 NCTC *in vitro* passages. A) Isolates cultured at a temperature of 42°C. B**)** Isolates cultured at a temperature of 37°C. M = molecular weight marker.

Table 1. LPS profiles from *C. freundii* E9750 isolates obtained from *in vitro* passes at two culture temperatures.

Temperature	Profile LPS		Total (%)
	With variation** (%)	Non-variation* (%)	
37	34 (64)	19 (36)	53 (100)
42	19 (30)	44 (70)	63 (100)
Global	53 (46)	63 (54)	116 (100)

*Profile A, **Profiles B, C, D, E, and F. Chi-square test: p = 0.0003.

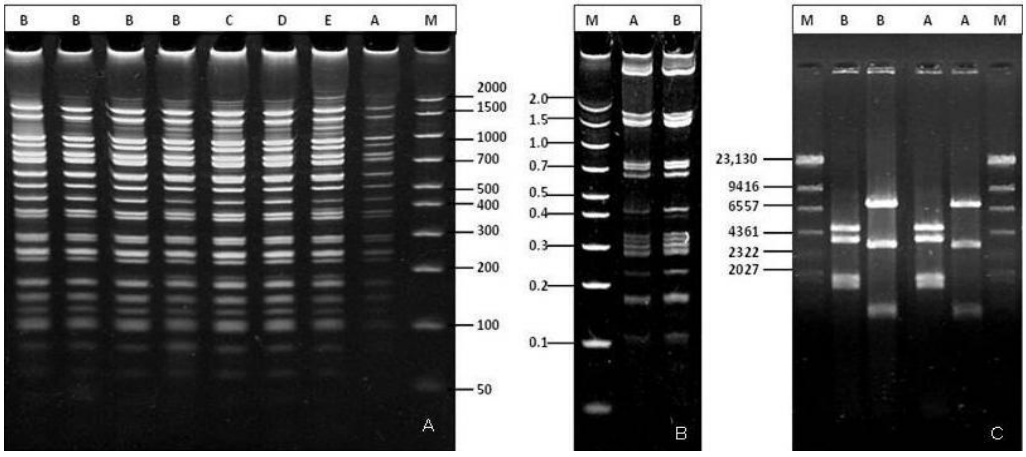

Figure 2. PCR - RFLP. O-antigen gene cluster of individual colonies isolated from *C. freundii* E9750 NCTC *in vitro* passages. A) Gene cluster *rfb* after *Mbo*II cleavage. B) Gene cluster *rfb* after *Hha*I cleavage C) Gene cluster *rfb* after *Hind*III and *Eco*RI, respectively. M = molecular weight marker.

Enterobacteriaceae, this has been demonstrated among isolates of *Citrobacter freundii* (Chart et al., 1993a).

In this current study, the reaction of *C. freundii* E9750 Oanti serum from isolates obtained during *in vitro* passages, showed heterogeneity, a high variability withits own O-anti serum, and a reaction to *Salmonella senftemberg* O-anti serum, suggesting variability in the composition or conformation of O-antigen epitopes. The heterogeneity of this serologic reaction against its own serum is related to a low concentration of LPS carbohydrates.

Environmental conditions during growth have been shown to affect the composition of the LPS wall of Gram negative Enterobacteriaceae. Previously, quantitative

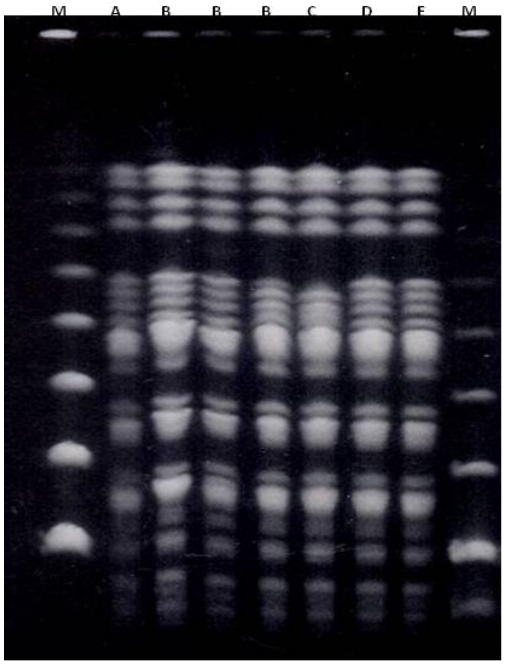

Figure 3. PFGE patterns obtained of different insolated from *C. freundii* E9750 *in vitro* passages. Bacterial DNA was digested with *Xba*I, M = molecular weight marker.

differences in the amounts of amino sugars and unknown reducing components were found in the LPS of *Citrobacter freundii* when batch cultures were grown in glucose or arabinose at 5 or 45°C (Korczynski and Wheat, 1970).

Growth conditions have been shown to influence the expression of O-antigenic LPS molecules. McGroarty and Rivera (1990) demonstrated a very marked decrease in the length of the O-specific LPS of *P. aeruginosa* cells grown under a number of stress conditions (pH, temperature, Mg and carbon starvation etc.).

Similarly, in enteric bacteria, temperature is a parameter that affects the size, distribution and abundance of various molecular types of LPS (Guard-Petter et al., 1995).

Results also showed a migration of LPS SDS-PAGE, which in this case is related to the number and size of repeating oligosaccharide units in the long-chain LPS, in that the profile band represents progressively larger concatemers of the repeating oligosaccharide units. Profile differences between isolates occur due to a variation between the repeating oligosaccharide units present in the LPS, therefore, as the LPS chain length increases, the difference becomes more noticeable and the “rungs” of the “ladder” profiles appear to be out of phase (Lawson et al., 2002).

In the present study, the heterogeneity of LPS was demonstrated by the migration of LPS in polyacrylamide gel with six distinct profiles being identified. The different profiles from *Citrobacter freundii* E9750 appear to be in phase but somewhat shifted (Figure 1).

However, tests of individual immunoblot assays for isolates over subsequent subcultures produced a stable LPS structure. Immunostaining clearly demonstrated differences in LPS mobility between the different isolates and confirmed that the same profiles were present in subcultures of the same isolated strain (Figure 4).

In addition, considering the polymorphism of the O-antigen gene cluster, it was predicted that different restriction patterns would be obtained for some isolates. However, clearly identifiable and reproducible patterns were obtained for each isolate following the *Mbo*II, *Hha*I, *Hind*II and *EcoR*I digestion of amplified products (Figure 2).

Therefore, PCR-RFLP of *rfb* and PFGE profiles were carried out to ensure that these isolates were not mixed populations; the results suggest homogeneous populations (s 2 and 3). All patterns of PCR-RFLP of *rfb*, and PFGE shared identical profiles, with clearly identifiable and reproducible RFLP O-patterns being obtained (Figure 2). Although the isolates appeared to have the same genetic origins, variation in the expression of LPS was observed.

Cross-reactions between O-serogroups made serotyping difficult; a method likely to fail when strains are rough or belong to an undescribed serotype (Coimbra et al., 2000). It is suggested that the *rfb*-RFLP in *Citrobacter freundii* provides the molecular method that most closely approaches the resolution of full serotyping to establish the identity of isolated strains (Figure 2).

The *rfb* restriction patterns (O-patterns) of *C. freundii* in this study suggest such a method to be feasible. There are no reports concerning the PCR-RFLP of *C. freundii rfb* clusters; further studies are needed to assess this method for typing *C. freundii.* The high frequency in the changes presented in the ladder profiles (SDS-PAGE) of LPS, the high variability of the agglutination test results, it’s the reactivity with the O-anti sera of *Salmonella senftenberg*, and the high variability of the test results with *C. freundii* O-antiserum for the isolates, all suggest the expression of different components of the O antigen.

The antigenic variation generated and the precise nature of each of the biochemical factors, which generates these different patterns, is unknown. As yet, no chemical analyses have been carried out on the antigens described in this study. Due to the fact that charides are important factors in virulence and also determine the bases for serotyping, the importance of the high frequen-cy of intra-strain O-antigen heterogeneity in *C. freundii* E9750 should be highlighted. Further studies are needed to assess the biological relevance of variations in O-antigen expression in *C. freundii* strains. Previous re-search with other organisms has indicated that size variability of LPS is attributable to changes in the structure of LPS carbohydrates.

Lipopolysaccharides, found in the outer membrane of Gram-negative bacteria, are a family of phosphorylated lipoglycans, which are generally considered to be toxic

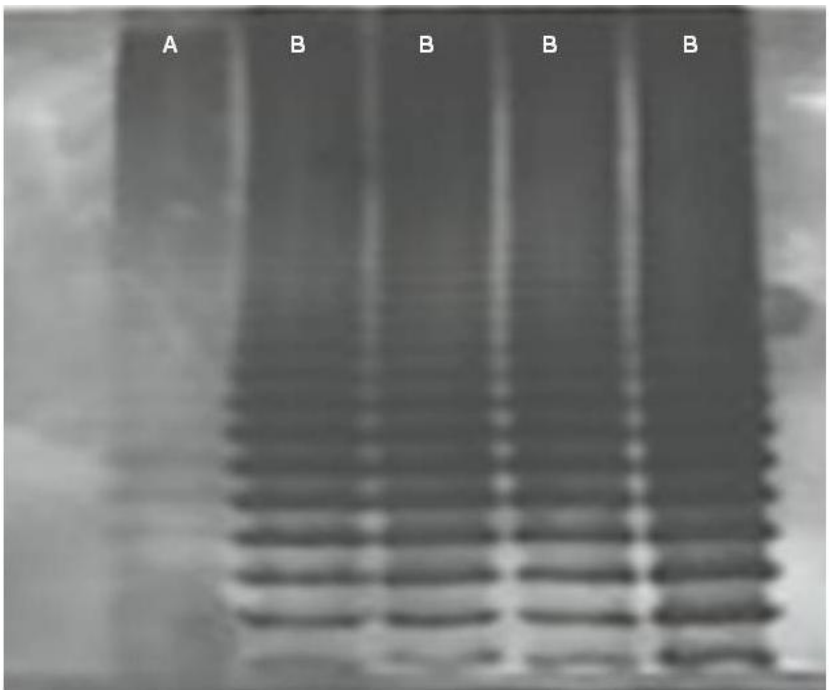

Figure 4. Immunoblot profiles of LPS. A: Refence strain. B: Profiles of individual colonies from *C. freundii* E9750 NCTC *in vitro* passages, reacted with O-antigen-specific polyclonal antiserum *S. senftenberg* 74210.

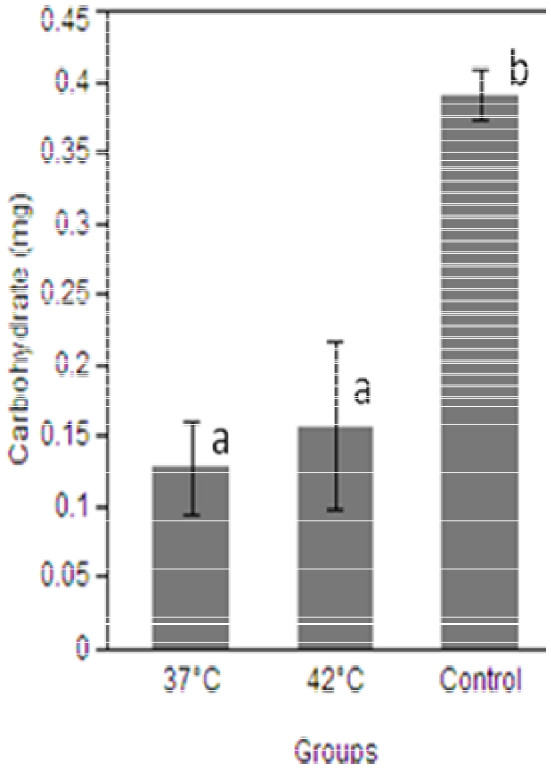

Figure 5. the carbohydrate content of the LPS's of isolated groups at two temperatures 37°C, 42°C. Control group= control strain. (Kruskal Wallis rank sums test: p=0.0011; Bonferroni correction: p<0.05).Groups not connected by same letter are significantly different at the level p<0.05

overlooks the important role played by LPS in the stability and functioning of the bacterial outer membrane, as well as their contribution to the interaction of the bacterial surface with the microbial niche of a given bacterial species.

The bacterial persistence of many colonizers of the gut, including commensal bacteria and pathogens, have developed mechanisms to vary LPS structure particularly those associated with the O-chain and lipid A components, which may prevent recognition by innate immune receptors. This underlines the importance of LPS variability as a surface structure and immune recognition component (Miller et al. 2005; Moran, 2007).

Therefore, knowledge and understanding of these changes is essential in studying the composition of bacterial walls or LPS. It also seems possible that control of changes in LPS composition might be useful in relating structure to antigenic response.

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