Comparison of *S. aureus* Proteomic Profiles from Biofilm and Planktonic Growth Conditions using 2D- Gel Electrophoresis.

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Abstract:

Bacteria growing as biofilms are distinct from the same bacteria growing as planktonic cells. Biofilms cells show increased resistance to antimicrobial, immunological, predatory, and chemical attack than planktonic cells. Most studies on bacterial diseases use planktonic bacteria. The objective of this study was to identify expressed proteins that are unique to Staphylococcus aureus biofilm mode of growth. S. aureus was grown in tryptic soy broth and Dulbecco's modified eagle medium at biofilm and planktonic growth conditions. Protein samples were cleaned up and separated according to their electrophoretic mobility using 7 cm IPG strips (pH 3–10 and pH 4–7) on 2D gel electrophoresis. Expressed proteins of both growth conditions were compared. Data analysis revealed that the expression of S. aureus proteins from planktonic and biofilm was higher in TSB media than DMEM media. Biofilm growth condition showed higher intensity of expressed proteins and new expressed proteins were observed. One protein was found to be upregulated in planktonic growth condition. Additionally, the majority of the proteins were clustered in the area of acidic region (pH 4–7). 2D-gel electrophoresis is a powerful and widely used method for the proteomic analysis. Biofilms represent a realistic representation of bacterial behavior and organisms are capable of altering their physiology in the surrounded environments. The results could help to illustrate the differences in pathogenesis between biofilm and planktonic cells in any model of disease. This will identify biological markers to improve the diagnostics, treatment, and prevention of S. aureus biofilms.

Keywords: *Antagonism, biological control, grapevine decline, fungi, Iraq.*

Introduction

S. aureus is one of the major pathogens of humans; it causes various suppurative diseases, food poisoning, pneumonia, and toxic shock syndrome (Foster, 2005). In most natural and clinical environments, microorganisms aggregate together on a surface or air/liquid interface and grow within communities where they encase themselves within extracellular polymeric substances composed of proteins, lipids, and polysaccharides (Flemming and Wingender, 2010). Such an existence is now defined as a biofilm (Stewart and Costerton, 2001, Branda *et al.,* 2005). Bacteria growing as biofilms are genetically and physiologically distinct from the same bacteria growing as free-swimming planktonic cells (Sauer *et al.,* 2002, Resch *et al.,* 2005). Bacteria growing as biofilms are significantly less susceptible to antibiotics and host cell defenses than are planktonic forms. Further advantages of biofilm growth include increased metabolic efficiency, substrate accessibility, enhanced resistance to environmental stress and inhibitors and an increased ability to cause infection and disease (Schierle *et al.,* 2009). Biofilm activities include the upregulation of virulence factors and secretion of extracellular polymers (Branda *et al.,* 2005). A study identified over 160 genes that were expressed at significantly higher levels under biofilm growth conditions, which included binding factors, polysaccharide intracellular adhesion (PIA), and peptidoglycan (Resch *et al.,* 2005).

The inability of chronic wounds to heal has now been associated with the presence of microbial biofilms (Gjodsbol *et al.,* 2006, James *et al.,* 2008). Shierle *et al*. (2009) demonstrated that presence of Staphylococcal biofilms delayed the reepithelialization process in a murine model. It was demonstrated that greater transcriptional activity in human epithelial keratinocytes (HEKa) cells exposed to biofilm conditioned media compared to HEKa cells exposed to planktonic conditioned media (Tankersley *et al.,* 2014). Recent evidence reveals that soluble products from *S. aureus* biofilms and soluble products from planktonic *S. aureus* differentially affect viability and inflammatory cytokine production by human keratinocytes and fibroblasts (Secor *et al.,* 2011, Kirker *et al.,* 2012). Unfortunately, most studies often use planktonic bacteria to study the host/pathogen interactions. The identification and characterization of *S. aureus* biofilm specific secreted proteins will allow elucidating the relationship between *S. aureus* biofilms and

different aspects such as inflammation and impairing wound healing at the molecular level. The goal of this study is to identify secreted proteins that are unique to *S. aureus* biofilm mode of growth by using two-dimensional gel electrophoresis to compare secreted protein profiles between biofilm secreted products (BSP) and planktonic secreted products (PSP). This will identify biological markers to improve the diagnostics, treatment, and prevention of *S. aureus* biofilms.

Material and methods

Bacteria and culture conditions.

Staphylococcus aureus ATCC 6538, (American Type Culture Collection) provided by Dr Robert E. Brennan, were incubated statically at 37^{o}C for 24 hrs in 5 ml of tryptic soy broth (TSB) and Dulbecco's modified eagle medium (DMEM). The biofilm and planktonic conditioned media were prepared as following:

A- Biofilm-conditioned Medium

Biofilm-conditioned medium (BCM) was produced based on previously described method (Tankersley *et al.,* 2014). Briefly, the 10 mm diameter tissue culture inserts (NalgeNunc International, Rochester, NY) were placed in a 24 well Nunc™ Cell-Culture plate (Thermo Scientific) and inoculated with 10 µl of overnight culture and 500 µl of TSB and inoculated at 37^{o}C for 72 hrs. Every 24 hrs during that 72 hrs period the TSB supernatant was removed, the inserts were moved to new wells in the 24 well plates, and 500 µl of fresh TSB was added to the wells. At the end of 72 hrs period the TSB and DMEM was removed and 500 µl of phosphate buffered saline pH 7.4 (PBS) was added and left for 1 hr to wash the remaining TSB or DMEM from the tissue culture insert. After the removal of the PBS, 500 µl of TSB or DMEM was added and incubated for 24 hrs at 37^{o}C. The new biofilm conditioned media (BCM) was then removed from the well and filtered with 0.45 µm syringe and collected in 15 ml centrifuge tubes. This BCM collecting and filtering procedure was repeated every 24 hrs for 3 days. The collected BCM (secreted protein) was then pooled and frozen at -20^{o}C until use.

B- Planktonic-conditioned Medium

Planktonic-conditioned medium (PCM) was prepared by using previously described methods (Tankersley *et al.,* 2014). An overnight culture was created by inoculating a colony of *S. aureus* into 5 ml of TSB or DMEM for 24 hours at 37^{o}C on a rotary shaker set at 150 rpm. After incubation the *S. aureus* culture was centrifuged for 7 minutes at 1500 rpm. The supernatant was then replaced with PBS, and pellet was re-suspended by thoroughly mixing with pipette. The *S. aureus* was then centrifuged for 7 minutes at 1500 rpm and the PBS was decanted. Five milliliters of TSB or DMEM was then added to the washed *S. aureus* culture and mixed thoroughly with pipette. The *S. aureus* in the TSB or DMEM was then incubated for 24 hours at 37^{o}C on a rotary shaker set at 150 rpm. After 24 hours the culture was centrifuged at 1500 rpm for 7 minutes and the supernatant (secreted protein) was decanted and filtered with a 0.45 µm syringe and stored at -20^{o}C until use.

Protein sample manipulation

In order to get of derbies, Secreted protein samples from planktonic and biofilm *S. aureus* were cleaned up using ReadyPrep™ 2-D Cleanup Kit (Bio-Rad, USA) according to the manufacturer's instructions. Protein (resuspend in 2-D dehydration sample buffer) concentration was determined using Nanodrop spectrophotometry (Thermo) by measuring the absorbance at 280 nm. 2-D dehydration sample buffer used as a blank.

Two-dimensional gel electrophoresis

Protein samples were separated according to their electrophoretic mobility using ReadyPrep™ 2-D Starter Kit according to the manufacturer's instructions (Bio-Rad, USA). This technique separate proteins in two steps, according to two independent properties: the first-dimension is isoelectric focusing (IEF), which separates proteins according to their isoelectric points (pI); the second-dimension is SDS-PAGE, which separates proteins according to their molecular weights (You and Wang, 2006, Matsumoto *et al.,* 2012). The process included three main steps.

A- Loading the protein samples on IPG strip (7 cm)

One hundred twenty five µl (100 µg) of the protein sample were loaded on rehydration/equilibration trays and then the 7 cm immobilized pH gradient (IPG) strips gel (pH 3–10 and pH 4–7) was placed onto the sample and the strips overlaid with 2 ml of mineral oil to prevent evaporation during the rehydration process. To rehydrate the IPG strips and load the protein sample, the rehydration/equilibration tray

was covered with the plastic lid and left on bench (room temperature) for 14 hrs then it was used directly or stored at -80°C. After incubation, the oil was removed (by vertically holding it for 5 sec).

B- Isoelectric focusing of the protein sample

Isoelectric focusing for the protein sample was performed using protean IEF cell (Bio-Rad). A paper wick was placed at both ends of the channels covering the wire electrodes of the PROTEAN IEF focusing tray and 8 µl of nanopure water was added onto each wick. The IPG strip was transferred to the channel in the focusing tray (the gel side down) and the strip was covered with 2 ml of mineral oil. The focusing tray was placed into the PROTEAN IEF cell and the electrophoresis performed using the appropriate 3-step protocol provided in the kit manual.

C- SDS-PAGE electrophoresis

After completion of the electrophoresis, the IPG strip removed from the focusing tray and washed with buffer I and buffer II (in each step the strip was washed for 10 min at room temperature with shaking). The IPG strips were dipped briefly into the 1× Tris/glycine/SDS running buffer and then IPG strips were run in the second dimension on 12.5% SDS-polyacrylamide gel electrophoresis (PAGE). SDS-PAGE electrophoresis was run using Mini-PROTEAN (Bio-Rad) in 200 V, constant condition for 40-50 min. Gel was washed for 2 min and stained with Brilliant Blue R-250.

Results

Visual observation of protein generated on 2D gel electrophoresis revealed that *S. aureus* protein spots were at higher level of expression on TSB media than DMEM media (Fig 1 A and B).

In general, the protein banding patterns generated by 2D gel maps of *S. aureus* cells were found to be similar. However, the observations revealed that certain spots appeared to have intensity differences. Visual analysis of the images indicated that there is a significant variation in the expression of proteins in the biofilm state had higher intensity than in the planktonic state. Furthermore, new expressed proteins (spots) were observed in *S. aureus* cells that were grown under biofilm conditions (Fig 1). Interestingly, one specific protein was observed to be upregulated in *S. aureus* cells that were grown under planktonic conditions on TSB media (Fig 1A).

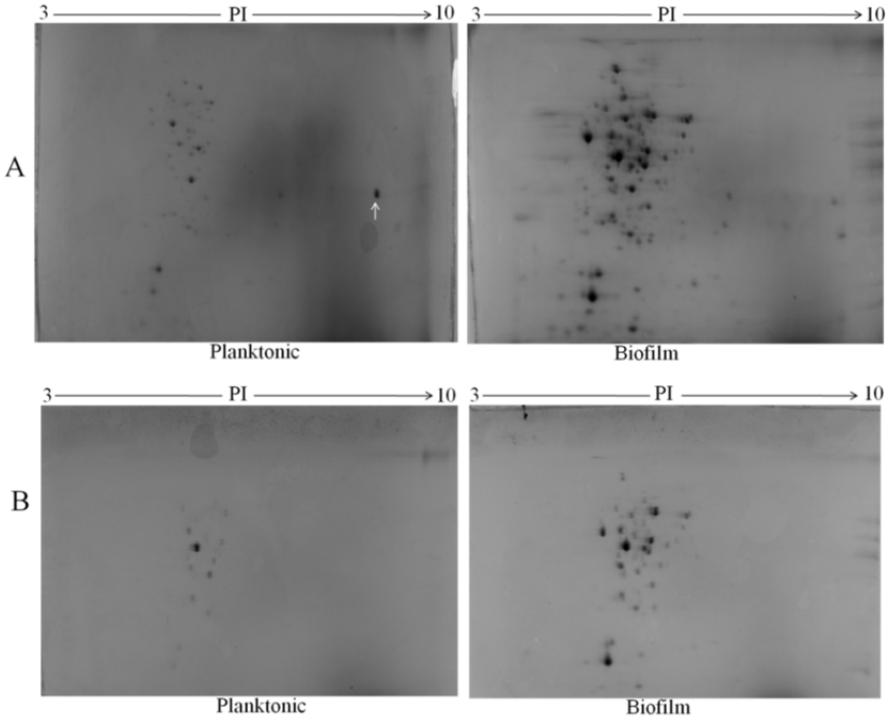

Figure (1): 2D-gel electrophoresis of the *S. aureus* profile protein from biofilm cells and from planktonic cells. Bacteria were grown in TSB media (A) and DMEM media (B). 125 µl (100 µg) of the protein was loaded on pH 3–10 isoelectric point (PI) strips and proteins were separated on SDS-PAGE and followed by staining with Brilliant Blue R-250. Arrow in figure A shows the upregulated protein in planktonic cells.

2D gel electrophoresis analysis of the expressed proteins from planktonic and biofilm cells, using pH gradient (pH 3 to 10) strips, showed that the majority of the proteins (spots) were centralized in the area of acidic region (pH 4–7); therefore, to get better separation of proteins, narrow pH range strips (pH 4–7) were used. *S. aureus* cells grown on TSB showed higher intensity of expressed proteins and also many new proteins were expressed when cells grown under biofilm condition as shown in figure 2.

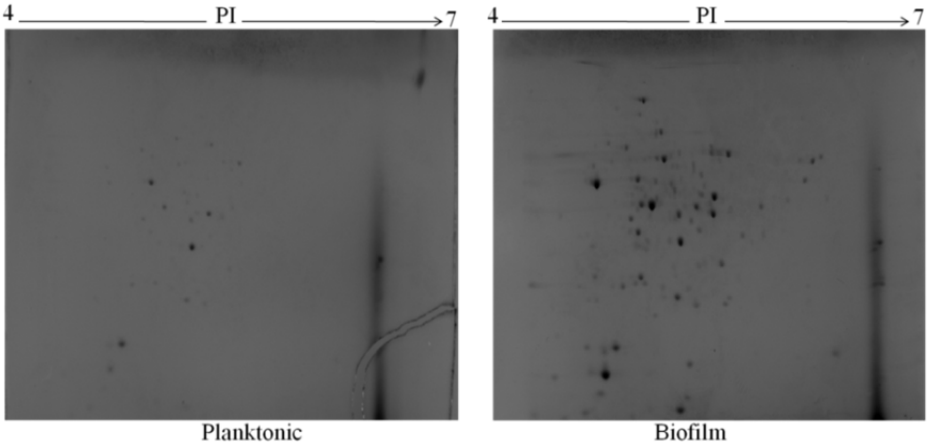

Figure (2): 2D-gel electrophoresis of the *S. aureus* profile protein from biofilm cells and planktonic cells. Bacteria were grown in TSB. 125 µl (100 µg) of the protein was loaded on pH 4–7 isoelectric point (PI) strips and proteins were separated on SDS-PAGE and followed by staining with Brilliant Blue R-250.

Discussion

TSB media showed higher level of protein expression. This is could be due to the fact that TSB medium is suitable medium for expression of some proteins. It is known that the gene expression is different according to the surrounding environment (medium) (Cheung *et al.,* 2004, Oogai *et al.,* 2011). In this regard, Hogt *et al*. (1983) explained that TSB was the ideal medium for production of slime layer with *S. epidermidis* but not suitable for expression of cell surface hydrophobicity.

In biofilm cell, it is found that spots were of higher intensity and many new proteins were expressed. This is due to the fact that there are many genes and proteins which are expressed in greater folds in biofilm state than the planktonic state. For example, Beenken *et al*. (2004) identified 48 genes, associated with informational pathways, metabolism, cell envelope, and cellular processes, that were expressed by at least two-fold greater in *S. aureus* biofilms compared to planktonic *S. aureus*. Studies on proteomic differences between planktonic and biofilm *S. aureus* identified about 37 *S. aureus* biofilm-specific soluble proteins. The majority of the proteins identified were proteins associated with metabolism, translation, and stress response, but in each case few proteins were found with no known function (Brady *et al.,* 2006, Secor *et al.,* 2011).

In this study, one specific spot was highly upregulated in planktonic growth condition. This could be due to the fact that some genes and proteins are upregulated in planktonic state. In this regard, Rexch *et al*., (2005) identified various genes encoding toxins and proteases were upregulated under planktonic growth conditions. The impact of biofilm formation on *S. aureus* virulence is controversial. In one study, virulence factor gene expression in *S. aureus* cells within a biofilm was downregulated when compared to planktonic *S. aureus* cultures (Resch *et al.,* 2005). On the other hand, another study showed that biofilm formation had no effect on the virulence of *S. aureus* (Kristian *et al.,* 2004). Oogai *et al*. (2011) identified one gene that was drastically downregulated in biofilms, which was *spa*, the gene that encodes protein A.

The majority of the *S. aureus* proteins in both biofilm and planktonic cells were clustered in the acidic region. It is well known that the majority of *S. aureus* proteins clustered between pH 4 and 6 (Cordwell *et al.,* 2002, Brady *et al.,* 2006). In Gram-negative and Gram-positive bacteria the proteins are highly phosphorylated therefore they appear as highly acidic. The *S. aureus* proteins that clustered between pH 4 and 6 includes many cellular proteins, such as chaperones, biosynthetic, and metabolic

enzymes (Rosen *et al.,* 2004). For this reason, better separation of proteins was performed by using pH 4–7 strips. 2D gel electrophoresis showed more intensive expressed protein (spots) in biofilm cells compared to planktonic cells. Biofilm formation by *S. aureus* has been shown to be highly dependent on the staphylococcal accessory regulator (*sarA)* (Beenken *et al.,* 2004) and to a certain degree on the (*icaABCD)* operon (Beenken *et al.,* 2003, Valle *et al.,* 2003), and the *walRK* operon (Delaune *et al.,* 2012). It has become clear that biofilms represent a far more realistic representation of bacterial behavior outside of the laboratory setting. Bacteria are capable of radically altering their physiology to cope with stressful environments (Stoodley *et al.,* 2002, Otto 2008).

It is of interest to identify secreted proteins specific to *S. aureus* biofilm mode of growth, bacterial biofilms proteins are a major barrier to healing chronic wounds.

Further studies are needed to include proteins with enzymatic functions such as proteases, lipases, and nucleases as well as proteins containing secretion signal sequences, as these types of proteins are well known virulence factors. This will help to change the focus of treatment approaches from treating the symptoms to treating the causes. The impact of these results will begin to establish what *S. aureus* biofilm/host products are involved in *S. aureus* pathogenesis, such as inflammatory responses, which inhibit reepithelialization.

In conclusion, *S. aureus* protein expression was at higher level on TSB media and the expression was upregulated in the biofilm growth condition except one specific protein that upregulated under planktonic conditions. Furthermore, the majority of the proteins cluster was centralized in the area of acidic region (pH 4–7). Collectively, further proteomic analyisis is needed for this study and other studies which could help to explain the differences in pathogenesis between bacterial biofilm and planktonic cultures.

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Conflicts of Interest

The author declares no conflict of interest.

References

Beenken, K.E.; Blevins, J.S., and Smeltzer, M.S. (2003) Mutation of sarA in *Staphylococcus aureus* limits biofilm formation. Infect. Immun., 71: 4206-4211.

Beenken, K.E.; Dunman, P.M.; McAleese, F.; Macapagal, D.; Murphy, E.; Projan, S.J., and Smeltzer, M.S. (2004) Global gene expression in *Staphylococcus aureus* biofilms. J. Bacteriol., 186: 4665-4684.

Brady, R.A.; Leid, J.G.; Camper, A.K.; Costerton, J.W., and Shirtliff, M.E. (2006) Identification of *Staphylococcus aureus* proteins recognized by the antibody-mediated immune response to a biofilm infection. Infect. Immun.,74: 3415-3426.

Branda, S.S.; Vik, S.; Friedman, L., and Kolter, R. (2005) Biofilms: the matrix revisited. Trends Microbiol., 13: 20-26.

Cheung, A.L.; Bayer, A.S.; Zhang, G.; Gresham, H., and Xiong, Y.Q. (2004) Regulation of virulence determinants in vitro and in vivo in *Staphylococcus aureus*. FEMS. Immunol. Med. Microbiol., 40: 1-9.

Cordwell, S.J.; Larsen, M.R.; Cole, R.T., and Walsh, B.J. (2002) Comparative proteomics of *Staphylococcus aureus* and the response of methicillin-resistant and methicillin-sensitive strains to Triton X-100. Microbiology, 148: 2765-2781.

Delaune, A.; Dubrac, S.; Blanchet, C.; Poupel, O.; Mader, U.; Hiron, A., and Msadek, T. (2012) The WalKR system controls major staphylococcal virulence genes and is involved in triggering the host inflammatory response. Infect. Immun., 80: 3438-3453.

Flemming, H.C., and Wingender, J. (2010) The biofilm matrix. Nat. Rev. Microbiol., 8: 623-633.

Foster, T.J. (2005) Immune evasion by staphylococci. Nat. Rev. Microbiol., 3: 948-958.

Gjodsbol, K.; Christensen, J.J.; Karlsmark, T.; Jorgensen, B.; Klein, B.M., and Krogfelt, K.A. (2006) Multiple bacterial species reside in chronic wounds: a longitudinal study. Int. Wound. J., 3: 225-231.

Hogt, A.H.; Dankert, J., and Feijen, J. (1983) Encapsulation, slime production and surface hydrophobicity of coagulase-negative staphylococci. FEMS. Microbiology Letters, 18: 211-215.

James, G.A.; Swogger, E.; Wolcott, R.; Pulcini, E.; Secor, P.; Sestrich, J., and Stewart, P.S. (2008) Biofilms in Chronic Wounds. Wound. Rep. Reg., 16: 37-44.

Kirker, K.R.; James, G.A.; Fleckman, P.; Olerud, J.E., and Stewart, P.S. (2012) Differential effects of planktonic and biofilm MRSA on human fibroblasts. Wound Repair Regen., 20: 253-261.

Kristian, S.A.; Golda, T.; Ferracin, F.; Cramton, S.E.; Neumeister, B.; Peschel, A., and Landmann, R. (2004) The ability of biofilm formation does not influence virulence of *Staphylococcus aureus* and host response in a mouse tissue cage infection model. Microb. Pathog., 36: 237-245.

Matsumoto, H.; Haniu, H.; Kurien, B.T., and Komori, N. (2012) Two-dimensional gel electrophoresis: glass tube-based IEF followed by SDS-PAGE. Methods Mol. Biol., 869: 267-273.

Oogai, Y.; Matsuo, M.; Hashimoto, M.; Kato, F.; Sugai, M., and Komatsuzawa, H. (2011) Expression of virulence factors by *Staphylococcus aureus* grown in serum. Appl. Environ. Microbiol., 77: 8097-8105.

Otto, M. (2008) Staphylococcal biofilms. Curr. Top. Microbiol. Immunol., 322: 207-228.

Resch, A.; Rosenstein, R.; Nerz, C., and Gotz, F. (2005) Differential gene expression profiling of *Staphylococcus aureus* cultivated under biofilm and planktonic conditions. Appl. Environ. Microbiol., 71: 2663-2676.

Rosen, R.; Becher, D.; Buttner, K.; Biran, D.; Hecker, M., and Ron, E.Z. (2004) Highly phosphorylated bacterial proteins. Proteomics, 4: 3068-3077.

Sauer, K.; Camper, A.K.; Ehrlich, G.D.; Costerton, J.W., and Davies, D.G. (2002) *Pseudomonas aeruginosa* displays multiple phenotypes during development as a biofilm. J. Bacteriol., 184: 1140-1154.

Schierle, C.F.; De la Garza, M.; Mustoe, T.A., and Galiano, R.D. (2009) Staphylococcal biofilms impair wound healing by delaying reepithelialization in a murine cutaneous wound model. Wound Repair Regen., 17: 354-359.

Secor, P.R.; James, G.A.; Fleckman, P.; Olerud, J.E.; McInnerney, K., and Stewart, P.S. (2011) *Staphylococcus aureus* Biofilm and Planktonic cultures differentially impact gene expression, mapk phosphorylation, and cytokine production in human keratinocytes. BMC Microbiol 11: 143.

Stewart, P.S., and Costerton, J.W. (2001) Antibiotic resistance of bacteria in biofilms. Lancet, 358: 135-138.

Stoodley, P.; Sauer, K.; Davies, D.G., and Costerton, J.W. (2002) Biofilms as complex differentiated communities. Annu. Rev. Microbiol., 56: 187-209.

Tankersley, A.; Frank, M.B.; Bebak, M., and Brennan, R. (2014) Early effects of *Staphylococcus aureus* biofilm secreted products on inflammatory responses of human epithelial keratinocytes. J. Inflamm., 11: 17.

Valle, J.; Toledo-Arana, A.; Berasain, C.; Ghigo, J.M.; Amorena, B.; Penadés, J.R., and Lasa, I. (2003) SarA and not sigmaB is essential for biofilm development by *Staphylococcus aureus*. Mol. Microbiol., 48: 1075-1087.

You, S.A., and Wang, Q.K. (2006) Proteomics with two-dimensional gel electrophoresis and mass spectrometry analysis in cardiovascular research. Methods Mol. Med., 129: 15-26.

كورتيا ليّكولينىّ:

ئەو بەكتريا گەشە دكەن ب پردين زيندو (Biofilm) جياوازن ژ هەر وىّ جورىّ بەكتريا گەشە دكەن ب (Planktonic). خانيّت بەكتريا پردين زيندو دياردكەت كو پتر بەرگيرى هەيە بو درمانين دژى بەكتريا و بەرگيرى و نيجيرىّ و هندەك موادين كيمياو جياوازە ژ خانين بەكتريا (Planktonic). پەتريا ڤەكولينين لەسەر نەساخيا بەكتريا هاتيە بەكارئينان ب بەكتريا (Planktonic). ئەرمانج ژ ڤىّ ڤەكولينىّ ئەو بەدياركرنا وىّ پروتينى تيت جيكرن و كو يا تايبەتە ب بەكتريا *Staphylococcus aureus* كو كەشە دكەت بشيوىّ پردين زيندو. هاتيە خودان كرن بەكتريا *Staphylococcus aureus* لەسەر (مرق تربسين الصويا و وسگ دوبلبيكو ايكل المعدل) او ژىّ لەسەر وان بارىّ گەشىّ كو بوكتريا پردين زيندو و (Planktonic) تيدا بشين بژين. نمونين پروتينا هاتنە پاقژكرن و ژيك جدا كرن لەسەر ڤەكوهاستنا كهربائىّ و ئەو ژىّ بەكارئينانا هندەك IPG كو 7cm(3pH–10و4–7) لە (2D gel electrophoresis). پروتينىّ هاتيە جيكرن لە هەردوو بارىّ گەشىّ بەكتريا تيدا دژيت هاتيە هەلسگاندن لەگەل ئيك. هاتيە ديار كرن لە شلوڤەكرنىّ دا او پروتينىّ هاتيە جيكرن لە بەكتريا *Staphylococcus aureus* گەلەك پترە لە (الوسگ الغژائى مرق تربسين الصويا (TSB)) هەلسگاندن لەگەل (وسگ دوبلبيكو ايكل المعدل (DMEM)). باريت گەشىّ كو بەكتريا تيدا دژيت لە پردين زيندو دا ديار كر چريكا بلند يا پروتينىّ هاتيته جيكرن و هەروسا دياره بىّ كو پروتينه كىّ نوى يە. ئيك ژ وان پرونينا هاتين ديتن بشيوەيەكىّ بلند وريك وپيك بىّ لە بارى گەشا (Planktonic). زيدەكرن لەسەر هندىّ، باهرا پتر پروتينين كەتينە سەر ئيك لە ناوجيّت گرش (7-4 pH). ڤەگوهاستنا كهربائىّ (2D gel electrophoresis) گرنگيەكا بلند و ريكا كارئينانا وىّ بشيوەيەكىّ بەرفروان بو شلوڤەكرنا پروتينا. پردين زيندو كارتيكرنەكا راستەقينە هەيە سەر پەروردا بەكتريا و زيندەورين زيندو شيان هەيە بگوهورينا فسلجا خو لە دوروبەرين ژينگەهىّ تيدا دژين. ئەنجامىّ مە باشن بو ديار كرنا جياوازين نەخەشيا ناڤ بينا خانين پردين زيندو و (Planktonic) لە بو هەر نموونوكا نەخوشىّ. ئەڤا هە دىّ بو مە دەست نيشان كت نيشانيت زيندىّ بو باشكرنا تشخيص ، جارەسەرىّ و نەهيلانا پردين زيندو *Staphylococcus aureus*.

الخلاصة

البكتريا التي تنمو كأغشية حيوية (Biofilm) تختلف عن نمو نفس البكتريا التي تنمو كعوالق بكتيرية (Planktonic) . خلايا الاغشية الحيوية تظهر زيادة في مقاومتها للمضادات البكتيرية، المناعية، الافتراسية والكيميائية مقارنة بخلايا العوالق البكتيرية. أغلب الدراسات عن الامراض البكتيرية تستخدم العوالق البكتيرية. ألهدف من هذه الدراسة هو تحديد البروتينات المنتجة والتي هي مميزة لبكتريا المكورات العنقودية ذات نمط النمو كأغشية حيوية. تم نمو بكتريا المكورات العنقودية على مرق تربسين الصويا ووسط دوبلبيكو ايكل المعدل وذلك في ظروف نمو كاغشية حيوية و معلقات بكتيرية. عينات البروتين نظفت وفصلت اعتمادا على تنقلها الكهربائي باستخدام شرائط ال IPG ذات 7 سم (حموضة 3–10 و 4–7) على هلام الهجرة الكهربائية ثنائي الابعاد. البروتينات المنتجة من كلا ظروف النمو قورنت مع بعضها البعض. كشف تحليل البيانات ان البروتينات المنتجة من قبل المكورات العنقودية كان اعلى في الوسط الغذائي مرق تربسين الصويا (TSB) مقارنة بالوسط الغذائي ووسط دوبلبيكو ايكل المعدل (DMEM). ظروف النمو على الاغشية الحيوية اظهرت كثافة اعلى من البروتينات المنتجة ولوحظت برويتنات منتجة جديدة. احد البروتينات وجد بانه نضم بشكل اعلى في ظرف النمو كعوالق. بالاظافة الى ذلك، اغلب البروتينات تكدست في المنطقة الحامضية (حموضة 4–7). هلام الهجرة الكهربائية ثنائي الابعاد ذو اهمية عالية وطريقة تستخدم بشكل واسع في تحليل البروتينات. الاغشية الحيوية تمثل التمثيل الحقيقي لسلوك البكتيريا وان الكائنات الحية قادرة على تغير فسلجتها الحيوية في البيئات المحيطة بها. ان النتائج قد تفيد في توضيح الاختلافات في الامراضية بين خلايا الأغشية الحيوية والعوالق الخلوية في اي نموذج من الامراض. هذا سوف يحدد العلامات الحيوية من أجل تحسين التشخيص، العلاج ومنع الأغشية الحيوية للمكورات العنقودية.