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العدد 19

مجلة التربوي

مجلة علمية محكمة تصدر عن كلية التربية

جامعة المرقب

العدد التاسع عشر

يوليو 2021م

- **المجلة ترحب بما يرد عليها من أبحاث وعلى استعداد لنشرها بعد التحكيم .**
- **المجلة تحترم كل الاحترام آراء المحكمين وتعمل بمقتضاها .**
- **كافة الآراء والأفكار المنشورة تعبر عن آراء أصحابها ولا تتحمل المجلة تبعاتها .**
- **يتحمل الباحث مسؤولية الأمانة العلمية وهو المسؤول عما ينشر له .**
- **البحوث المقدمة للنشر لا ترد لأصحابها نشرت أو لم تنشر .**

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يشترط في البحوث العلمية المقدمة للنشر أن يراعى فيها ما يأتي :

- أصول البحث العلمي وقواعده .
- ألا تكون المادة العلمية قد سبق نشرها أو كانت جزءا من رسالة علمية .
- يرفق بالبحث تزكية لغوية وفق أنموذج معد .
- تعدل البحوث المقبولة وتصحح وفق ما يراه المحكمون .
- التزام الباحث بالضوابط التي وضعتها المجلة من عدد الصفحات ، ونوع الخط ورقمه ، والفترات الزمنية الممنوحة للتعديل ، وما يستجد من ضوابط تضعها المجلة مستقبلا .

تنبيهات :

- للمجلة الحق في تعديل البحث أو طلب تعديله أو رفضه .
- يخضع البحث في النشر لأولويات المجلة وسياستها .
- البحوث المنشورة تعبر عن وجهة نظر أصحابها ، ولا تعبر عن وجهة نظر المجلة .

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The relationship between *slyA* DNA binding transcriptional activator gene and *Escherichia coli* fimbriae and related with biofilm formation

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ABSTRACT*:* In this study, we demonstrate that the conjugative pET28a plasmid influences biofilm formation by increasing the biomass of *E. coli.* The results showed that, when pET28a was inserted into *E. coli*, biofilm formation was significantly increased compared with the strains without plasmid. The findings revealed that the Congo red agar (CRA) aerobic culturing of 28°C and 37°C incubation was the same indicator of biofilm production. Nevertheless, other findings revealed that the *E. coli* MG1655, MG1655Δ*slyA* were isolated from Congo red plates as a single colony and then regrooved again at 37°C and 28°C, MG1655 wild kind produced a red morphotype whereas MG1655Δ*slyA* strain created a white morphotype. Collective finding recommended that the temperature has effect on biofilm formation owing to the expression of the *E. coli slyA* gene is temperature-regulated. Moreover, these finding indicate that MG1655 strains and strains have plasmids (pET28a) have ability to enhanced sedimentation level when comparing with Δ*slyA* strains. Finally, this study indicates that conversion of MG1655 with pET28a vector within the existence or nonexistence of *slyA* transcriptional activator gene did not influence cell aggregation.

INTRODUCTION

Bacteria form biofilms by adhering to biotic or abiotic surfaces. This phenomenon causes several problems, including a reduction in the transport of mass and heat, an increase in resistance to antibiotics, and a shortening of the lifetimes of modules in bio industrial fermenters. *slyA* is shown to control gene expression via competition with H-NS. Although *slyA* antagonises H-NS inhibition of most of the genes it regulates in both *E. coli* and *Salmonella* species, altering the structure of the H-NS protein enables expression of the K5 capsule gene cluster in *E. coli*. Since SlyA's antagonism of the repressive effect of H-NS on the expression of fimB has been demonstrated, *slyA* can be described as a novel activator of type 1 fimbriae production in *E. coli*. Moreover, Mcvicker *et al.*,(2011) stated that *slyA* is shown to bind to two operator sites (*OSA1* and *OSA2*), situated between 194 and 167 base pairs upstream of the *fimB* transcriptional start site (fig.8). *fimB* expression is derepressed in an *hns*(H-NS gene) mutant and diminished by a *slyA* mutation in the presence of H-NS only.

METHODS

Bacterial Strains

E. coli MG1655 wild type (WT), mutant strains (Δ*slyA*), MG1655:pET28a and MG1655:pET28a: *slyA* have been used in this study and were kindly provided by Mel Lacey (Sheffield Hallam University). The mutants were *E. coli* K-12 MG1655 *slyA* mutant (Δ *slyA*).

Biofilm assay. Biofilm assays were performed using 96-well plates essentially as described by Tagliabue et al. [27] using M9 minimal medium with 20% (w/v) glucose and 50 µg ml kanamycin. Wells containing 200 µl of medium were inoculated (1:10) from an overnight culture of E. coli K-12 MG1655 pET28a or E. coli K-12 MG1655 pGS2468 and then incubated for 16 h under aerobic conditions at 37°C. Growth of cultures was monitored by measuring OD600. The planktonic cells were removed and the remaining biofilm was stained for 5 min with 200 µl 1% (w/v) crystal violet solution. Excess stain was removed by three washes with deionized water before the plate was air-dried. To quantify the extent of staining, 200 µl ethanol:acetone (4:1) was added to each well, and after incubating for 20 min the amount of biofilm was estimated by measuring A600. Adhesion units were calculated by dividing the A600 values for crystal violet-stained adhered cells by the OD600 values for the corresponding planktonic cells.

Congo red

To detect the production or formation of curli/cellulose, L-broth agar combined 0.004% Congo red was utilised. Next, the relevant strains were streaked onto the plates and left at the desirable conditions to incubate overnight. Color differences amongst the stains were assessed by sight (Spiers*et al.*, 2003).

Sedimentation assay

The sedimentation tests were done using certain strains which had been developed under anaerobic conditions, in L-broth at either 37ºC or 25ºC for 16 hours within a 16 ml centrifuge tube. The formed cultures were then left for a whole 24 hours at room temperature, whilst the level of sedimentation was then assessed by sight.

Yeast aggregation assay

To complete the yeast cell aggregation tests, the E. coli strains underwent tests to test their effectiveness at aggregating yeast cells. The test was done using readily available baker's yeast (Saccharomyces cerevisiae) and was suspended in phosphate-buffered saline (PBS; 5 mg [dry weight]/ml). PBS was used to wash E. coli strains, which were then re-suspended so that they reached an optical density of 530nm (OD530) of 0.4, before being mixed with the yeast cell suspension in PBS, either with or without 1% D-mannose. Through visual monitoring, the aggregation was recorded, with the titer being the last dilution to achieve a positive aggregation reaction.

Growth curve

The inoculation of 20 mL of L- broth medium in a 50-mL flask, featuring a single colony of E. coli is done before the time point collection. This is then incubated overnight at temperatures of 37°C and 28^0C with shaking. A stationary phase population of 10^9 CFU/m is observed in *E. coli*. . Following the incubation period, the inoculate 200µL of the overnight culture is poured into 20mL of (LB) into a 50 mL flask and is thoroughly combined. The remainder should be incubated at 37°C with shaking, before setting and calibrating the spectrophotometer. This is achieved by setting the wavelength dial (at the top of the instrument) to 600nm. Blank the spectrophotometer. This can be done by inserting a cuvette holding the medium (also known as a 'blank') into the holder. The blank should then be taken from the instrument. Organisms are re-suspended by swirling the culture flask and should be followed by carefully pouring out 1ml of the *E. coli* culture. The culture's transmittance and optical density (OD) should then be recorded by %.

Results and Discussion

Growth curve

The fitness of the *E.coli* strains was assessed by determining the growth rates of the strains in a nutrient LB medium as well as in two temperatures as this parameter also could contribute to environmental adaptability. Growth curve was performed to check the differences in the growth rate of *E.coli* MG1655 wild type, *E. coli* MG1655Δ*slyA*, *E. coli* MG1655: pET28a and *E.coli* MG1655: pET28a:*slyA*. All strains showed similar growth curves in LB medium (Fig. 10, 11). Furthermore, the growth curves were also similar when the bacteria were cultivated in LB medium at different temperatures including 28°C and 37°C. In addition, It was found that *E. coli* with Δ*slyA* gene can grow at the same rate as WT does in planktonic cells at 37°c while at 28°c the growth rate was slightly different which is that growth of *E. coli* with Δ*slyA* gene was differently at stationary phase. Moreover, all four strains exhibited faster growth in rich media, as stationary phase was reached after 8 h for all strains in LB at both temperatures. Corbett et al., (2007) showed that expressed level of *E. coli slyA*gene which is temperature-regulated,was higher at 37 °C than at 20 °C and this mean that the regulation of this gene is independent of H-NS. Navasa *et al*., (2011) observed that the expression levels of transcriptional thermoregulatory genes which including *rfaH*, *hns*, *slyA* and *dsrA* was a higher at 37°C than at 19°C in *E. coli* K92.

biofilm formation assay

Since *slyA* protein triggers *fimB* transcription that serves to contribute growing in expression of category 1 fimbriae that significant element in biofilm creation. To investigate the relationship between*slyA* DNA binding transcriptional activator gene and *E. coli* fimbriae and related with biofilm formation, for strains including *E. coli*

MG1655 wild type, *E. coli* MG1655Δ*slyA*, *E. coli* MG1655: pET28a and *E. coli* MG1655: pET28a: *slyA* were compered by using different assays as described in the experimental section. The capacity of MG1655 and its removal mutants to perform biofilm creation was evaluated by contrasting the biomasses of surface-bound and planktonic cells, as approximated by determining the optical thickness at 600 nm. The difficulty of ordinary OD readers is that they determine the OD simply at one spot within the centre of the well. Therefore, if the density of the biofilm at that position considerably varies from the other part of the well, the dimension will not be precise. Nevertheless, harmonized resolubilization of the dye attached to the bacterial cells within the biofilm cover obtained through the suggested protocol facilitates indirect but accurate dimension of the biofilm creation. As demonstrated within figure 12, *E. coli* MG1655Δ*slyA* strain at 37°C demonstrated reduced biofilm creation when contrasted with WT strain whereas at 28°c; Δ *SlyA*strain revealed additional lessened biofilm creation when contrasted with WT strain. Additionally, *E.coli* MG1655:pET28a and MG1655:pET28a:*slyA*showed extra biofilm mass when contrasted with WT strain and Δ *slyA* strain respectively. *slyA* and H-NS (histone-like nucleoid structuring protein) are involved in regulating the transcription of the *E. coli* K5 capsular polysaccharide gene cluster and that*sly*A expression is autoregulated, independent of H-NS but dependent on temperature.*slyA* is shown to control gene expression via competition with H-NS. Although slyA antagonises H-NS inhibition of most of the genes it regulates in both E. coli and Salmonella species, altering the structure of the H-NS protein enables expression of the K5 capsule gene cluster in E. coli (Corbett et al., 2007). As a result of the *E. coli* K-12 strains capable to transport conjugative plasmids. These plasmids support biofilm creation by supporting cell aggregation (Van Houdt, and Michiels, 2005). In addition, the plasmid pET28a that transferred the curli- and style 1 fimbriae-related gene was constructed. When pET28a was transformed into MG1655, the capability of biofilm formation augmented within MG1655 strain. Ghigo (2001) noticed that in an *E. coli* strain bearing a conjugative plasmid, known as a strong adhesion factor the presence of flagellum was dispensable for biofilm formation. Moreover, MG1655:pET28a:*slyA* at 37°C revealed reduced biofilm formation when compared with *E.coli* MG1655:pET28a whereas at 28°C the extent of biofilm formation of pET28a: *slyA* mutant strain was in excess of MG1655:pET28a strain.

Congo red assay

Congo red dye agar experiment was foremost applied by Surgalla and Beasly (1969) for segregation of non-virulent and virulent Pasteurella (now Yersinia) pestis. Consequently, it was applied as phenotypic indicator of colisepticaemic (invasive) and non-coliseptecaemic*E. coli* within poultry by Berkhoff and Vinal (1986). CR has been employed widely in microbiological researches to score the creation of microbial amyloids together with polysaccharides and other extracellular structures and has been applied as a selective medium to differentiate curli-producing microbes from non-

curliated microbes when CR-binding is established to be curli-dependent. The CRA technique is quick, reproducible, and offers a benefit: the colonies stay feasible within the medium for additional study. As a result, the process was selected in an effort to enhance its capability to recognize biofilm creation within *E. coli* strains through creating alterations in the formula and regulating diverse physical parameters. The technique is simple to perform and the findings are normally founded on the colony colour generated that scopes from white for non-biofilm–generating strains to red for biofilm-generating strains. Originally, the strains were cultured in streaks onto the CRA plates to envision the entity colonies, but this streaking can as well obscure the categorization because of disparities between the colonies colours seen alongside the streaks.

During the present research, of four strains which are *E. coli* MG1655 wild type, *E. coli* MG1655Δ*slyA,E. coli* MG1655: pET28a and *E. coli* MG1655: pET28a: *slyA* were cultured at different parameters including two incubation temperature which were evaluated.Surprisingly, the findings revealed that the CRA aerobic culturing of 28°C and 37°C incubation was the same indicator of biofilm production as shown in (fig.14 A, B, C, D). Nevertheless, other findings revealed that the *E. coli* MG1655, MG1655Δ*slyA* were isolated from Congo red plates as a single colony and then regrooved again at 37°C and 28°C as shown in figure 14(E and F), MG1655 wild kind produced a red morphotype whereas MG1655Δ*slyA* strain created a white morphotype.Da Re and Ghigo (2006) showed that in *E. coli*, Congo red binds to both celluloseand curli and have found that the curli-deficient strains of *E. coli* which arecsgB, csgBompA and csgD mutants had white colonies. This suggests that slyA activator protein have role in curliformation that enhances the biofilm formation.

The MG1655 laboratory strain of *E. coli* is adept in curli formation, as established through its red phenotype on agar medium complemented with Congo red stain (CR medium); the red phenotype of MG1655 on CR medium is completely eliminated through inactivation of the*slyA* gene, encoding the main subunit of curli. *E. coli* bring together extracellular adhesive amyloid fibres termed curli that mediate cell-surface and cell-cell interfaces and acts like an structural and adhesive scaffold to enhance biofilm assemblage and other sets behaviour (Barnhart, and Chapman, 2006). Amyloid fibres act to promote adhesion and biofilm creation in *E. coli* (Olsén, Jonsson, and Normark, 1989). Curli and other amyloid fibrils have significant functions in improving the viscoelastic attributes of biofilms. This feature has been recognized in rheological researches of natively created curli-containing pellicle (biofilm created at the air-liquid edge) (Wu, Lim, Fuller, and Cegelski, 2012) and in researches of in vitro created biofilm-like materials (Lembré, Di martino, and Vendrely, 2014).

Sedimentation assay

Given that the several protein triggers curli and cellulose creation, which are aspects that are engaged in biofilm creation and during cell-cell interface, the likelihood that is the *slyA* transcriptional stimulator gene might as well play a function

in these procedures were experimented. Cell-cell interaction was established by a cell collective sedimentation experiment. The collective findings showing that the quantity of sedimentation was different between two temperature 28°C and 37°C which was more at 28°C As shown in Fig.13 (C and D), these finding recommended that the temperature has consequence on biofilm formation owing to the expression of the *E. colislyA* gene is temperature-regulated, positively autoregulated, and independent of H-NS (Corbett, 2007) Moreover, Gally (1993) and Sohanoal (2004) stated that there are many different environmental cues including temperature, availability of certain amino acid nutrients (alanine, leucine, isoleucine and valine), N-acetylneuraminic acid (Neu5Ac) and N-acetylglucosamine (GlcNAc) that can influence the activation of the site-specific recombinases, *fimB* that is regulated by *slyA* transcriptional activator gene , which control the expression of type 1 fimbriae via the inversion of a DNA "switch. In additionally, as shown in fig. 13 A the rate of sedimentation is lesser in *E.coli* MG1655. B shows *E.coli* MG1655:pET28a gave the sedimentation levels was lesser when comparing with *E.coli* MG1655:pET28a: *slyA*while at 28°C the rate of sedimentation is higher in *E.coli* MG1655 as shown in fig. 13 C. D shows *E.coli* MG1655:pET28a gave the sedimentation rates were higher when comparing with *E.coli* MG1655:pET28a:*slyA*. These finding indicate that MG1655 strains and strains have plasmids (pET28a) have ability to enhanced sedimentation level when comparing with Δ*slyA* strains asindicated in previous study which is the presence of plasmids in *E. coli* strains is influential factor in biofilm formation (Roder*et al*., 2013).

Yeast aggregation assay

The consequence of *slyA* transcriptional activator gene on *E.coli* biofilm formation was experimented by means of yeast aggregation test that was performed through the *E.coli* MG1655 strains including *E. coli* MG1655, *E. coli* MG1655Δ*slyA*: pET28a and *E.coli* MG1655: pET28a: *slyA*. Cell aggregates that were clearly detectable in our aggregation assay as shown in Fig. 15. Biofilm formation is enhanced in *E. coli* K-12 strains transferring conjugative plasmids. These plasmids support biofilm creation through enhancing cell aggregation (Gonzáles Barrios et al., 2005; Reisner et al., 2003; Van Houdt and Michiels, 2005). However, Conversion of MG1655 with pET28a vector within the existence or nonexistence of *slyA*transcriptional activator gene did not influence cell aggregation. This suggests that*slyA* transcriptional activator gene may does not have role in fimbriae creation. In contrast, Duncan et al., (2005) indicated that there are major subunit and at least three minor subunits which are composition of *E. coli* type 1 fimbriae and the minor subunit which is FimH is responsible for the sugar specificity of type 1 fimbriae.

Figure legends

FIG. 1. Growth curves for *E. coli* with LB medium at 37°C. Comparison between *E. coli* MG1655 wild type, *E. coli* MG1655Δ*slyA*, *E. coli* MG1655: pET28a and *E. coli* MG1655: pET28a: *slyA*. Bacterial strains were grown for 24 h, and the optical

densities at 600 nm were recorded hourly. The data used is an average of three replicates of each strain.

In this graph, *E. coli* MG1655: pET28a: *slyA* shows the best growth characteristics with a maximal optical density OD_{600} = 0.72. *E. coli* MG1655: pET28a shows a slightly lower growth due to the higher replication stress. *E. coli* MG1655 showing 10% decreased maximal cell density and *E. coli* MG1655Δ *slyA*shows a further decreased growth which was 11 % reduced in comparison to *E. coli* MG1655: pET28a: *slyA.*

FIG. 2. : Growth curves for *E. coli* with LB medium at 28°C. Comparison between *E. coli* MG1655 wild type, *E. coli* MG1655Δ*slyA*, *E.coli* MG1655: pET28a and *E. coli* MG1655: pET28a: *slyA*. Bacterial strains were grown for 20 h, and the optical densities at 600 nm were recorded hourly. The data used is an average of three replicates of each strain.

In this graph *E. coli* MG1655 shows the best growth characteristics with a maximal optical density OD_{600}= 0.49. *E. coli* MG1655Δ*slyA* shows lowest growth due to the higher replication stress. *E. coli* MG1655: pET28a showing 11% decreased maximal cell density and *E. coli* MG1655: pET28a: *slyA* shows a further slightly decreased growth which was 10 % reduced in comparison to *E. coli* MG1655.

FIG. 3. Biofilm formation of *E. coli* MG1655, MG1655Δ*slyA*, *E. coli* MG1655:pET28a and *E. coli* MG1655:pET28a: *slyA* were estimated using biofilm assay at 37°C and 28°C.A shows biofilm formation for *E. coli* with LB medium at 37°C.Comparison between *E. coli* MG1655 wild type, *E. coli* MG1655Δ*slyA*. In this graph, the level of biofilm formation of Δ*slyA* mutant strain was slightly less than MG1655 wild type. The statistical analysis has not shown any significant results between *E. coli* MG1655 wild type, *E. coli* MG1655Δ*slyA* at 37°C. B shows biofilm formation for *E. coli* with LB medium at 28°C. Comparison between *E. coli* MG1655 wild type, *E.coli* MG1655Δ*slyA*. In this graph, the level of biofilm formation of Δ*slyA* mutant strain was less than MG1655 wild type. The statistical analysis has shown high significant results between *E. coli* MG1655 wild type, *E. coli* MG1655Δ*slyA* at 28°C. C shows biofilm formation for *E. coli* with LB medium at 37°C. Comparison between *E. coli* MG1655:pET28a, *E.coli* MG1655:pET28a: *slyA*. In this graph, the level of biofilm formation of pET28a: *slyA* mutant strain was less than MG1655:pET28a strain. The statistical analysis has shown more high significant results between *E. coli* MG1655:pET28a, *E.coli* MG1655:pET28a:*slyA*at 37°C. D shows biofilm formation for *E. coli* with LB medium at 28°C. Comparison between *E. coli* MG1655:pET28a, *E. coli* MG1655:pET28a: *slyA*. In this graph the level of biofilm formation of pET28a: *slyA* mutant strain was more than MG1655:pET28a strain. The statistical analysis has

shown also more high significant results between *E. coli* MG1655:pET28a, *E.coli* MG1655:pET28a: *slyA* at 28°C.

FIG. 4. sedimentation level was determined for all strains including *E. coli* MG1655 and MG1655Δ*slyA*, MG1655:pET28a and MG1655:pET28a: *slyA*at 37°C and 28°C.In this image, A shows the rate of sedimentation is lower in *E.coli* MG1655. B shows *E.coli* MG1655:pET28a gave the sedimentation levels were lower when comparing with *E.coli* MG1655:pET28a:*slyA*, C shows the rate of sedimentation is higher in *E.coli* MG1655. D shows *E.coli* MG1655:pET28a gave the sedimentation rate was higher when comparing with *E.coli* MG1655:pET28*a: slyA*.C and D were incubated at 28°C.

FIG. 5. Comparing colony colour of *E. coli* strains including MG1655 wild type, *E.coli* MG1655Δ*slyA*, *E. coli* MG1655: pET28a and *E.coli* MG1655: pET28a: *slyA*which are grooved onto Congo red agar .Two plates were grown at 37°C for 24h. A show the growth characteristics of *E.coli* MG1655 wild type and MG1655 Δ *slyA*strains which is no deference between them. B represents the growth characteristics of MG1655:pET28a and MG1655:pET28a: *slyA* that present no deference as well. Two plates were grown at 28°C for 24h.C shows the growth characteristics of MG1655 wild type and Δ *slyA* strains which are no deference between them. D represents the growth characteristics of MG1655:pET28a and MG1655:pET28a: *slyA* that present no deference as well. Two plates were isolated from Congo red plates as a single colony then re cultured again at 37°C and 28°C respectively for 24h. In this image E and F show two distinctly different morphotype which were observed in the colonies formed by the two *E. coli* strains, MG1655 wild type produced a red morphotype while MG1655Δ*slyA* strain produced a white morphotype (Fig.14 E and F).

FIG. 6. yeast aggregation was determined using yeast aggregation assay for all strains including *E. coli* MG1655 wild type, *E. coli* MG1655Δ*slyA*, *E. coli* MG1655: pET28a and *E. coli* MG1655: pET28a:*slyA.* (A and B were incubated at 37°C while I and J were incubated at 28°C, all these cuvettes contained 500µl yeast and 500µl *E.coli*. C and D were incubated at 37°C while K and L were incubated at 28°C; all these cuvettes contained 500µl yeast, 250µl *E.coli* and 250PBS. E and F were incubated at 37°C, M and N were incubated at 28°C, and all these cuvettes contained 500µl yeast, 100µl *E.coli* and 400 PBS. G and H were incubated at 37°C while O and P were incubated at 28°C; all these cuvettes contained 500µl yeast, 50µl *E.coli* and 450 PBS.

FIGURE 1

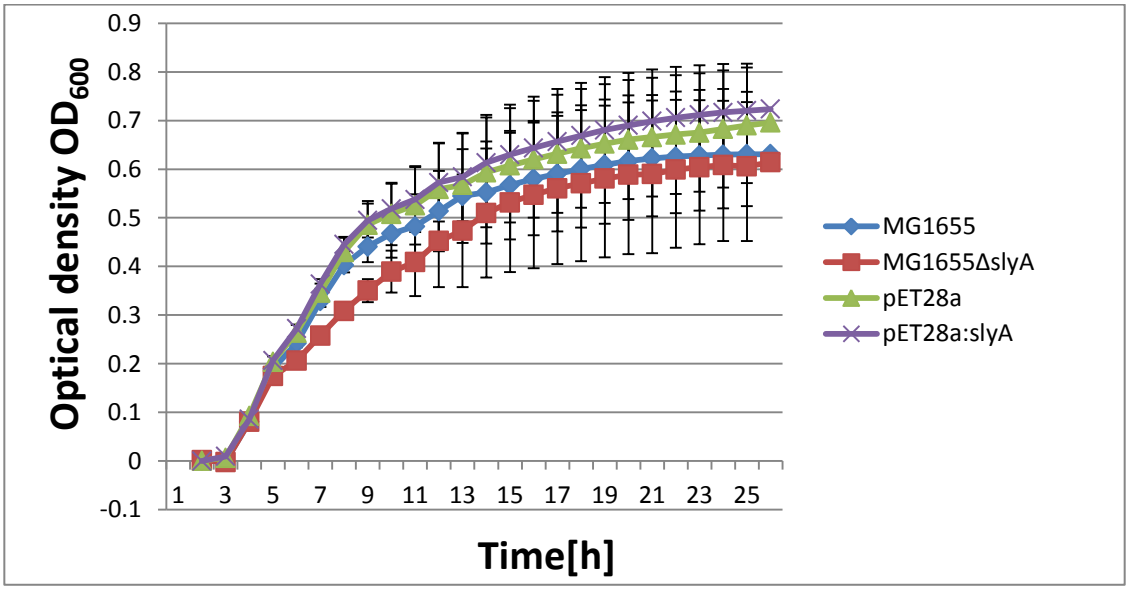


FIGURE 2

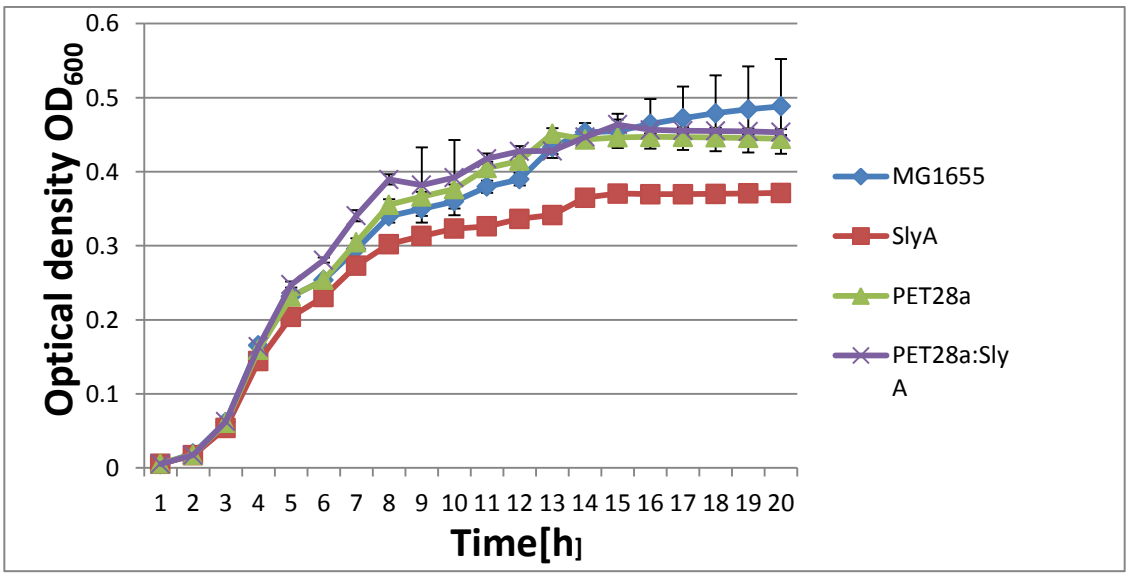


FIGURE 3

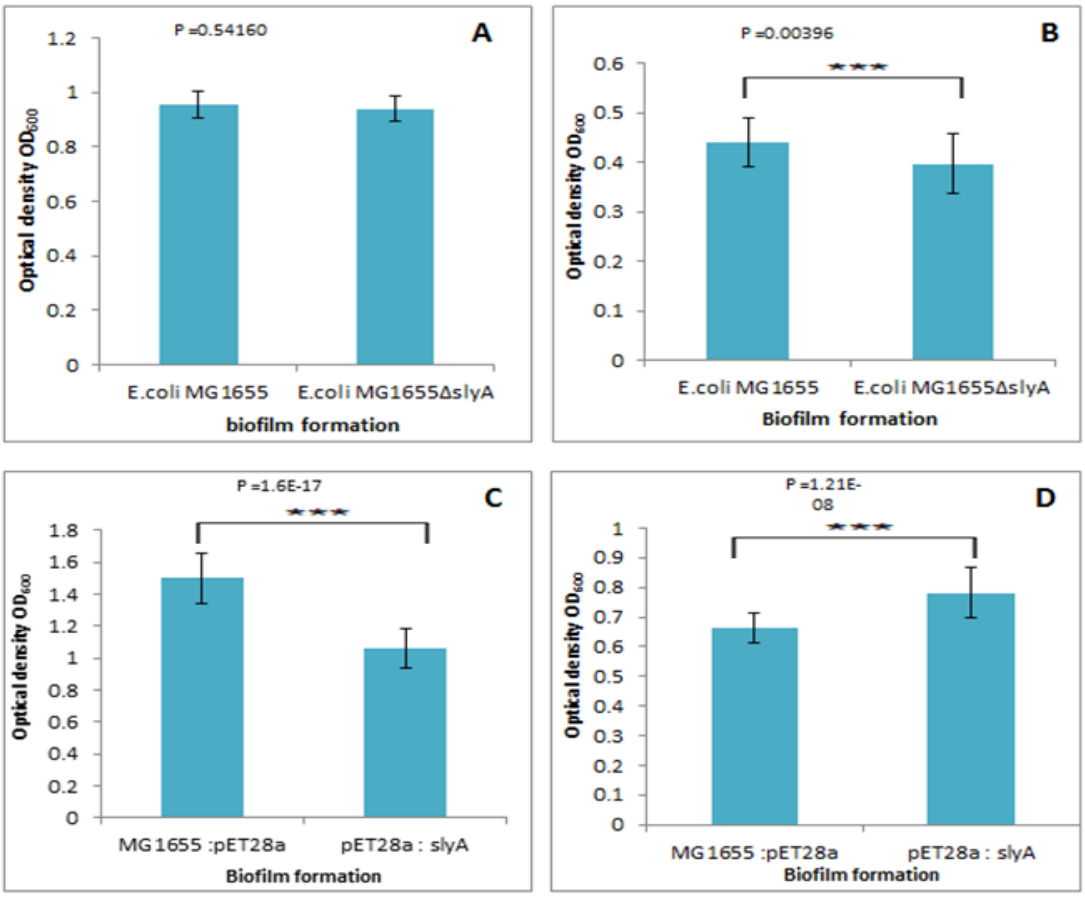


FIGURE 4

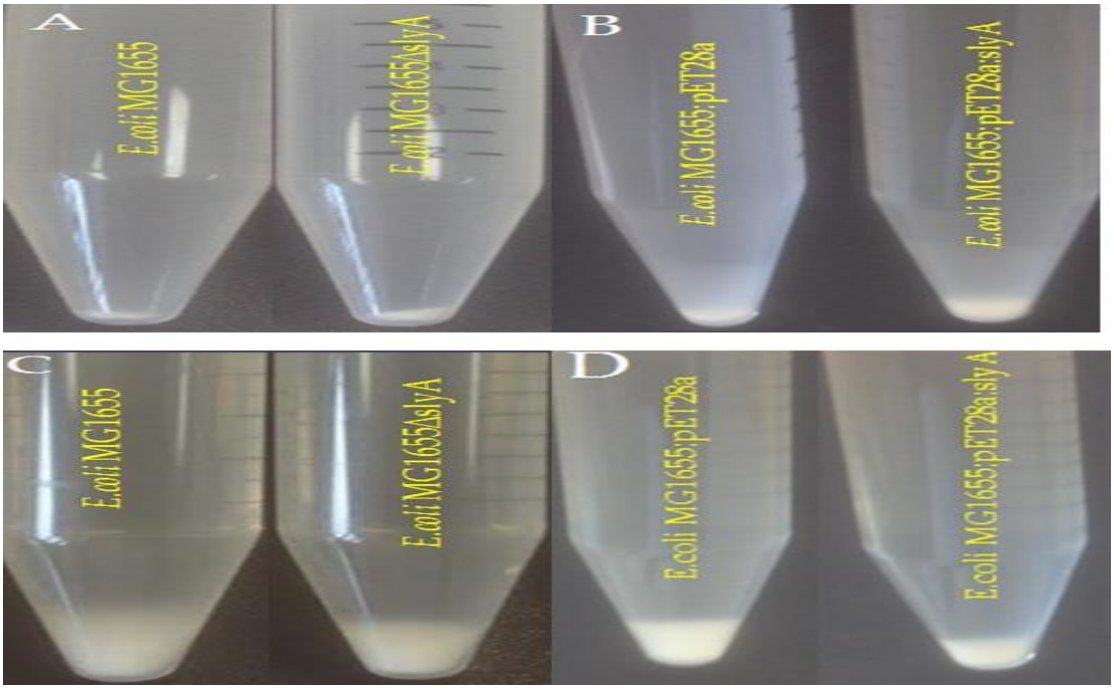


FIGURE 5

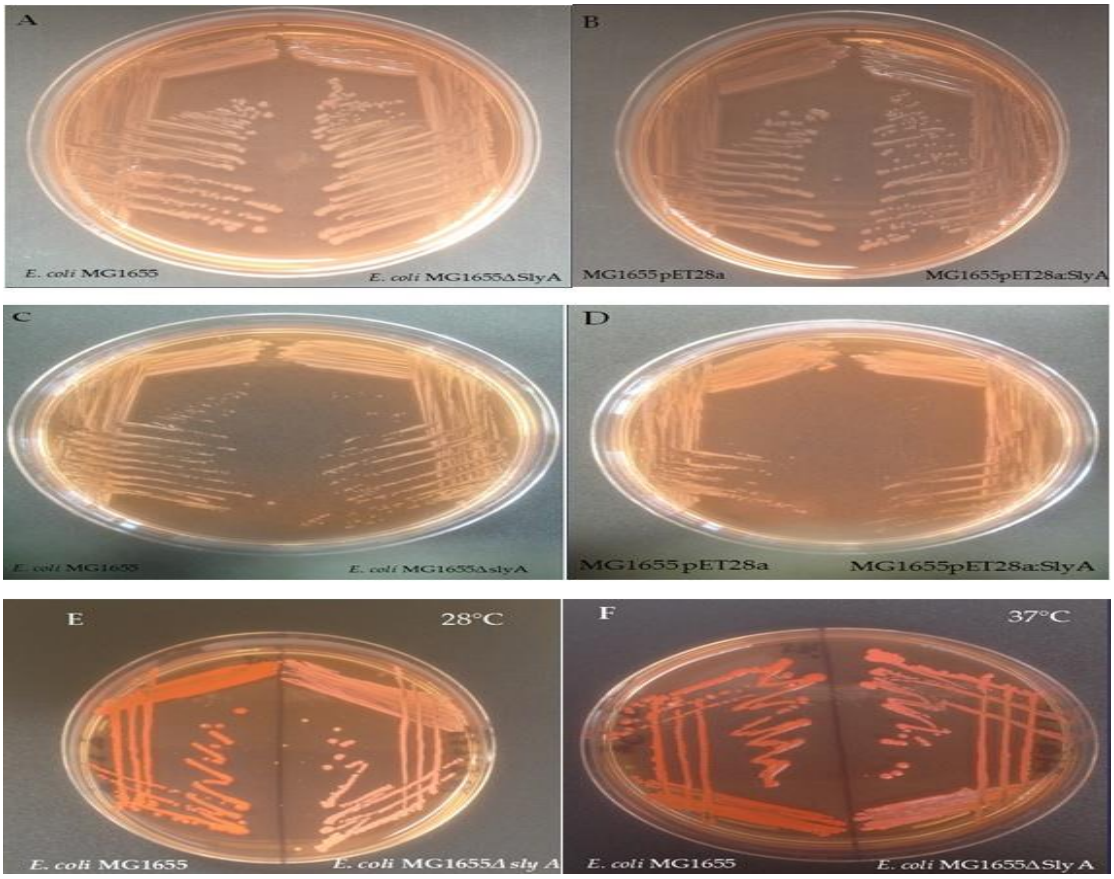
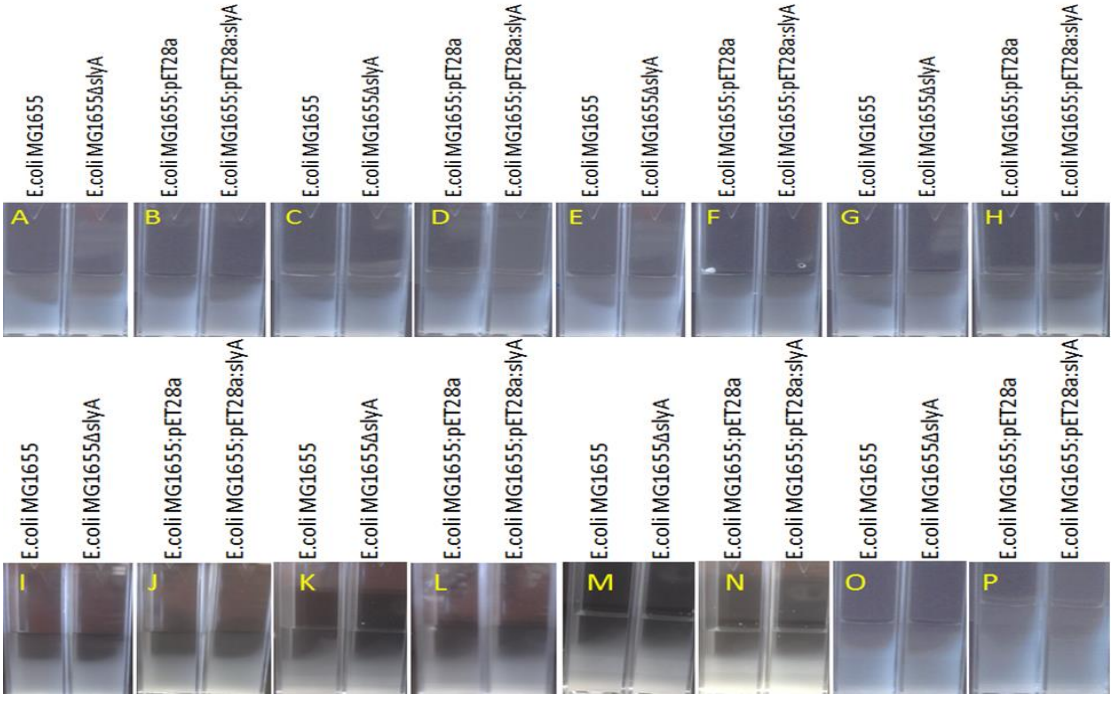


FIGURE 6



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