Identification and Molecular Analysis of Cultivable and Uncultivable Bioprospects Capable of Producing Biofilm

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Abstract

Microbial biofilm formation can provide structural and functional advantages to the microbial community including tolerance to drastic changes in pH, temperature and presence of antimicrobial agents. Nowadays biofilm studies have taken focus in understanding its role in bioremediation, industries contamination and biomedical research, specifically in nosocomial infections due to antibiotic resistance. Nosocomial infections are the fourth leading cause of death in the U.S. with 2 million cases annually and the cost of the treatment related with this event has an average of $2,100 per case. Also, biofilm has shown impact in the medical devices industry and its application in bioremediation, specifically in the field of wastewater in which have been used in biodegradation, bioaccumulation, biosorption and biomineralization in municipal water treatments. This research seek to determine the ability of Purple Non-sulfur Bacteria (PNSB) from Macro and Micro environments in Puerto Rico, and clones from Metagenomics libraries to produce biofilms. The Microtiter Dish Biofilm Formation (BF) Assay was used to detect the BF in the isolates, and the presence of known BF genes was determined by PCR. A total of 4 PNSB isolated from bromeliad phytotelmata were positive for BF assay. The culture dependent genetic analysis suggests that some of the genes responsible for BF are similar to icaA from Staphylococcus aureus. This result support the importance of searching for BF activities in novel environments combining culture dependent and independent approaches to understand and identify novel genes associated with biofilm formation with potential biomedical and biotechnological applications.

Introduction

- Biofilms are sessile microbial communities that are embedded in a self produced matrix composed by extracellular polymeric substance (EPS).
- This EPS matrix can serve as a shock absorber on hostile environmental changes like pH, temperature and presence of antimicrobial agents.
- The biofilm formation has been identifying as one of the main causes of nosocomial infections, antibiotic resistance and industry contamination.
- On biotechnological applications, biofilms have demonstrated their potential use on wastewater treatments and industrial facilitator due to the capability to increase biotechnological products and long activity.
- This study focuses on the potential of biofilm formation of PNSB and metagenomics libraries isolated from different environnment around the island of Puerto Rico.

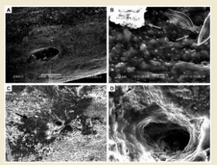


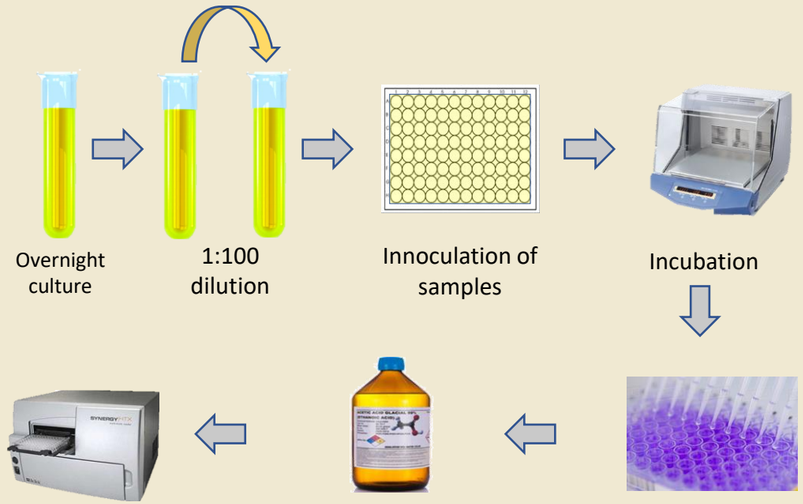
Figure 1. SEM images of biofilm surface and pores . A) Biofilm on inner pore surface. B) Higher magnification of A, showing individual bacterial cells and EPS. C) Biofilm surrounding surface pore, and D) Higher magnification of C, showing biofilm on inner pore surface. (Peterson, 2010)

Objectives:

- Identify clones from metagenomics libraries responsible for biofilm formation in the *E. coli* surrogate strain.
- Identify Purple non Sulfur Bacteria capable of producing biofilm.
- Search for known biofilm formation genes encoding by PCR and transposon mutagenesis.

Methodology

Biofilm assay was performed according to O'Toole (2011) to obtain qualitative and quantitative results. Metagenomic libraries and PNSB were tested with this assay.



These results were analyzed statically using InfoStat.

Molecular analysis

- To identify the possible genes responsible for the biofilm formation in metagenomic libraries and PNSB, PCR reactions were performed.
- Primers were designed from genes related to biofilm production identified previously from *S. aureus, P. aeruginosa* and *R. palustris.*
- The genes related with the biofilm production used in this study are *ndv*B, *tol*A, *ica*A, *upp*C and *upp*E.
- The genes ndvb and tolA were isolated from Pseudomonas aeruginosa.
- The gene icaA was isolated form Staphylococcus aureus.
- The genes *upp*C and *upp*E were isolated from *Rhodopseudomonas palustris.*

Results

Biofilm Assays:

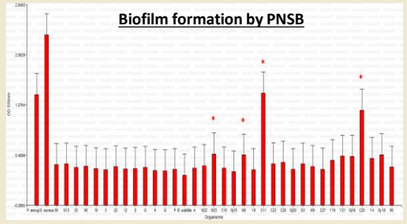


Figure 2. Quantitative results of PNSB absorbance at 550nm. Samples with numbers, letters and letter with numbers were isolated from water bodies, heliconia and bromeliads phytotelmata respectively. The ones with asterisk have significant minimum difference in compare with the control.

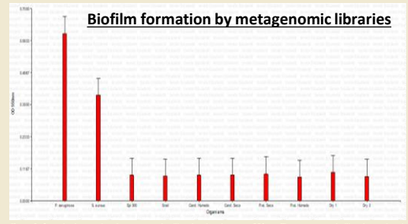


Figure 3. Quantitative results of metagenomics libraries absorbance at 550nm. The metagenomic libraries analyzed on this assay were isolated from Snail, microbial matts from Candelaria and Fraternidad, and from the dry forest of Guánica.

Molecular analysis:

lcaA:

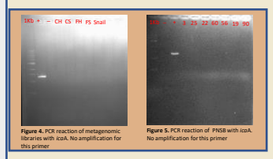


Figure 4. PCR reaction of metagenomic libraries with *icaA*. No amplification for this primer

Figure 5. PCR reaction of PNSB with *icaA*. No amplification for this primer

ndvB:

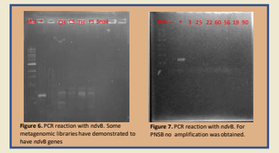


Figure 6. PCR reaction with *ndvB*. Some metagenomic libraries have demonstrated to have *ndvB* genes

Figure 7. PCR reaction with *ndvB*. For PNSB no amplification was obtained.

tolA:

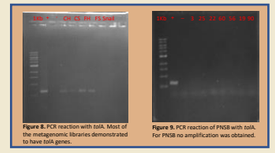


Figure 8. PCR reaction with *tolA*. Most of the metagenomic libraries demonstrated to have *tolA* genes.

Figure 9. PCR reaction of PNSB with *tolA*. For PNSB no amplification was obtained.

uppC:

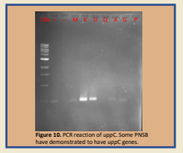


Figure 10. PCR reaction of *uppC*. Some PNSB have demonstrated to have *uppC* genes.

uppE:

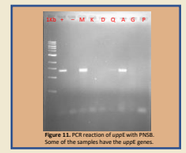


Figure 11. PCR reaction of *uppE* with PNSB. Some of the samples have the *uppE* genes.

Summary and Future Work

- The quantitative results of the microtiter biofilm assay suggest the presence of PNSB capables of producing biofilm but not in the metagenomic libraries.
- Despite that metagenomic libraries does not show any biofilm formation on the assay, the molecular analysis suggest that some of these libraries have the presence of biofilm genes like *ndv*B and *tol*A. These molecular results suggest that environmental factors can induce or repel the biofilm formation.
- Some PNSB have shown the presence os the genes *upp*C and *upp*E.
- For the future work PCR reactions with *upp*C and *upp*E needs to be performed with the metagenomic libraries.

Cited literature

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