BIOSTUDIO: PRINTING AND DYEING USING ACTINOBACTERIA

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ABSTRACT

Biodesign employs living organisms on the composition of products and services offered to society or their application for manufacturing process. Based on this idea, and faced with the need to promote innovation in textile dyeing processes and in surface design, this research appears with the objective of relating Biodesign and Fashion through the staining of natural fibers and creation of patterns using actinobacteria strains. The laboratory results were positive, but still lack some analysis on the microorganism interaction with the fabric and adherence methods.

INTRODUCTION

Biology and design have interacted with one possibility to find new solutions for services and products, such as in the Bionic and Biomimicry fields.

The principal of applying Biomimicry to Design is to observe nature and create analogies for product projection, with the goal of finding possible, unexpected and viable solutions. (LACERDA, SORANSO, FANGUEIRO, 2012)

In the other hand, Bionics has a proximity with mechanizing organic methods, where natural processes are observed and converted to automated ones for industrial purpose or to repair an organic mechanism that doesn`t function anymore, such as in prosthetics

The observation of nature to make formal and functional analogies was established as Biomimicry Method in 1957, terminology first used by Otto H. Schmidt. Janine Benyus, researcher in the field and founder of the Biomimicry Institute, separates Biomimicry in three study areas: nature as a model, where it is inspired or copies biological models to solve human problems; nature as a measure, where natural patterns are used to evaluate and balance new inventions; and nature as a mentor, process where nature serves and is valued as a guide.

According to Benyus (2012), "Living beings maintain a dynamic balance, using natural resources without waist.", therefore, man could follow that example and rethink the industrial process as ecosystems, where all the leftovers become raw material for use in other processes.

Biomimicry projects applied to clothing are generally developed by textile technology based on analogies of well resolved natural models, but not always from organic products, focusing only on the esthetic and functional aspects of the fabric. It is necessary to reorganize this production model in suitable conditions for life, in room temperature, without changing pressure and not utilizing aggressive chemical products.

According to Lacerda (2012), biomimicry will perform a fundamental part in a new industrial revolution process, which will focus on the way a product is manufactured and its contribution to sustainability.

More recently, the Biodesign field appeared from the interaction between Biology and Design, characterized by Design projects that use living organisms as part of the production process and final product, adding nature`s top technology to find contemporary solutions for life (MYERS,2012).

According to Lasky (2013), one of the greatest advantages of Biodisign is that the final product, after its use is worn out, can be returned to nature entirely and reabsorbed by decomposition of its components in nature`s nutrient cycle.

Antonelli (2012) believes that the consequences and results of the release of these projects will be a new way of thinking and making design, also changes in the human trajectory, and the pathway from the information era to the biology era. It can also serve to supply the demand for cleaner technology and the possibility for the user to create products without leaving his home.

Numerous Biodisign researches are presented by Myers (2012), which evidence these studies in this area have increased even more, showing the United States of America having approximately 2% of its GDP (Gross Domestic Products) attributed to genetically modified products. Designers are not wasting this opportunity and are summoning researchers to Biodisign.

When related to clothing, the Biodisign research is generally restricted to the development of textile materials based on the observation of form and function of several living species. Otherwise, the present work has the goal of incorporating bacteria in the production of different fabrics, or in the improvement of processes, like surface design, in the production of localized engraving (template) through the stencil method, looking for innovation for the textile and fashion industry.

More specifically, the research tries to include bacteria in fabric composition; identify different bacteria strains that can be used as coloring agents for fabric and localized engraving production; produce different engraving patterns/bacterial colonies in fabric and verify its legibility; test different organic fabrics as a base for coloring and adherence of the bacterial colony; verify if it is possible to perform washing and ironing of the material after incorporating the bacteria.

Because it presents a disciplinary and exploratory character, this work presents a flexible methodology, in some occasions experimental and in other moments descriptive, with qualitative data analysis

The research technique was a direct, intense and systematic observation in laboratory with photographic record. The chosen organism was observed during its growth for characterization and variable survey. The results obtained were analyzed and the variables adjusted to obtain more resistant and practical fabric for utilizing in clothing.

The choice of bacteria for the achievement of these experiments is due first for being a ubiquitous microorganism, with a relatively short growth time and specifically the actinobacteria for having good relations with humans; produce pigment, present texture and having nonpathogenic species.

The choice for the engraving and coloring process was due to the production of pigment by the bacteria. It is an important theme in textile production because a major problem in the fashion industry is pollution and also the waste of water and energy during the dyeing and printing of fabrics. Thinking about sustainability with biomimicry eyes, involves interdisciplinary context to provide innovation to industry.

For what concerns the fabric coloring universe, several factors are important, mainly the use of water, a preponderant and sensitive factor, when thinking about sustainable processes.

According to Silva (2012), fabric coloring is a practice that has been around for thousands of years. The ongoing coloring technology consists of several steps and are chosen according to the fabric`s nature, structure characteristics, classification and dye availability, compatible adherence to the material, price and other various criteria.

During the coloring, the adherence of the dye, water proportion and the need of heating are critical during the process. An ideal sustainability way would be the use of a dye that requires a less amount of water, high adherence, diminishing the amount of auxiliary chemicals in the process and no need of heating, minimizing the energy used in the process.

Another complicated fact in the coloring process is the reuse and disposal of the dye baths. Some processes allow the dye to be reused, depending on the type of fiber and dye used, although returning these baths to nature depend on physical and chemical reactions that make the process expensive.

The ideal would be to produce a dye that could be reused, and when exhausted, be disposed to nature without causing any harm. In the case of dyes originating from bacteria present in soil shared with plants, the innocuous/coloring of a fabric would be the beginning/innocuous of another batch of fabric, and when the coloring is finished, the disposed material could be reused as fertilizer in plantations, not causing waste or inappropriate disposal.

Natural dyeing is a challenge due to difficulty of color reproducibility, large scale production and color stability, but according to Fletcher (2011), this kind of dyeing does not intend to accommodate the patterns that the industry imposes.

Besides that, according to Silva (2001), many developed countries already use natural dyes in their food and cosmetic industry, due to high toxicity of synthetic dyes that have accumulative effects in the organism, causing several diseases, contrary to natural dyes that easily decompose and have low collateral effect rates.

Shifting to print works, we face the same problems and factors involving dyeing, but with some worsening. But why think about sustainable print work? Very simple, besides avoiding synthetic dyes, less chemical agents for adherence are used, less use of water and energy, less creation of toxic waste and once we print an organic fabric, production line or localized print work with synthetic dye, we will never be able to separate these two materials, making organic fabric a great pollutant, too.

Print work is a localized dyeing process, creating figurative or abstract forms. During this process, more dye adherence are made and therefore more use of water and chemicals for print adherence. Some printed fabric washing are done to avoid the discoloration and formation of stains by the user.

In the sustainable print work process, the use of natural dyes, like in this work, with the use of bacteria, this print work process is less controllable due to variability of dye concentration and the randomization of bacterial growth, even under controlled conditions.

The chosen bacteria, Actinobacteria, is a phylum of important Gram-positive, aerobic bacteria that are most nonpathogenic organisms founded on soil and vegetal material, and have an economic importance in the production of antibiotics and enzymes including various cellular ones. (MADIGAN, 2010).

In this work, most of the microorganisms belong to the *Streptomyses* genus, that has ramified filaments. Due to its rapid spread and ramification, these microorganisms form filament nets called mycelium, similar to the mycelium formed by filament fungus.

One of the important characteristics of the actinobateria, is the fact that it produces spores when in extreme situations, adding protection, and it is exactly in this same moment that pigments and antibiotics are produced by this bacteria. Spores usually don`t occur in liquid cultures, but can happen with the formation of a film on the surface of the culture when not subjected to shaking.

The identification of *Streptomyces* in the midst of solid cultures is simplified by its colony opaque, wrinkled aspect and its compact and coloration nature as well.

For the experiments of this project, six strains of actinobacterias (G27, G28, G29, G78, G85 e JUA183), from the soil of the Caatinga in Pernambuco, Brazil, that produce stain during the spore creation process, were isolated and used.

According to Giulietti and Ferreira (2004, 2012, apud CORRÊA, 2014, p. 15) the Caatinga biome has species that present adaptations to the hydro deficiencies, although there are few studies about the techno biological potential from this extreme conditions region, existing the hypothesis that the microorganisms also develop adaptation mechanisms to survive adverse conditions, like the plants from this region.

Roots of plants from the Caatinga empower the growth of bacteria and fungus that colonize the rhizosphere by altering the surrounding soil, working as substrate for the selective growth of soil microorganisms, capable of using certain substrates efficiently (SILVEIRA; FREITAS, 2007 apud CORRÊA, 2014, p. 20).

Streptomycin is little nutritionally demanding, making use of a big variety of carbon sources. It is strictly aerobe, intensely enhancing its growth when subjected to shaking. This characteristic makes its use a lot easier, because it can be grown in simple culture medium and in contact with air.

Another Streptomycin strong characteristic is the large production of antibiotics. Some species produce more than one antibiotic. In addition, a same antibiotic can be produced by more than one organism.

Streptomycin`s ecology is still little known, but it is believed that the antibiotic production is related to the spore creation process, because this phenomena occurs in media with depletion of nutrients, the organisms form spores and produce antibiotics to diminish the competition and increase the perpetuation of the specie (MADIGAN et al., 2010).

This allows this research to use and conserve the microorganism for other experiments, also providing the user of the developed print work these spores, giving the chance to reinsert the microorganism in the culture media to grow again, creating new print work from the last ones.

After these selected actinobateria were received from the microorganism collection of the antibiotic laboratory of UFPE (Universidade Federal de Pernambuco), the preserved lineage were previously inoculated (placed for growth) in 50 milliliters (ml) pre-inoculums of ISP-3 liquid medium (20 grams of oat flour, 1 ml of salt trace solution and 1000 ml of distilled water), and cultivated under shaking of 180 rotation per minute (rpm) for five days in at 37 degrees Celsius (ºC).

The experiments were divided in two groups, one group to dye natural fibers in liquid and solid medium, (silk, cotton and linen), and another group to create patterns to print the same textiles through surface design using stencil.

The dyeing process was performed in liquid and solid IPS-3 medium, and grown for five days at 37ºC in incubator, in contact with the natural textiles. Different inoculation methods were tested and the best results were washed with neutral soap and ironed, but the color remained solid. The samples were analyzed by observation and optical microscopy.

In the second stage of development of prints with actinobacteria, the technique of stencil patterns were developed making use of acetate cut with figurative drawings of low complexity. After obtaining the prints/actinobacteria colonies, fabrics were washed with neutral soap and ironed, but they remained unchanged and impregnated into the fabric even after these processes.

1. Dyeing test

The study began through the analyses of the fabric and actinobacteria interaction. Since the bacteria is filamentous, we believed the fabric`s fibers could be associated with the ones of bacteria, endowing them with some properties such as color and resistance to other bacteria growth.

These first tests were performed using cambric fabric (100% cotton), in contact with the selected actinobateria and grown in liquid and solid medium, with or without shaking, and direct or indirect contact with the fabric.

After the first results, a selection of one of the techniques that obtained a considerable coloration were repeated, but using different kinds of organic fabric.

Pictures were taken and microscopy was performed to evaluate the interaction between the bacteria and the fabric`s fibers. The experiments generated a diversity of coloration types on the fabrics, also distinct growth rate in each case.

After the bacteria grew, every fabric from all experiments went through a drying process that included the withdraw of the fabric from the culture media, insertion between two layers of 100% cotton fabric, manual pressing with the aid of sterilized Petri dishes and heated in incubator at 40ºC for 24 hours.

1.1 Dyeing in solid medium in Petri dish

The six isolated actinobacteria selected, after the pre-inoculums were performed, as previously mentioned, were inoculated with the aid of a Drigalski spatula in 90 mm Petri dishes, prepared in two different ways, separated in this subchapter as TOP experiment and SAND experiment.

These experiment terminologies were given due to the microorganism`s inoculation place, on top of the culture medium (TOP) and in between layers of the culture medium, as in a kind of sandwich (SAND).

Both were of 20 ml ISP-3 culture medium with 1,6% agar, an 80 mm diameter fabric (Cambric) and a second layer of ISP-3 medium with 0,7% of agar. The difference between the two experiments was the place where the 100 µl (microliters) of pre-inoculums collected bacteria sample was deposited. In the TOP experiment, the bacteria was inoculated under the second medium layer, on top of the dish; Now, in the SAND experiment the inoculums were performed over the fabric, between the two layers of medium. The dishes` illustration for both experiments can be seen on figure 1.

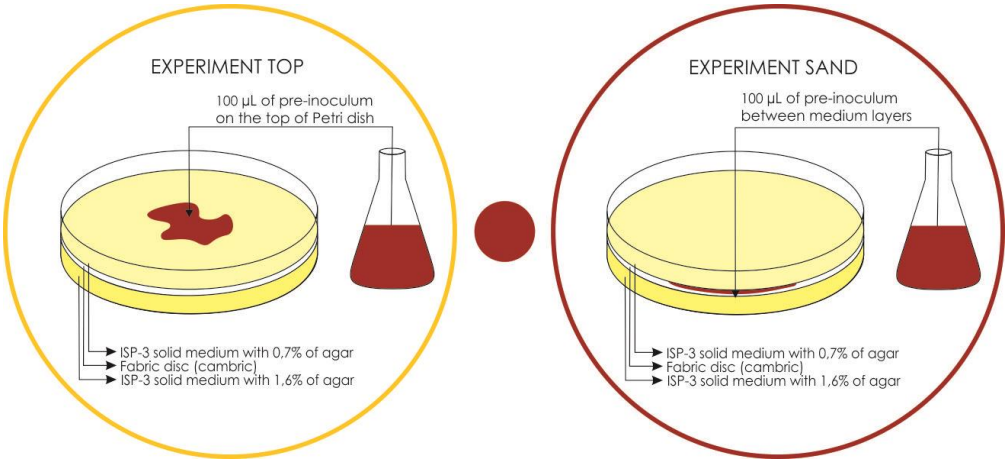

Figure 1. Demonstration of inoculums on TOP and SAND experiments.

The dishes were grown for 5 days in incubator at 37ºC, enough time for the creation of spores and dye production by the actinobateria to occur.

1.2 Dyeing in liquid medium in erlenmeyer

A second coloration and interaction test between the actinobateria and the fabric was performed in an ISP-3 liquid medium in a 500 ml erlenmeyer.

On this experiment, 100 ml of culture medium was placed in a vial, 1 ml of pre-inoculums with bacteria, and a 90 mm cambric fabric that was constantly in contact with the bacteria and the culture media during 5 days. This experiment was performed under constant movement on a shaking table at 180 rpm (PLAY experiment) and static in incubator (STOP experiment), both at 37ºC (see figure 2).

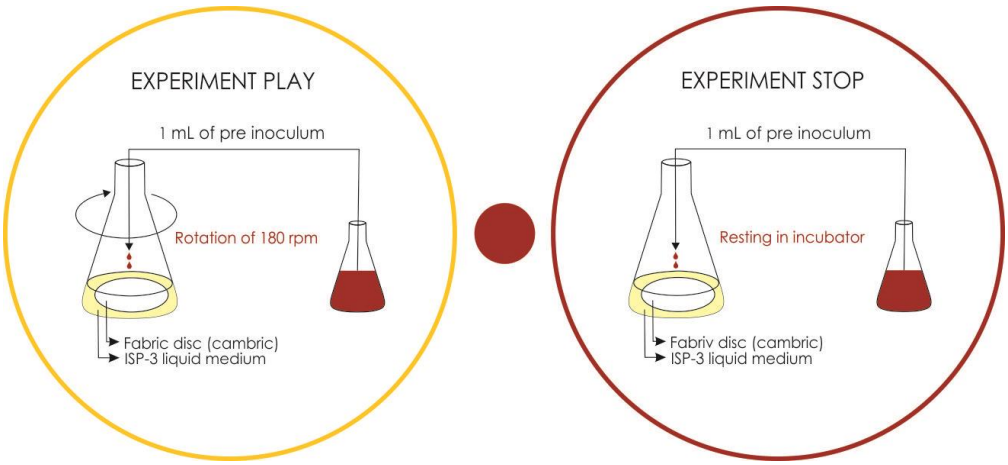

Figure 2. Inoculums in the PLAY and STOP experiments.

1.3 Dyeing in liquid medium in Petri dish

A last test was performed in Petri dishes, but with liquid medium inside. An 80 mm diameter cambric fabric was placed inside the dish, humidified with 10 ml of liquid ISP-3 and inoculated with 100 µl of pre-inoculums.

A great concern with this experiment was the possibility of the medium drying out and the bacteria ceasing its growth, which is why a piece of sterilized hydrophilic cotton was added to each dish, increasing the humidity and nutrients available. In addition, 1ml of medium was added to the dishes every day during the five days growth in incubator at 37ºC.

This experiment was named COTTON (figure 3). Also to avoid the dishes to dry due to the growth temperature, the humidity inside the incubator was controlled with distilled water.

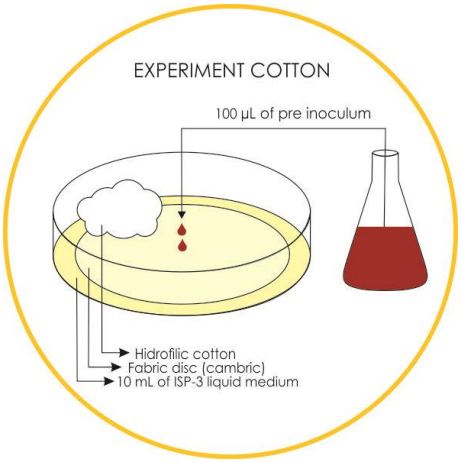

Figure 3. Inoculums in the COTTON experiment.

1.4 Dyeing diferent kinds of fabric

After obtaining good results with the Petri dishes in the COTTON experiment, as it will be demonstrated next, the experiment was repeated without the cotton ball and with different kinds of natural fabric fibers (tricoline, laise, linen, toweling, silk, guipure, and silk georgette) and only two isolated bacteria, that had better growth and coloration were used, G27 and G85, as it will be shown bellow on the results.

The initial amount of liquid ISP-3 culture medium remained the same, 10 ml, and the fabric were cut in 80 mm diameter dishes as in the first experiment. Only 1 ml of medium was added to the dishes twice during the five day growth, instead of the daily supply added previously. The samples were grown in incubator at 37ºC.

All the fabric obtained was submitted to optic microscopy to analyze the interaction between fabric and bacteria.

2. Print test

The tests for the possibility of actinobacteria use for Print Work were performed with the stencil technique. In first instance the microorganism inoculation technique was tested in solid culture medium, with (experiment A) and without (experiment B) the presence of 200 string percale fabric, with 100% cotton composition. A second experiment (experiment C) was performed to test the possibility of using more complex drawings for patterns. The fabrics that had better results were subjected to washing and ironing to evaluate the adherence to the fabric.

To begin the experiments, from the pre-inoculums previously performed, inoculations were done in 150 mm Petri dishes according to the description below.

2.1 Print test in solid medium with fabric

On experiment A, the inoculated dishes presented four consecutive layers of material. The first layer corresponded to 40 ml of solid ISP-3 medium with 1, 6% of agar, the second had a disc of 140 mm diameter of 200 string Percale fabric (100% cotton), the third 5 ml of solid ISP-3 medium with 0, 7% of agar, and the last layer had a disc of 140 mm diameter sterile acetate, hollowed with stars and squares, cut with a paper punch, as shown on figure 4.

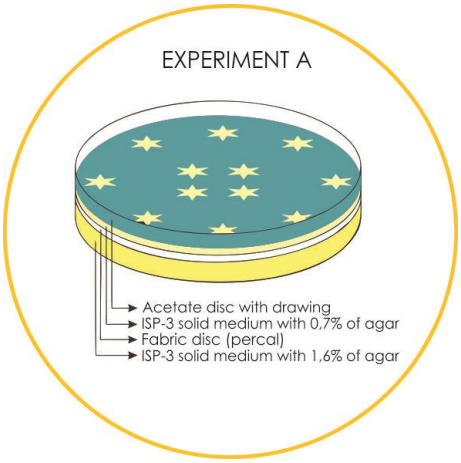

Figure 4. Dish layers from experiment A.

After the dish preparation, 1 ml of each one of the six lineages was inoculated with the help of a Drigalski spatula over the acetate. The samples were duplicated. These dishes were cultivated in incubator during 7 days at 37ºC.

When fully grown, the samples from experiment A went through a drying process, where the acetate was removed, the culture medium discarded, and the percale fabric with the print/actinobacteria colony was subjected to a 24 hour drying between two layers of cotton fabric in incubator at 40ºC.

After dried, experiment A samples that had better results, were cut in half with one of the halves submitted to 1 hour washing in Erlenmeyer containing 100 ml of distilled water and 5ml of neutral detergent, under 100 rpm shaking. After dried, the prints were ironed with a domestic iron without vapor at 200ºC.

2.2 Print test in solid medium without fabric

The inoculations of dishes in experiment B were performed this time without the use of the fabric disc and from the same pre-inoculums used on experiment A. After the microorganism was grown in the print shape, an attempt to transfer the print from the culture media to the cotton fabric discs was performed, through the use of distilled water and drying in incubator.

This way, the dishes on experiment B only presented two layers, being the first with 40 ml of ISP-3 solid medium with 1,6% of agar and the second one with the disc of 140 mm diameter of sterile acetate, hollow with stars and squares forms, cut with a paper punch (figure 5).

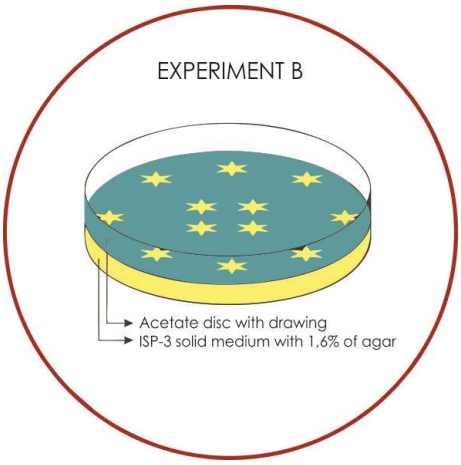

Figure 5. Dish layers from experiment B.

After grown, the samples from experiment B had the acetate removed and the culture media with the print/actinobacteria colony was dried in contact with a 140 mm diameter disc of 200 string Percale fabric (100% cotton) saturated in distilled water, between two external layers of 100% cotton fabric, in incubator for 24 hour at 40ºC.

2.3 Figurative print work with fabric

At the end, experiment A was repeated, with the two lineages that showed better results (G27 and G85) originating experiment C. This time the same method as in experiment A was used, but with 90 mm diameter Petri dishes, 80 mm diameter cambric discs (100% cotton), and acetate discs of 80 mm diameter, with figurative drawings with more details. The samples were only grown for five days.

RESULTS AND CONCLUSIONS

3. Results and coloring process debate

Among the experiments performed in Petri dishes using solid culture medium, the one that presented better coloring and growth results was experiment TOP as seen on figures 6 and 7 below.

The 5 day growth period was ideal for the pigmentation process, as well as the 37ºC temperature, where on experiment TOP, besides obtaining the fabric`s coloration, the isolated also produced the culture medium coloration. Among the isolated, the only one that did not obtain a differentiated and considerable coloration was the isolated G183.

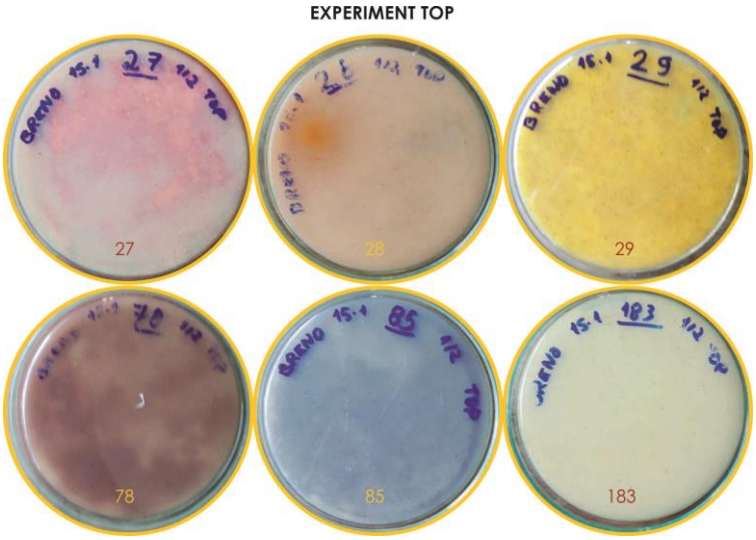

Figure 6. Reverse of the isolated dishes showing the media and fabric`s pigmentation color on experiment TOP.

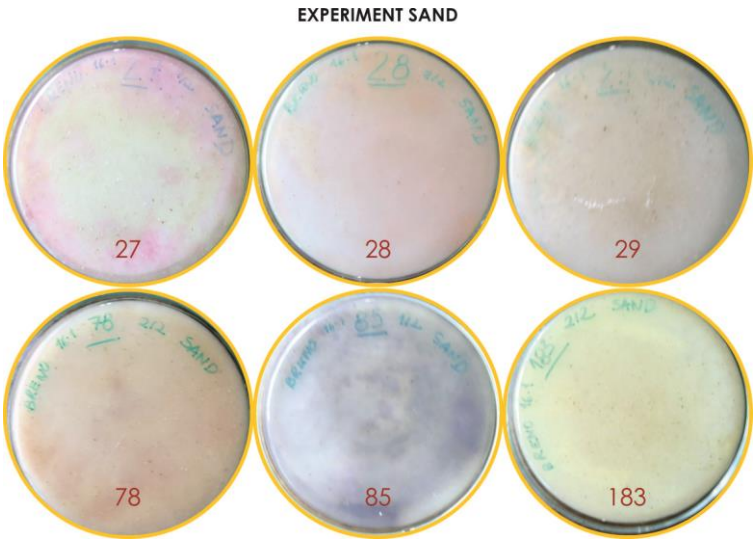

Figure 7. Reverse of the isolated dishes showing the small bacteria growth and consequently little fabric pigmentation on experiment SAND.

The obtained colorations were the following:

- G27 Burgundy
- G28 Orange
- G29 Yellow
- G78 Purple
- G85 Greenish gray
- G183 Beige

After the drying process, it can be seen that when using the solid medium, the bacteria dye the fabric in a moderate way, maybe for being more intensely attached to the right side and not the reverse side of the fabric.

It can also be observed that the bacteria layer formed on top of the fabric, after drying, becomes crumbly and slowly detaches from the fabric.

After dried, experiment TOP samples that had better results, were not subjected to any coloration adherence process. These fabrics were cut in half, with one of the halves submitted to 1 hour washing in Erlenmeyer containing 100 ml of distilled water and 5ml of neutral detergent, in 100 rpm shaking. The samples were soaked in distilled water and dried under shade.

There was a great loss of the fabric`s coloration to the water, although the fabrics were still colored, but the color was a little faded. Therefore, new adherence possibilities must be researched.

Among the isolated, those that appeared to have more pigmentation loss to the water during the washing were G29 and G85. In a way, the result was already expected, once the pigment adherence process is part of the textile dyeing process.

Now, the fabrics that were placed in Erlenmeyer and grown with bacteria, with and without shaking, characterized by experiments PLAY and STOP, had very distinct results among them and of the experiments performed in solid medium.

The ones that were grown under shaking (experiment PLAY), it coud be seen that the culture media became very blur. Among the yellow and brown tones, they had a material deposit rings above the height of the culture medium and agglomeration over the fabric, too.

Therefore the experiment was the best test results of all dye experiments for cambric fabric coloring, where was observed a strong pigment color, as it can be seen on figure 8. Isolated G27, G29 and G85 presented a much darker coloration, with high concentration.

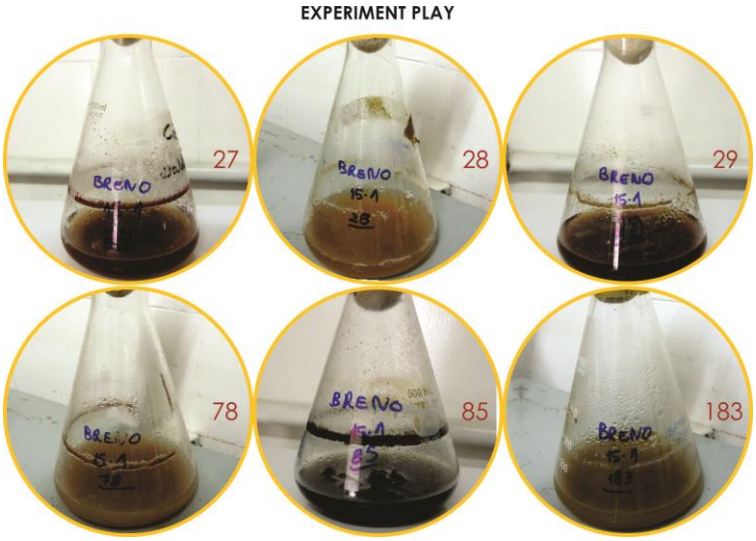

Figure 8. Results from experiment PLAY. Notice that isolated G27, G29 and G85, were the ones that more intensely produced the coloration.

On the other hand, on experiment STOP, where bacteria was grown in liquid medium, but static in incubator at 37ºC, another phenomenon can be observed, the formation of a layer on the surface of the culture media with a predominant whitish characteristic and a slight glow formed by the bacterial spores, that can be more evidently seen in the isolates G29 and G85 on figure 9.

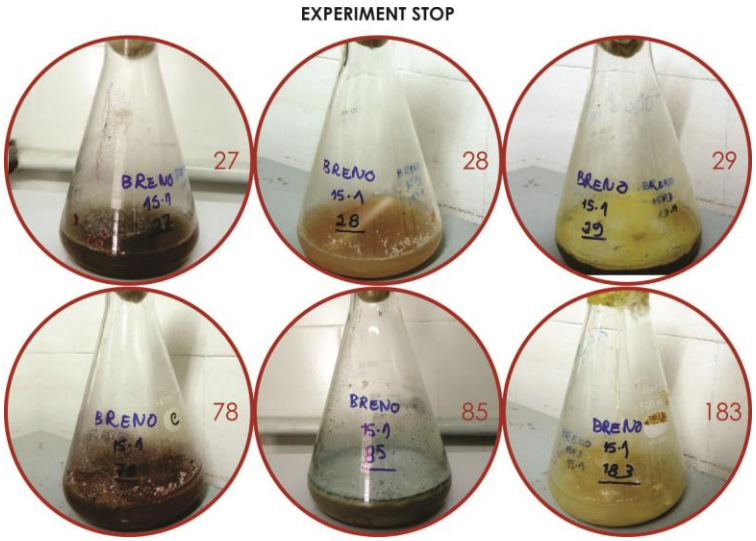

Figure 9. Results from experiment STOP after 5 day growth. The formation of a supernatant layer on isolates G29 and G85.

Although it might appear to have had a good growth, the dye did not occur in a efficient manner in the STOP experiment, probably due to the lack of rotation which makes better the contact between the pigment and the fabric which was slightly dyed.

Comparing experiments PLAY and STOP we can see how distinct they are only because of the lack of agitation and consequent aeration. It is possible to infer that, for a more efficient production of pigment, a good supply of oxygen is important.

In the last dye testing performed in Petri dishes, but in liquid medium, that characterized experiment COTTON, a moderate result was obtained, because it did not present a large production of pigment. Besides, it was verified that it was an experiment that needed adjustments, because the amount of culture medium response on the dish was very big. The presence of cotton that should have aided on the preservation of humidity, messed up the dyeing, because it ended up pulling the pigment through capillarity. The colony`s morphology also presented a different aspect, more isolated, when on the solid medium it generally formed a cellular layer (figure 10).

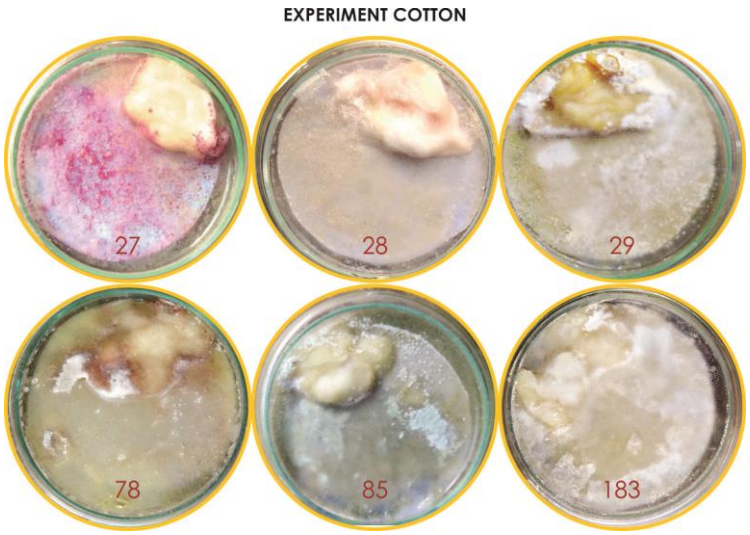

Figure 10. Results from experiment COTTON, which did not obtain a very strong coloration and presented isolated colonies.

These result from the Petri dish experiments, either with solid or liquid media, were compared with the aid of a cellular counter, that has magnifying lens with approximately a 5X zoom capacity and a light coming from underneath the dish, that makes evident the colony`s shape and the color of the pigment produced (figure 11).

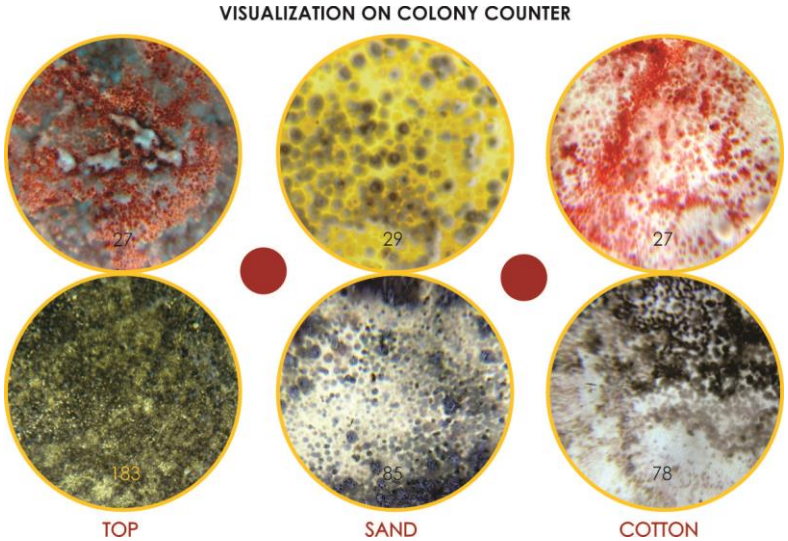

Figure 11. Comparison of TOP, SAND and COTTON experiments, performed with a cellular counter, showing colonies and isolates coloration.

Figure 11 also shows how much the coloration of isolates **G27** and **G29;** burgundy and yellow become more visible in relation with other isolates. It could also be seen that the amount of colonies was bigger in experiment **TOP** than in the SAND and COTTON experiments. The culture medium and fabric`s background coloration was a lot larger in the TOP experiment than in the rest of them.

In the last stage, the COTTON experiment was redefined and performed again, but using seven different kinds of natural fiber fabric and only two isolates that presented rapid growth and good pigmentation, isolates G27 and G85.

The experiment was redone with the removal of the cotton and diminishing of the response media amount, which presented excellent results during the days, allowing the microorganism's faster growth and better pigmentation.

Among the fabrics, 100% cotton (tricoline, toweled, guipure and laise), 100% silk (silk georgette and pure silk), and 100% linen (pure linen), the one that presented better growth and fabric pigmentation was linen, as shown on figure 12.

Tricoline, that has less texture and thinner organized fibers presented a larger spore production and good pigmentation. Now, the silk fabrics had a superficial dyeing, in pastel shade, very smooth and delicate.

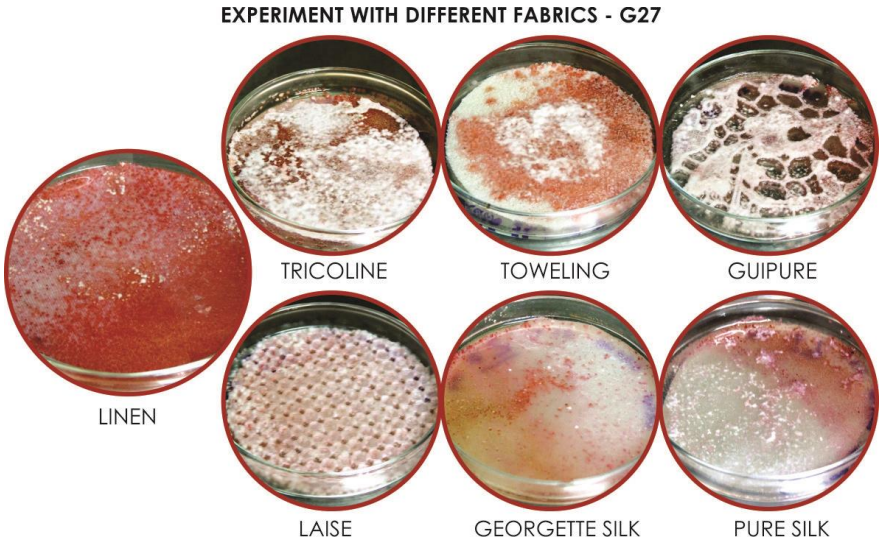

Figure 12. Comparison of different fabrics grown in Petri dish with liquid media, and the G27 isolate, highlighting good growth result on linen.

In the same experiment, but using the G85 isolate, we get a similar result in dyeing terms, but different in relation to growth.

When analyzing the fabric`s final dyeing, linen was also the one that had better coloration at the end of the drying process. Isolated G85 had an excellent growth on all fabrics and also formed different textures on the embossed fabrics, such as laise, guipure and toweling (figure13).

A negative point of isolated G85 is the fact that it has a greenish aspect, which is similar to the fungus that we commonly call mould, not presenting a pleasant color, but presents diversified and very interesting textures for surfaces.

Figure 13. Comparison of different fabrics grown in Petri dish with liquid medium, and the G85 isolate, highlighting good growth result on linen.

Some of these samples were analyzed with optic microscopy with 200X zoom and photographed to analyze the microorganism` morphology. Depending on the fabric, this visualization was jeopardized, mainly on fabrics that presented very thick fibers, which prevent the passage of light.

First analyzing linen, because this fabric obtained the best coloration with isolates G27 and **G85**, it presents very thick fibers, being difficult to visualize the microorganisms (figure 14).

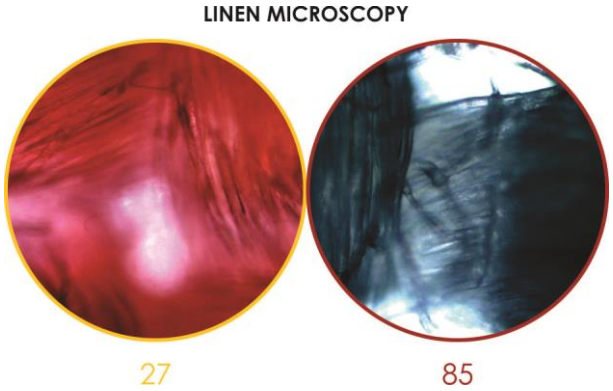

Figure 14. Optic Microscopy of isolates G27 and G85, with 200X zoom on linen.

Now, on other cotton fabrics (figure 15), with the exception of toweled, which is too textured, it is a lot easier to see the actinobacteria, especially with isolate G85, characterized by small dark groups with several united spirals.

It can be seen that the location of these isolates are in between the strings, possibly being the adherence region of the microorganism. The reddish coloration of isolate G27 becomes more clearly evident, and despite the coloration of isolate G85 be shown as bluish, the color seen on the fabric with naked eye is a grayish green.

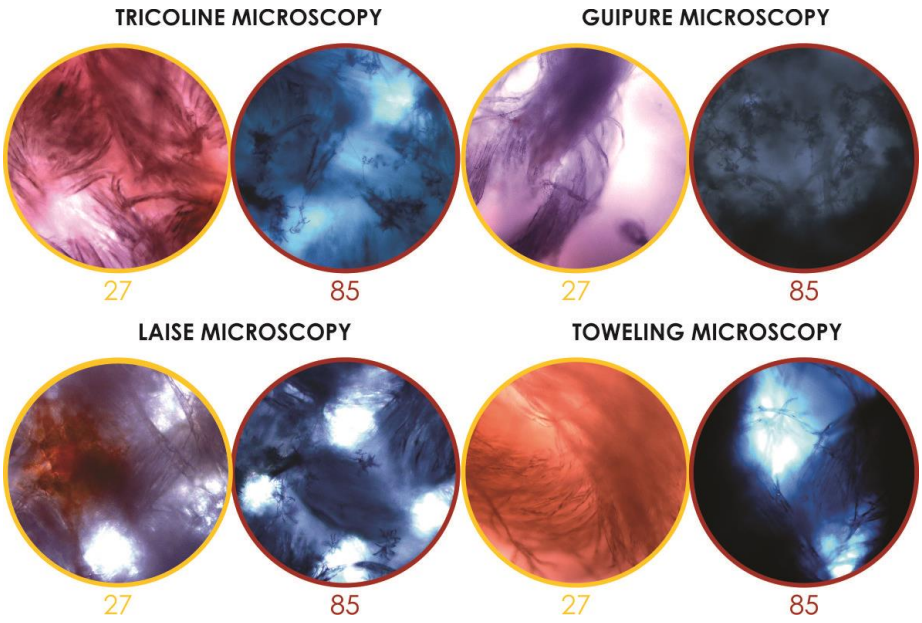

Figure 15. Optic Microscopy of isolates G27 and G85, with 200X zoom on fabrics with 100% cotton composition.

And finally silk fabric, that present much thinner and light strings, especially georgette silk, clearly presents its spiral morphology on isolated **G85**. The easiness to visualize the georgette is also due to the low twisting of the string and larger spacing of the strings, (figure 16).

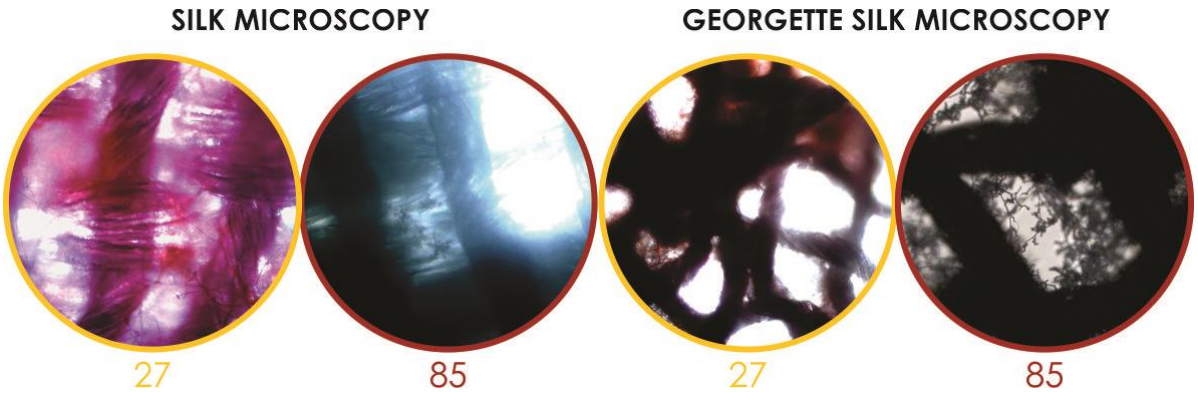

Figure 16. Optic Microscopy of isolates G27 and G85, with 200X zoom on fabrics 100% silk.

Summarizing, the best way to perform the coloration of organic fabric with actinobacteria isolates is with liquid growth medium under shaking, where a higher production and concentration of dye occur, in addition to the increased contact of the pigment with the fabric.

In relation to the color of the pigments produced, the ones that presented higher saturation were isolates G27, G29 and G85, in opposition to G183 that practically doesn`t present coloration.

The washing process showed that, despite the possibility of dyeing the fabrics, another process for adherence of the color is necessary, since all dyed the water during the washing. As the research primes for sustainable processes, possible natural color adherence processes will be analyzed in the future.

Analyzing a larger diversity of fabric samples, the ones that better dye are the ones composed of linen, followed by cotton and silk. Therefore, linen will be used in future tests, as well as tricoline that showed a good connection with isolates G27 and G85.

4. Results and print work process debate

After the bacteria inoculation, the growth was observed during 3, 5 and 7 days. It could be verified that a large growth from the third to the fifth day occurred, although, after the fifth day the print`s growth is stabilized, not occurring considerable changes in the color nor in the morphology of the print/colony, only needing 5 days for the bacteria growth under the determined conditions of 37ºC temperature.

Comparing the bacteria growth in experiments A and B, it was verified that there is no difference in the print in terms of morphology and coloration, if the material is inoculated with or without the fabric, probably due to the fabric`s permeability and the thin layer of culture media deposited over the fabric, as it can be seen in figure 17. The coloration only appears to be stronger in experiment B due to the absence of fabric, which makes the picture more opaque.

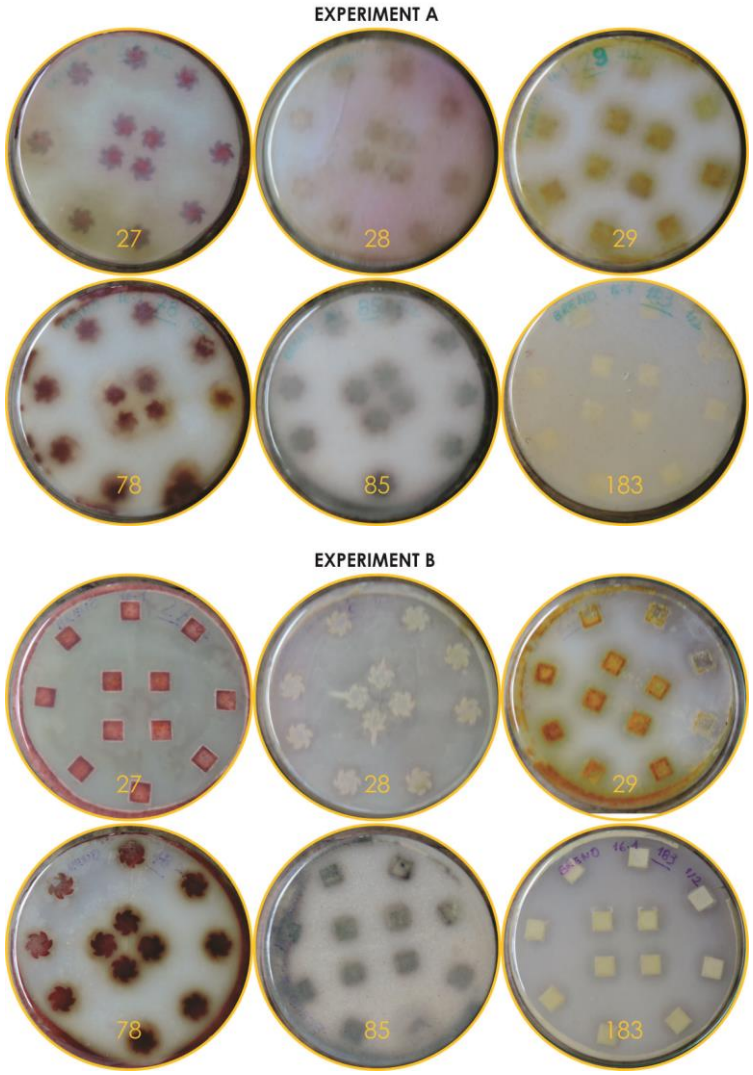

Figure 17. Print/colony`s morphology of all 6 lineages with seven days of growth in experiments A and B.

In experiment A we verified that the G27 and G85 lineages presented better print development, as well as a better growth and more solid coloration.

The biggest success was obtained with experiment A, mainly due to the drying process. While it is possible to remove the fabric just with a thin layer of culture medium in experiment A, experiment B needs to be dried, unavoidably, with all the culture medium`s volume, which

makes the fabric full of contaminants and too dense. After the drying process, in experiment A, the print adheres to the faric, while in experiment B the print still remains adhered to the culture medium and is lost in a layer superposed to the fabric.

During the washing process of the resulting fabrics and prints from experiment A, a certain amount of pigment from the print was released in the water, not jeopardizing the print that kept its tonality, only removing the contaminants from the fabric. In this case, the print becomes fragile, notwithstanding it isn`t damaged, as it can be seen in picture 18 which shows the comparison of fabrics before and after washing of the prints produced with lineage G27.

Now, after drying under shade, the fabric was ironed with a domestic iron. The fabric didn`t go through any alteration while being ironed on the right side, as well as on the reverse side, as it can also be seen in figure 18, of G27`s lineage print.

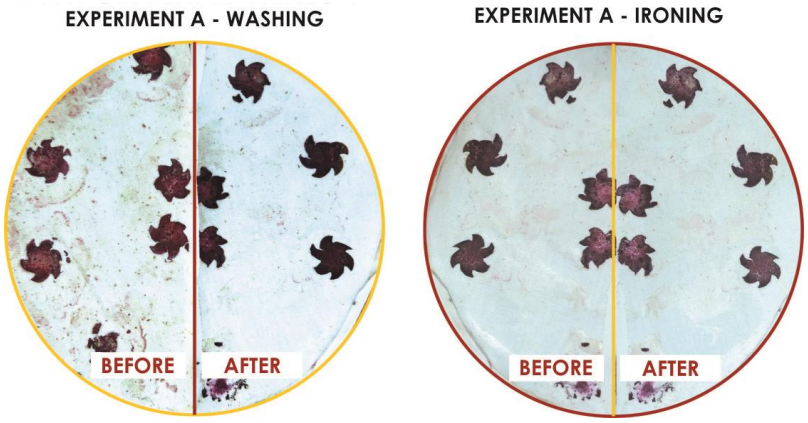

Figure 18. Experiment A`s print, before and after washing and drying.

In experiment C, the ideal conditions obtained with experiment A were repeated with lineages G27 and G85, it can be seen that it is possible to make prints using am acetate cut with more complex pictures, acquiring different drawings, still with good results. It can be seen in figure 19, which shows the inoculated dishes, the prints obtained and the fabric ready for drying.

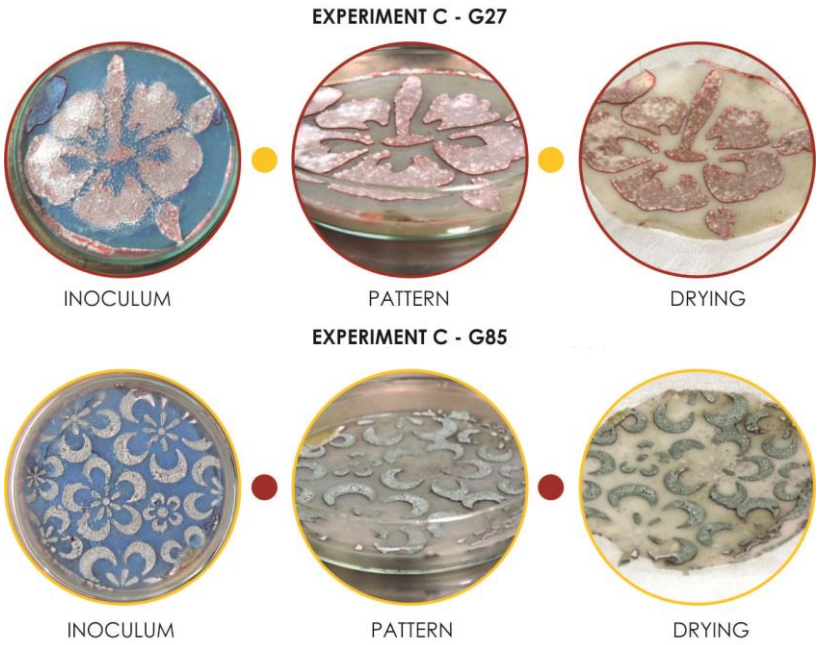

Figure 19. Inoculums, print and drying of lineages G27 and G85 performed during experiment C, where print with more number of details were performed.

The changing of fabric didn`t cause any significant alteration to the experiment, probably because it still is a 100% cotton fabric.

It was verified that the production of prints through the stencil method is possible using lineages of actiobacteria as a raw material, instead of ink. Of the lineages used, the ones that presented better results in terms of morphology, as well as adherence and resolution, were lineages G27 and G85.

To bring to completion, the ideal drying process to obtain prints with the less number of contaminants is when the inoculums are performed with the fabric united with the culture medium, obtaining cleaner prints with better resolution and adherence. Prints obtained in more complex ways were also possible.

After the print production, the drying and ironing process did not damage nor modified the placement or form of the print, being possible to perform them successfully.

The present work can be used in the future for industrial processes for obtainment of localized prints on surface design, as long as the obtainment method is adjusted to a line production process.

The next research steps will be oriented to better adherence techniques to the print after washing, making it more durable. It is also in our interest, investigate the extraction of the pigments produced by these actinobateria lineages for direct use in dyeing and printing.

Besides, it is salutary to analyze the interaction between print and the user`s skin to verify if there could be any kind of allergic reaction on the skin and more specific specie determination of the lineages used.

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