WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 7.421
Volume 8, Issue 4, 1490-1498 Research Article ISSN 2278 – 4357

ENHANCED *STAPHYLOCOCCUS AUREUS* PIGMENT FORMATION UNDER THE INFLUENCE OF GUM ACACIA

M. T. Nurjahan[1], Sneha Saha[1], Asha Ninan[1] and Satadal Das[2]*

[1]*Dept. of Microbiology, the Oxford College of Science, Bangalore, University of Bangalore.
[2]Peerless Hospital & B. K. Roy Research Centre, Kolkata.

Article Received on
18 Feb. 2019,
Revised on 10 March 2019,
Accepted on 31 March 2019,
DOI: 10.20959/wjpps20194-13531

***Corresponding Author**
Prof. Satadal Das
Dept. of Microbiology, the Oxford College of Science, Bangalore, University of Bangalore.

ABSTRACT

Most *Staphylococcus aureus* strains produce the orange colored carotenoid pigment, staphyloxanthin. In this study we find an enhanced pigment formation of *S. aureus* in presence of gum acacia particularly after exposure to sunlight. This enhanced pigment formation was found at a concentration of 1g/dl and the pigment formation was decreased at 2g/dl concentration possibly due to antimicrobial action of gum at higher concentration. We have also tested another gum – gum tragacanth, but it did not show any changes of pigment formation in comparison to the controls. Thus we conclude that gum acacia at a concentration of 1g/dl may be used for enhanced pigment formation of *S. aureus* which may be used for commercial exploitation for production of carotenoids.

KEYWORDS: *Staphylococcus aureus*, gum acacia, gum tragacanth, carotenoid pigment.

INTRODUCTION

Natural colors are generally extracted from fruits, vegetables, roots and microorganisms and are often called bio-colors because of their biological origin.[1] Microorganisms produce various pigments which they are responsible for the yellow, orange, red and purple colours in a wide variety of plants, animals and microorganisms.[2] There are many reasons for bacteria to produce pigments such as photosynthesis, UV protection, defense mechanisms, secondary metabolites for storage of energy, stress which varies with environment.

These pigments play an important role as protective agents against oxidative damage.[3] Recently carotenoids have attracted greater attention due to beneficial role in human health. They are known to inhibit various types of cancer and elevates immune response in the

body.[4] Diseases which emanate due to "life style-related" habits such as cardiovascular disease and age related muscular degeneration can be prevented using carotenoids due to their antioxidant activity and pro vitamin A function.[5] A central role is played by carotenoids such as β-carotene in the metabolism of eye's macula and retina and in maintaining healthy vision.[6] They find an important industrial application as colorants in pigmenting salmon, poultry flesh, egg yolk and trout.[7]

Color is one of the most vital characteristic of any substance and is the first to be perceived by the senses.[8] It has a huge role in influencing the visual appearance of any product and hence the first parameter which is considered by the customer while purchasing a product.

The renewed interest to use more and more of natural products and minimizing chemical processes has led to a decline in the production of synthetic coloring agents or chemicals used as food additives.[9] The synthetic colorants may pose threat to human health due to their toxic effects such as mutagenecity and potential carcinogenicity and hence the focus has now shifted towards the development of pigments from natural sources.[10]

Most *Staphylococcus aureus* strains produce the orange colored carotenoid pigment, staphyloxanthin. The species epithet of *Staphylococcus aureus* can be differentiated from *Staphylococcus epidermidis*(formerly *Staphylococcus albus*) due to the distinguishing color of its colonies(S.aureus : golden).[11] Marshall and Wilmoth isolated the pigments from *S.aureus* as 17 intermediary products by chemical analysis and identified them as triterpenoid carotenoids having a C_{30} chain whereas C_{40} carotenoid structure was found in most other organisms.[11] α-D-glucopyranosyl1-0-(4,4'-diaopneurosporen-4-oate)-6-0-(12- methyl tetradecanoate) containing glucose esterified with both a triterpenoid carotenoid carboxylic acid and a C_{15} fatty acid was identified as the main component of the pigment, staphyloxanthin.[12]

The actual sale of carotenoids is estimated to be US $ 500 million approximately with an ever increasing market demand. Functional food is sought after right now hence inclining people towards more and more use of carotenoids resulting in its huge market value.[9]

Gum acacia also known as gum arabic is a natural gum consisting of the hardened sap of various species of the acacia tree. Gum arabic is collected from acacia species, predominantly *Acacia senegal* and *Vachellia seyal*. Whereas gum tragacath is obtained from the dried sap of

Middle Eastern Legumes of the genus *Astragalus* including *A.adscendens, A.gummifer, A.brachycalyx* and *A.tragacatha.*

The present study was aimed at isolation and determination of the enhancement in the production of staphyloxanthin pigment by *S.aureus* in the presence of gum Acacia and Tragacanth and spectrophotometric estimation of the carotenoid present in the isolated pigment and henceforth, planning a commercial exploitation of carotenoids.

MATERIALS AND METHODS

Bacteria: International strain (ATCC 29213) of *S.aureus* grown on blood agar media used in the experiment. The colonies appeared circular, 2-4mm in diameter, opaque,light golden yellow colored with β-haemolysis on the blood agar (Fig 1). For the experiment, Mueller Hinton media was prepared and autoclaved.

(a) ***(b)***

Figure 1: (a) ATCC strain 29213 Staphylococcus aureus on blood agar. (b)golden yellow colonies of S.aureus showing ß-haemolysis.

Gums: Gum acacia was procured from HiMedia[CAS No.- 9000-01-5, maximum limits of impurities: Ash- 5.0%, Acid insoluble ash- 0.5%, Insoluble matter- 0.5%, Loss on drying(at 105°C) – 15.0%] and gum tragacanth was procured from HiMedia(CAS no.-9000-65-1, Mol wt- 840,000, a complex mixture of polysaccharides including tragacanthin an bassorin). Gum acacia and gum tragacanth were prepared in both 1% and 2% concentrations in distilled water in four test tubes and vortexed properly for a homogenised mixture. All the tubes containing different chemicals were put for autoclaving. Sterilized cotton swabs were used to take the gum solution and slowly swabbed onto the individual Mueller Hinton agar plates. They were kept for drying in the incubator. After 10 minutes, the plates were taken out. Individual

colonies were picked from the blood agar plate and were streaked on the six Mueller Hinton media plates already having the gum surface coating on them (Table1). The plates were kept for overnight incubation at 37°C along with control plates.

Table 1: Different concentration of the gum coated on the Mueller hinton agar plates.

SL. NO. OF PLATES	ID NO. OF PLATES	GUM COATED	CONCENTRATION OF GUM COATED
1	A_1	Acacia	20 mg in 2 ml (1g/dL)
2	A_2	Acacia	40 mg in 2 ml(2g/dL)
3	T_1	Tragacanth	20 mg in 2ml(1g/ g/dL)
4	T_2	Tragacanth	40 mg in 2ml(2g/ g/dL)
5	C_1 control	-	-
6	C_2 control	-	-

The next day, the plates were taken out of the incubator and kept in sunlight for 10 minutes for the enhanced pigment production, under the influence of sunlight.

Extraction of carotenoid pigment

After the exposure to the sunlight, colonies equivalent to 0.002±0.001 gm weight from each of the six plates were picked up and added to 6 diferent eppendorf tubes containing 1 ml of methanol each. The tubes were vortexed for 30 seconds for better extraction. Then the tubes were centrifuged for 10 minutes at 14,000 rpm in a high speed centrifuge. The supernatant was taken and 100 µl from each tube was dispensed in microwells of an ELISA plate and the absorbance was analysed at 540 nm(complimentary wavelength of the color generated)in an "Thermo Multiskan EX" spectrophotometer and readings were obtained using Ascent software(Germany).

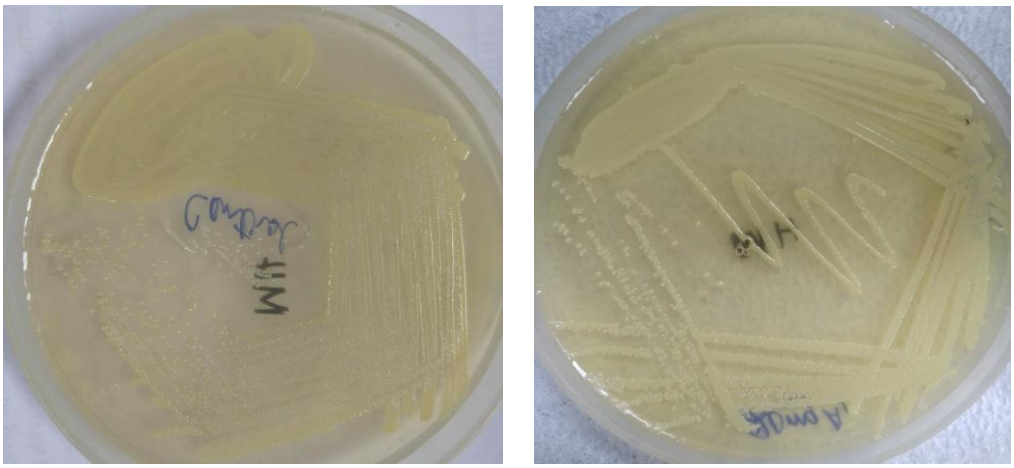
RESULTS

Examination of the colony characters on the Mueller hinton agar plates

After incubation, the colony characteristics were observed which were different. In control plates, colony morphology was observed as round, low convex, cream color and 1-4mmin diameter with entire edge (Fig 2). Colonies on both the concentrations of gum tragacanth were almost same as observed on control plates. In the first plate of gum acacia(1g/dL), colony appeared as round, low convex, orange colored and 1-4mm in diameter with an entire edge. While on the second plate(2g/dL), colonies appeared as round, low convex, pale orange and 1-2mm in diameter entire edge.

Plates on exposure to sunlight

The plates were exposed under sunlight for 10minutes to enhance the color development. The difference in the pigment production of gum acacia on 1g/dL plate was very much detectable with the naked eyes as the colonies turned brighter orange after sunlight exposure(Fig 2A), in comparison to the other plates and the control.



(c) ***(d)***

Figure 2: (c) S.aureus on Mueller hinton agar plate for control, (d) S.aureus on Mueller hinton agar coated with gum acacia concentration of 1g/dL.

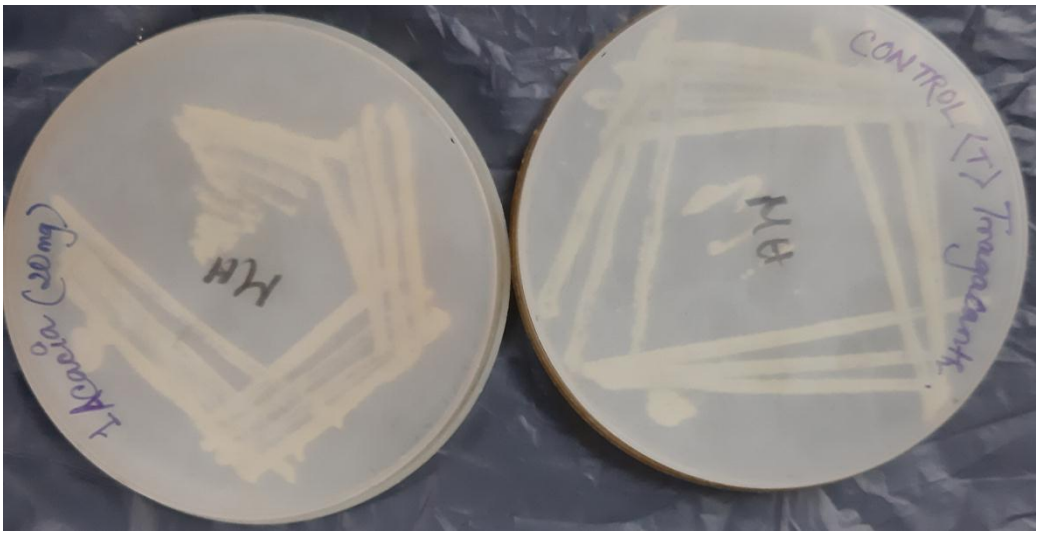


Figure 2A: The differences of pigments after exposure to sunlight gum acacia (left) and gum tragacanth (right).

Spectrophotometric readings of the extracted pigment

O.D taken at 540 nm in the Thermo Multiskan EX with different extracted materials (Table 2, Fig 3).

Table 2: Tabulated statistical data for the recorded absorbance of the different extracts at 540nm.

ID NO. OF TUBES	NO. OF STUDIES	ODVALUE MEAN±STANDARD DEVIATION±STANDARD ERROR OF MEAN
C	6	0.069333±0.016071±0.007187
A_1	3	0.119333±0.005508±0.003894
A_2	3	0.067333±0.002082±0.001472
T_1	3	0.059667±0.003055±0.00216
T_2	3	0.056667±0.019502±0.01379

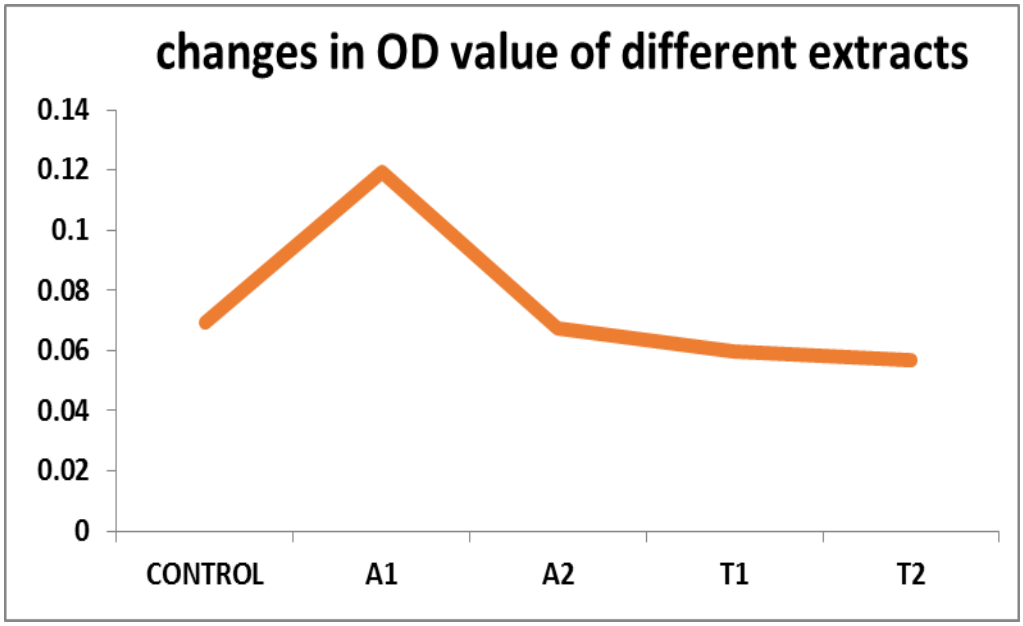


Figure 3: Graph showing change in OD value of different extracts(along X axis: ID no. of tubes, along Y axis: mean value of OD at 540nm).

STATISTICAL ANALYSIS

P value and t value calculation (Table 3)

1. Between control and 1g/dL of gum acacia(C X A1)

The two-tailed P value equals 0.0014

By conventional criteria, this difference is considered to be very statistically significant.

t = 5.0879

2. Between control and 2g/dL of gum acacia(C X A2)

The two-tailed P value equals 0.8415

By conventional criteria, this difference is considered to be not statistically significant.

t = 0.2075

3. Between control and 1g/dL of gum tragacanth (C X T1)

The two-tailed P value equals 0.3510

By conventional criteria, this difference is considered to be not statistically significant.

t = 0.9992

4. Between control and 2g/dL of gum tragacanth(C X T2)

The two-tailed P value equals 0.3302

By conventional criteria, this difference is considered to be not statistically significant.

t = 1.0462

5. Between 1g/dL and 2g/dL of gum acacia (A1 X A2)

The two-tailed P value equals 0.0001

By conventional criteria, this difference is considered to be extremely statistically significant.

t = 15.2957

6. Between 1g/dL of gum acacia and 1g/dL of gum tragacanth(A1 X T1)

The two-tailed P value is less than 0.0001

By conventional criteria, this difference is considered to be extremely statistically significant.

t = 16.4078

7. Between 1g/dL of gum acacia and 2g/dL of gum tragacanth

The two-tailed P value equals 0.0059

By conventional criteria, this difference is considered to be very statistically significant.

t = 5.3561

8. Between 2g/dL of gum acacia and 1g/dL of gum tragacanth

The two-tailed P value equals 0.0229

By conventional criteria, this difference is considered to be statistically significant.

t = 3.5915

9. Between 2g/dL of gum acacia and 2g/dL of gum tragacanth

The two-tailed P value equals 0.3996

By conventional criteria, this difference is considered to be not statistically significant.

t = 0.9419

10. Between 1g/dL and 2g/dL of gum tragacanth

The two-tailed P value equals 0.8054

By conventional criteria, this difference is considered to be not statistically significant.

t = 0.2632

Table 3: Matrix showing the p value for the different extracts (* = extremely significant, ** = very significant, *= significant)**

	C	A_1	A_2	T_1	T_2
C	-	0.0014**	0.8415	0.3510	0.3302
A_1	0.0014**	-	0.0001***	0.0001***	0.0059**
A_2	0.8415	0.0001***	-	0.0229*	0.3996
T_1	0.3510	0.0001***	0.0229*	-	0.8054
T_2	0.3302	0.0059**	0.3996	0.8054	-

DISCUSSION

Carotenoids have been demonstrated to increase tolerance to ultraviolet radiation. Under sunlight, more pigment production was observed. In *Staphylococcus aureus*, pigmentation is intracellular and is regulated by SOS stress response indicating a main function in UV tolerance. After exposure to sunlight, DNA damage occurs due to ultraviolet rays and thus, for the survival pigment formation increases to limit the DNA damage. SOS response is a global response to DNA damage in which the cell cycle is arrested and DNA repair and mutagenesis is induced.

Gum acacia being a complex mixture of natural polysaccharide and glycoproteins can act as an enhancer for the natural pigment production. 1g/dL concentration of gum acacia showed a comparable increase in the orangish pigment by the *S. aureus* colonies. The control plate with no gum coating seemed to have pale orange colored colonies which supports the aforesaid statement. While surprisingly, 2g/dL did not show much change in the pigment production compared to the control. Natural gums are known to show antimicrobial property and this maybe the reason for the negligible color change in the 2g/dL concentration plate at a higher concentration. The pigment production is directly proportional to the virulence of *S. aureus* and higher concentration of gum coating might have inhibited its growth and virulence of the bacteria.

REFERENCES

1. Pattnaik P, Roy U, Jain P, Biocolors: New generation additives for food, Indian food industry, 1997; 16: 21-22.

2. Wilhelm S, Helmut S, Lycopene: A biologically important carotenoid for humans?, Archives of biochemistry and biophysics, 1996; 336: 1-9.
3. Scolnik PA, Bartley GE, Nucleotide sequence of a pulative geranyl geranyl pyrophosphate synthase from Arabidopsis, Plant physiology, 1995; 104: 1469-1470.
4. Krinsky NI, Johnson EJ, Carotenoid actions and their relation to health and disease, Molecular aspects of Medicine, 2005; 26: 459-516.
5. Young AJ, Lowe GM, Antioxidant and prooxidant properties of carotenoids, Archives of Biochemistry and Biophysics, 2001; 385: 20-27.
6. Chidambaram KV, Perumalsamy L, An insightful overview on microbial pigment, Prodiogiosin, Electronic journal of biology, 2009; 5(3): 49-61.
7. Johnson EA, Schroeder WA, Microbial carotenoids, Advances in Biochemical engineering/Biotechnology, 1995; 11: 297-326.
8. Indra AP, Umamaheshwari S, Ranandkumar SG, Karthik C and Jayakrishna C, Screening of yellow pigment producing bacterial isolates from various eco-climatic areas and analysis of the carotenoid produced by the isolate, J Food Process Technol, 2014; 5.
9. Aberoumand A, A review article on edible pigments properties and sources as natural biocolourants in foodstuff and food industry, World Journal of Dairy and Food Sciences, 2011; 6: 71-78.
10. Domiguez-Espinosa RM, Webb C, Submerged fernentation in wheat subatrates for production of Monascus pigments, World Journal of Microbiology and Biotechnology, 2003; 19: 329-336.
11. Rosenbach, F. J, Microrganismen bei Wun dinfektionskrankheiten des Menschen, J.F Bergmen, Wiesbaden, 1884, Wiesbaden, Germany.
12. Wieland B, Feil C , Gloria-Maercker, E, Thumm G, Lechnar M, Bravo J.M, Poralle K and Gotz F, Genetics and Biochemical analysis of the biosynthesis of the yellow carotenoid 4,4' diaponeurosporene of S.aureus, Journal of Bacteriology, 1994; 176: 7719-7726.