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IMPACT OF VARIOUS FERMENTATION CONDITIONS ON THE PRODUCTION OF VIOLACEIN BY THE NOVEL ISOLATE CHROMOBACTERIUM VACCINII CV5

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ABSTRACT

The purple-coloured pigment violacein, produced mainly by bacteria of the genus *Chromobacterium*, had attracted and increased interest owing to its important biological activities and pharmacological potential. In the present study experiments were designed to investigate the effect of different fermentation conditions on the violacein production by novel isolate *Chromobacterium vaccinii* CV5. Different parameters were systematically manipulated in submerged fermentation to improve the yield of total violacein. The violacein production was maximum at Luria Bertani broth and also supported by the addition of glucose and tryptophan as carbon and nitrogen sources at 37°C with pH of 6.5 at 72 h incubation and agitation of 150 rpm.

KEY WORDS: Chromobacterium vaccinii, violacein, optimization, agitation, pigment

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INTRODUCTION

Chromobacterium sp, is a Gram-negative bacteria belonging to the Rhizobiaceae family, a genus of saprophytic, which has been generally isolated from the soil and water in tropical and subtropical areas. In most cases, it is a minor component of total microflora. *Chromobacterium* sp have occasionally been associated with rapid and lethal infections of humans [1-4] and other mammals. [5-6] It includes both pigmented and non-pigmented strains. The genus *Chromobacterium* has undergone numerous revisions, expansions and contractions since it was first described a century ago. [7,8] The current edition of Bergey's manual of systematic bacteriology lists only the type of the species, *Chromobacterium violaceum* [9] but some additional species have been proposed since 2007, based on the recognition of significant genetic, metabolic and ecological differences: *Chromobacterium subtsugae*,[10] *Chromobacterium aquaticum*, [11] *Chromobacterium haemolyticum*, [12] *Chromobacterium piscinae, Chromobacterium pseudoviolaceum* [13] and recently *C. vaccinii*. [14] Violacein is a bluish –purple pigment produced by *Chromobacterium* sp. It is a dimeric structure composed of 5-hydroxy indole, oxindole, and 2-pyrolidone subunits formed by condensation of two modified tryptophan molecules. [15] Violacein has several kinds of biological activity, including the broad-spectrum antibacterial activities against *Staphylococcus, Streptococcus, Bacillus, Mycobacterium, Neisseria* and *Pseudomonas.* [16] In addition, violacein has strong bactericidal, [17] antitumor, [18] anti-viral, [19] antioxidant [20] and anti-protozoan activities. [21-22] Violacein can also be used as a biological dye and coloring agent. [23] Therefore, it is of great importance to discover new microorganisms effectively producing violacein with better biological process. It has been reported that other than *Chromobacterium violaceum*, some other species such as *Chromobacterium fluviatile*, *Janthinobacterium lividum*, *Alteromonas luteoviolacea* [24] and *Pseudoalteromonas luteoviolacea* could produce violacein. Among them *C. violaceum* is well known. The advantages of microbial pigment include easy and fast growing in the cheap culture medium, independent from weather conditions, and colors of different shades. Hence, microbial pigment production is now one of the emerging fields of research. It also has immense potential for various industrial applications. [25] In this article an investigation on the effect of various physiochemical parameters on the production of violacein pigment from newly isolated strain *Chromobacterium vaccinii* CV5 is presented.

MATERIALS AND METHODS

Microorganisms

The *Chromobacterium vaccinii* CV5 isolated from the well water identified by morphologically and genetically was used in the present study. [26]

Pigment production and extraction

The production profile of crude violacein was obtained using a 500 mL flask containing 200 mL of nutrient broth. Fermentations were carried out at 37°C for 48 h with an inoculum size of 3% (v/v) 24 h old culture (OD 660 approximately 1). After 48 h of incubation period broth was taken for pigment extraction. For fast and simple assay of the violacein concentration, the crude violacein separated from the cells was measured as follow: (1) aliquots of 5 mL fermented broth were collected, then centrifuged at 10,000×*g* for 5 min. The supernatant was discarded; (2) the cell pellets were then rinsed with deionized water, followed by centrifugation (10,000×*g* for 5 min) and decanting of supernatant, (3) a 5 mL ethanol (with a purity of 99.7%) was added to the pellets, and the cells were disrupted by ultra-sonication (200 W and 10 min). The ethanol extract was then separated from the cells by centrifugation at 10,000×*g* for 5 min; (4) this extraction procedure was repeated until the cells were completely bleached and (5) all the supernatants were collected as crude violacein for measuring the violacein concentration. The absorbance of ethanol solution of crude violacein sample was measured by using an ultraviolet-visible spectrophotometer (Beckman DU800, USA) (extinction coefficient of violacein = 10.955 L/ (g cm) in ethanol at 570 nm). [27]

Effect of various media on violacein production

For optimization of the media for enhancing the yield of violacein, 1ml of overnight culture of *C. vaccinii* CV5 was inoculated into six different media such as nutrient broth (NB), luria bertani broth (LB), tryptic soy broth (TSB), peptone glycerol broth (PGB), brain heart infusion broth (BHI) and peptone broth (PB). The culture flasks were incubated at 37°C. After 48 h of incubation period broth was taken for pigment extraction, estimation as stated above. [28-29]

Effect of temperature

The influence of incubation temperature on pigment production by *C. vaccinii* CV5 was determined by incubating the inoculated broth at different temperature. The fermentation was carried out at 25°C to 52°C at an interval of 3°C, keeping all other conditions at their standard levels and then assayed for pigment production. The optimum temperature obtained by this step was fixed for subsequent experiments. [30]

Effect of incubation period on violacein production

To evaluate the optimum incubation period for pigment production, the fermentations were carried out for different at the time and the durations were from 12 to 96 h at an interval of 12 h. The optimum incubation period achieved by this step was fixed for subsequent experiments. [30]

Effect of inoculum size

To evaluate the effect of inoculum size on violacein production varied cell concentrations (1 to 10%) were added to different flasks containing luria bertani broth and then assayed for violacein production. The fermentation was carried out at 37°C keeping all other conditions at their optimum levels. The optimum inoculum level achieved by this step was fixed for subsequent experiments. [27]

Effect of pH on pigment production

The cultures were inoculated in five different LB containing test tubes and they were kept at different pH

(4-9 at an interval of 0.5) for 72 h incubation. The production of the pigments was estimated after incubation. The maximum pH, at which the maximum production of violacein takes place were observed, chosen and maintained for following studies. [27]

Role of different carbon and nitrogen sources

The selected medium was supplemented with different carbon sources to study its effect on the pigment production. The carbon sources chosen in the present study was dextrose, fructose, glucose, glycerol, maltose, lactose, sucrose and starch. After incubation in an optimal condition the violacein was quantified. Ammonium sulphate, ammonium nitrate, ammonium chloride, yeast extract, beef extract, tryptophan and peptone were chose to evaluate the effect of different nitrogen sources in violacein production. [30]

Effect of agitation

The effect of agitation was studied by incubating the inoculated medium at shaking incubator at 50 to 250 rpm and keeping the other one as static. After 72 h of incubation the violacein was extracted and quantified. [31]

Statistical analysis

The SPSS software (10.0 versions) was used for the major data processing throughout this work. All the results were expressed as the mean ± SD

RESULTS AND DISCUSSION

The optimization was carried out to increase the growth rate and pigment production of *Chromobacterium vaccinii* CV5. Among the physical parameters, the effect of temperature, pH, inoculum size, incubation period, agitation, carbon and nitrogen sources were studied with strain on selected media through submerged fermentation.

Effect of media on pigment production

Selection of a suitable media was crucial in the submerged fermentation. In the present work an attempt is taken to analyze the effect of media on pigment production (Fig. 1). Presently among six media, Luria Bertani (LB) media results in a favorably high pigment production of (1337µg/L) brain heart infusion broth (BHI) shows lower production of pigment (419.85 µg/L) by *Chromobacterium vaccinni* CV5. Luria Bertani broth was used for further studies. Ahmed *et al.* [28] studied the production of violacein in complex media. They got maximum production in nutrient broth and low production in tryptic soy broth. In the present study, LB give maximum production may be due to the capacity of isolates to utilize the tryptone and yeast extract. In BHI absence of tryptone, peptone and yeast extract may lead to the lower production. Drew and Demain, [29] report that the use of carbon sources results in a decrease cellular growth and an increase in the secondary metabolite production.

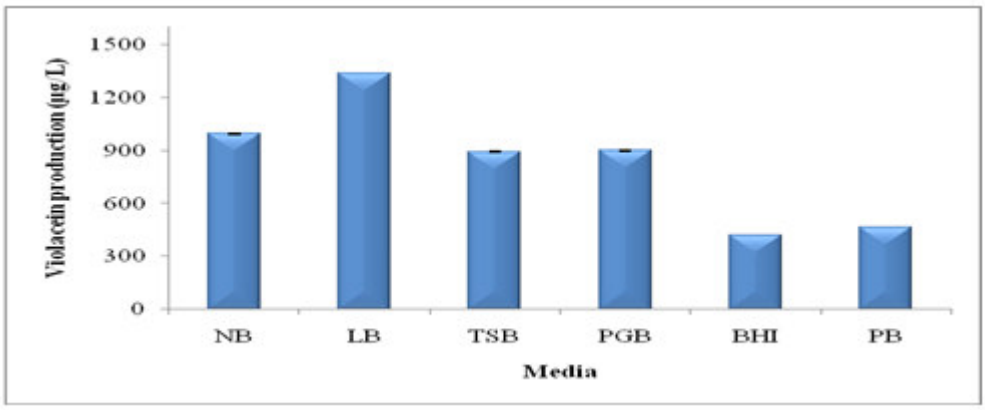

Figure 1
Effect of media on violacein production by C. vaccinii CV5

Effect of temperature

The temperature of incubation has got profound influence on the cell synthesis and pigment production. In the present study, the effect of fermentation temperatures in the range of 25°C to 52°C at an interval of 3°C is examined on the pigment in the production by *Chromobacterium vaccinii* CV5 (Fig.2). At 25°C and 52°C, the accumulation of pigments in media is markedly affected. The optimum temperature is found to be 37°C with maximum pigment production (1358 µg/L) of the selected isolates. In other studies with violacein producing bacteria, the optimum temperature for violacein production are reported at 28, 33, 25 and 20°C for *Chromobacterium violaceum* BB-78, [23] *C.violaceum* CCT 3496, [30] *Pseudoalteromonas* sp X82144 [31] and *Duganella* sp. DSM 19531 [27] respectively. Zhang *et al*. [32] study the optimization of fermentation conditions for violacein production by recombinant *Citrobacter freundii*. In their study, the maximum violacein production is observed at 37°C, this result is similar to the present study. Temperature strongly affects the fermentation process, which varies with respect to the organism involved. Even slight changes in the temperature can affect the pigment production. Temperature is one of the most important environmental factors affecting the growth of microorganisms and it causes changes in many biosynthetic pathways, such as carotenoid biosynthesis .[33] Higher than the optimal temperatures result in the enzyme denaturation and inhibition, excess moisture loss and growth arrest while lower temperatures lead to lower metabolic activities. [34]

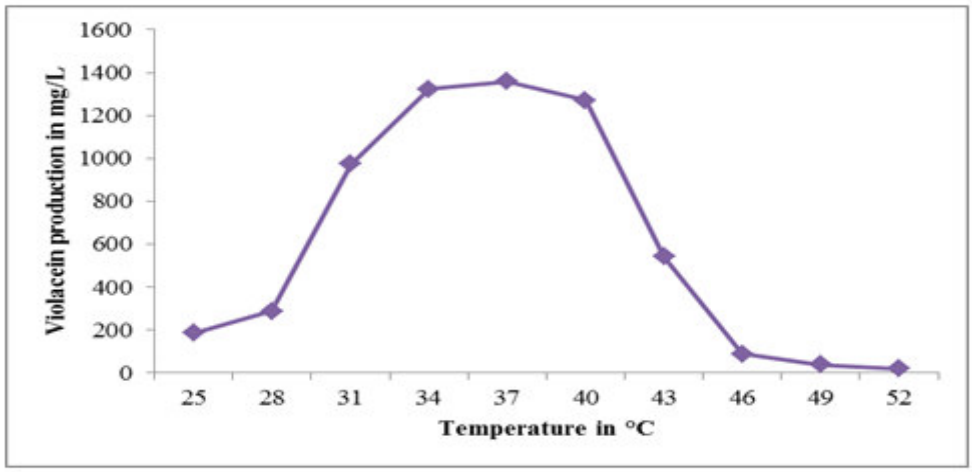

Figure 2
Effect of temperature on violacein production by C. vaccinii CV5

Effect of incubation period

Incubation time plays a substantial role in the maximum pigment production. The influence of incubation period in violacein production by *C. vaccinii* CV5 is studied at an interval of 12 h (Fig.3). It has been observed that the pigment production is increased with respect to increase in incubation time from 12 to 72 h and after the production is declined. The maximum production of violacein (1752 µg/L) is observed at 72 h of incubation. The lowest production is observed at 12 h of incubation. Wang *et al*. [27] in their study report that the maximum production of violacein is at the 32 h by a new strain *Duganella* sp B2. Sivaranjini, [35] reports the maximum production of violacein at the third day of incubation by *Chromobacterium* sp. JC1 in solid state fermentation. In Mendes *et al.* [30] study the highest crude violacein production is obtained after 36 h with *C. violaceum* CCT 3496 and after 40 h for recombinant *Citrobacter freundii* ATCC 05411 in the liquid cultures. Ahmad *et al*. [36] report the maximum production of violacein is at the 4th h after bacterial growth that is at the late exponential phase. Their results suggest that the pigments produced by bacteria are secondary metabolites. [37]

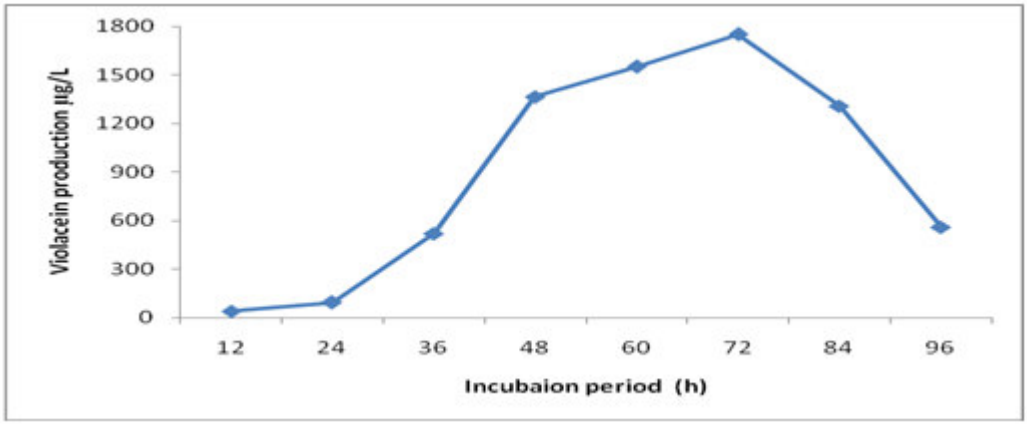

Figure 3
Effect of incubation period on violacein production by C. vaccinii CV5

Effect of inoculum size

The variation on inoculum size affects the biosynthesis of pigment. The effect of inoculum size on pigment production is tested. Inoculum of 8% gave maximum pigment production by *C. vaccinii* CV5 (Fig. 4). High inoculum sizes increase the biomass but decreases the pigment production, due to the inhibition of critical components of culture medium by increased bacterial biomass. [38] An increase in the inoculum size would ensure rapid proliferation and biomass synthesis. After a certain limit, the secondary metabolite production could decrease because of the depletion of nutrients due to enhanced biomass which could result in a decreased metabolic activity. Lower inoculum size requires longer time for the cells to multiply in the sufficient number to utilize the substrate and produce the metabolites. A balance between the proliferating biomass and available nutrient would yield an optimum at which the metabolite synthesis would be maximum. [39] In a study *Sivaranjini*, [35] reports the maximum biomass and violacein production by *Chromobacterium* sp. JC1 in wheat bran is attained at 20% (v/w) inoculum size above which it shows a decrease. At lower inoculum density, metabolite production drops and contamination risks increase due to an insufficient population. With the optimum inoculum concentration, there is a balance between the proliferating biomass and availability of nutrients that supports maximum production. [40]

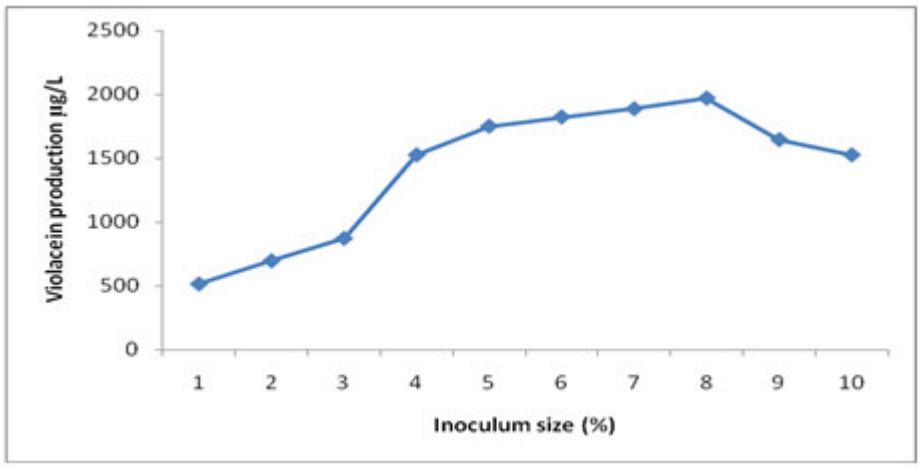

Figure 4
Effect of inoculum size on violacein production by C. vaccinii CV5

Effect of pH

The pH of the growth medium plays an important role by inducing morphological changes in microbes. The medium pH may affect cell membrane function, cell morphology and structure, the solubility of salts, the ionic state of substrates, the uptake of various nutrients and product biosynthesis. In general, cells can only grow within a certain pH range and metabolite formation is also often affected by pH. [41] The pH change observed during the growth of microbes also affects the product stability in the medium. [42] The violacein production at various initial pH (4-9) of the fermentation media is given in the Fig. 5. It is observed that the maximum pigment production is obtained at the pH 6.5. As the initial pH is increased from 4 to 5.5 the pigment production is found to be very low. The activity is found to decrease for further increase in initial pH beyond 7. The optimum initial pH 6.5 is chosen for further studies. Similar results are obtained in a study of Zhang *et al.* [32]

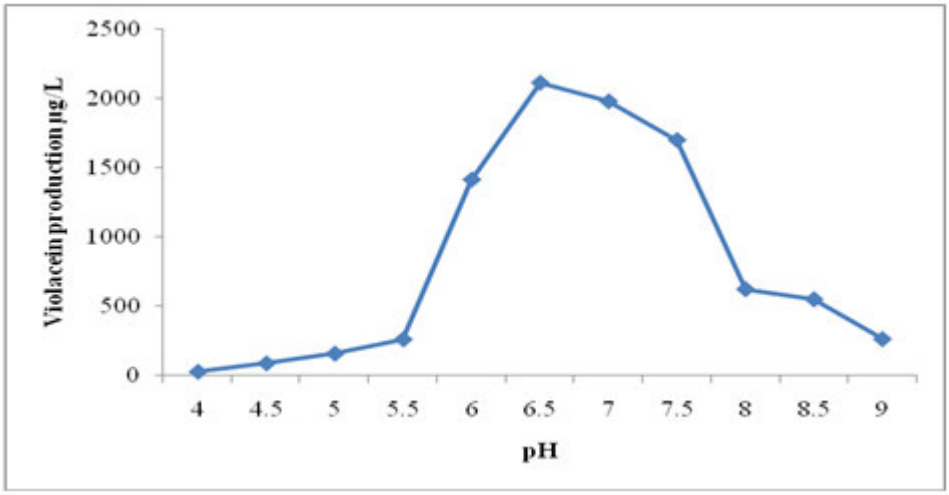

Figure 5
Effect of pH on violacein production by C. vaccinii CV5

Effect of carbon and nitrogen source

Microorganism growth and metabolic production are influenced by the organism's utilization of different carbon and nitrogen sources during the fermentation. The influence of carbon and nitrogen sources on the pigment production is observed by supplementing growth medium with 2% of different carbon sources (Fig.6) (glucose, dextrose, fructose, glycerol, maltose, lactose sucrose and starch), 2% of nitrogen sources (ammonium sulphate, ammonium nitrate, ammonium chloride, yeast extract, beef extract, tryptophan and peptone). In the present study, glucose is found to be the optimum carbon source for the violacein activity by the *Chromobacterium vaccinii* CV5 isolate are followed by dextrose, glycerol and maltose.

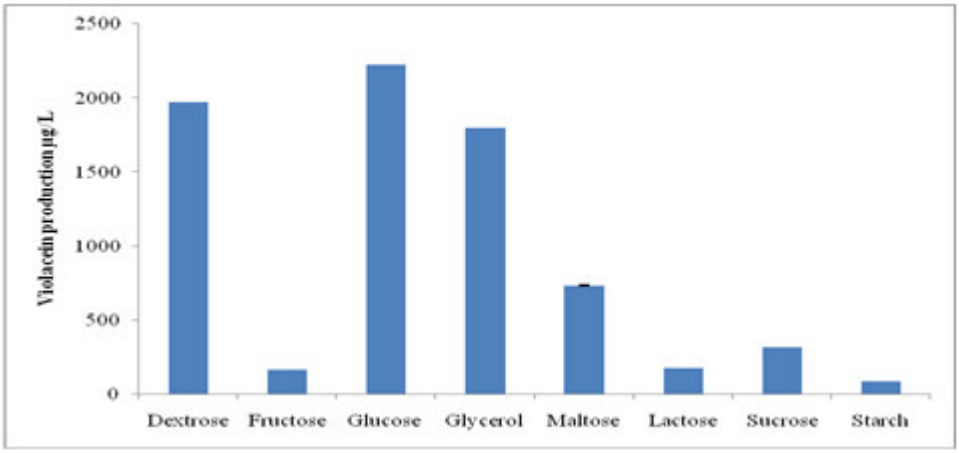

Figure 6
Effect of carbon sources on violacein production by C. vaccinii CV5

Varying the nitrogen sources in the culture medium affect the growth and production of pigment production. Tryptophan is found to be the best nitrogen source for augmenting the pigment production for the *Chromobacterium vaccinii* CV5. In this study tryptophan and yeast extract have a positive effect on pigment production, whereas ammonium sulphate, ammonium nitrate and ammonium chloride strongly inhibit the violacein production. (Fig.7).

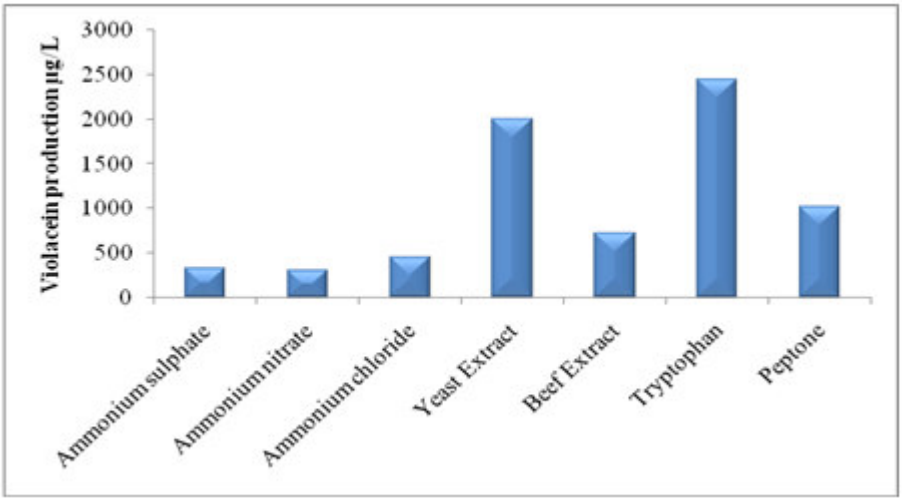

Figure 7
Effect of nitrogen sources on violacein production by C. vaccinii CV5

Effect of agitation

Agitation speed is a very important factor in the fermentation process since it will increase the amount of dissolved oxygen in the cultivation medium. [43] *C. vaccinii* CV5 shows increased growth and pigment production at shaking (150 rpm) incubation conditions (2720 µg/L) as compared to static conditions (2421 µg/L). The data suggests that the maximum growth and pigment extraction can be obtained at shaking conditions with 150 rpm (Fig.8). The violacein production is decreased on further increase in the agitation speed. The results are similar to the study of Yang *et al.* [31] they study the effect of agitation on violacein production in *Pseudoalteromonas luteoviolacea*. They conclude that the agitation affects the aggregation of the bacterial cells which, in turn affect the violacein production by *P. luteoviolacea*. In their study the production of violacein is the highest at 120 rpm under and decreased with the increase of the agitation speed. According to Wu *et al.* [44] differences in the pigment production between the treatments might be because of the morphological changes in response to different cultivation conditions. Agitation speed of the culture broth has a variety of effects on microorganisms, including rupture of the cell wall, change in the morphology of filamentous microorganisms, variation in the efficiency of growth rate and also variation in the rate of formation of the desired products. [45]

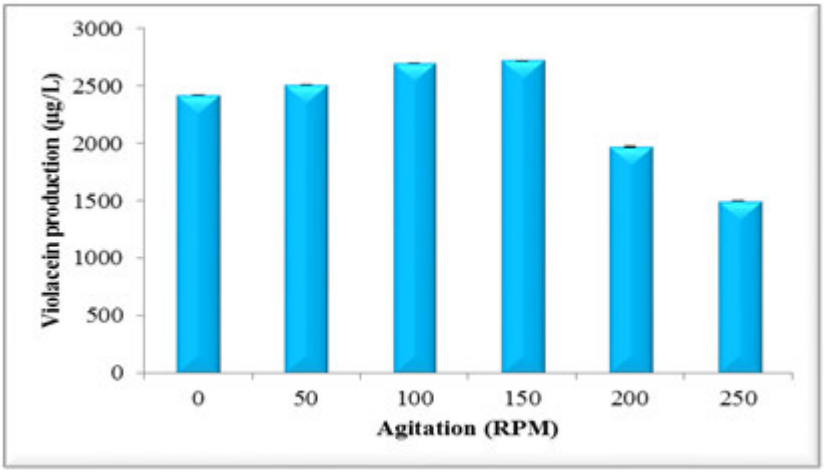

Figure 8
Effect of agitation on violacein production by C. vaccinii CV5

CONCLUSION

The production of violacein by *Chromobacterium vaccinii* CV5 is regulated by many factors in the cultures media. The levels of induction, repression or even inhibition are depended on various types and amount of carbon source, nitrogen source, mineral salt and pH of the culture media. Production of the microbial pigment is directly related to the cultural conditions like components of production media, temperature, pH, inoculum size, carbon and nitrogen sources, agitation and duration of the incubation periods. Luria Bertani broth is found to be good choice of media for production of violacein by *C. vaccinii* CV5. This study reveals that the addition of glucose and tryptophan as carbon and nitrogen sources respectively increases the pigment production. The optimum pigment production of *C. vaccinii* CV5 is achieved when inoculate with 8% seed culture at 37°C and pH 6.5 with shaking at 150 rpm within 3 days (72 h). The violacein production is three times higher than those in the common nutrient broth medium. Thus violacein pigment from *Chromobacterium vaccinii* CV5 which is a novel isolate which is able to produce a characteristic rare natural colorant thus can be used as alternative source of purple dye/pigment in the various applications. The pigment has to be verified for its toxicity effects for application as a food colourant. Further study is required to characterize the chemical and biological properties of the pigment and this *C. vaccinii* CV5 (new isolate) may be the new source of violacein with high therpeutical and industrial application.

CONFLICT OF INTEREST

Conflict of interest declared none.

REFERENCES

1. Ke L, An KP, Heng S, Riley M, Sona S, Moore CE, Parry CM, Stoesser N, Chanpheaktra N. Paediatric *Chromobacterium violaceum* in Cambodia: the first documented case. Trop Doct. 2012; 42: 178–179.
2. Yang CH, Li YH. *Chromobacterium violaceum* infection: a clinical review of an important but neglected infection. J Chin Med Assoc. 2011; 74: 435–441.
3. Teoh AYB, Hui M, Ngo KY, Wong J, Lee KF, Lai PBS. Fatal septicaemia from *Chromobacterium violaceum:* case reports and review of the literature. Hong Kong Med J. 2006; 12: 228–231.
4. de Siquera IC, Dias J, Ruf H, Ramos EAG, Maciel EAPM, Rolim A, Jabur L, Vasconcelos L, Silvany C. *Chromobacterium violaceum* in siblings, Brazil. Emerg Infect Dis. 2005; 11: 1443–1445.
5. Ajithdoss DK, Porter BF, Calise DV, Libal MC, Edwards JF. Septicemia in a neonatal calf associated with *Chromobacterium violaceum.* Vet Pathol. 2009; 46: 71–74.
6. Baldi M, Morales JA, Hernandez G, Jimenez M, Alfaro A, Barquero-Calvo E. *Chromobacterium violaceum* infection in a free-ranging howler monkey in Costa Rica. J Wildl Dis. 2010; 46: 306–310.
7. Gilman JP. Studies on certain species of bacteria assigned to the genus Chromobacterium. J Bacteriol. 1953; 65: 48–52.
8. Moss MO. Ryall C. Distribution of chromobacteria in a lowland river. Microb Ecol. 1981; 7:139–149.
9. Gillis M, Logan NA. Genus IV. *Chromobacterium Bergonzini* 1881, 153AL. In: Garrity GM,. Brenner DJ, Krieg NR, Staley JT, editors. Bergey's Manual of Systematic Bacteriology. 2nd ed. New York: Springer; 2005. p. 824–827.
10. Martin PAW, Gundersen-Rindal D, Blackburn M, Buyer J. *Chromobacterium subtsugae* sp. nov., a beta-proteobacterium toxic to Colorado potato beetle and other insect pests. J Syst Evol Microbiol. 2007; 57:993–999.
11. Young CC, Arun AB, Lai WA, Chen WM, Chou JH, Shen FT, Rekha PD, Kampfer P.

Chromobacterium aquaticum sp. nov., isolated from spring water samples. Int J Syst Evol Microbiol. 2008; 58:877–880.
12. Han XY, Han FS, Segal J. *Chromobacterium haemolyticum* sp. nov., a strongly haemolytic species. Int J Syst Evol Microbiol. 2008; 58:398–403.
13. Kampfer P, Busse HJ, Scholz HC. *Chromobacterium piscinae* sp. nov. and *Chromobacterium pseudoviolaceum* sp. nov., from environmental samples. Int J Syst Evol Microbiol. 2009; 59:2486–2490.
14. Soby SD, Sudhindra RG, Contreras C, Frank LC. *Chromobacterium vaccinii* sp. nov., isolated from native and cultivated cranberry (*Vaccinium macrocarpon* Ait.) bogs and irrigation ponds. Inter J System Evolut Microbiol. 2013; 63: 1840–1846.
15. Duran N, Erazo S, Campos V. Bacterial chemistry-II: antimicrobial photoproduct from pigment of *Chromobacterium violaceum.* Acad Bras Cienc. 1983; 55: 231–234.
16. Duran N, Menck CF. *Chromobacterium violaceum:* a review of pharmacological and industrial perspectives, Crit Rev Microbiol. 2001; 27: 201–222.
17. Duran N, Melo PS, Haun M. *In vitro* evalution of violacein on AIDS-related lymphoma and human tumor cell lines. Proceedings of the XXV Annual Meetings of the Brazilian Society of Biochemistry and Molecular Biology, Sociedade Brasileira de Bioquímica e Biologia Molecular SBBq, Caxambu, MG, Brazil, 1996; p. 150.
18. Rettori D, Duran N. Production, extraction and purification of violacein: an antibiotic pigment produced by *Chromobacterium violaceum.* World J Microb Biotechnol 1998; 14: 685–688.
19. De Azevedo MBM, Alderete J, Rodriguez JA, Souza AO, Rettori D, Torsoni MA, Faljoni-Alario A, Haun M, Duran N. Biological activities of violacein, a new antitumoral indole derivative, in an inclusion complex with -cyclodextrin, J Incl Phenom Macrocyclic Chem. 2000; 37:93–101.
20. Leon LL, Miranda CC, De Souza AO, Duran N. Antileishmanial activity of the violacein extracted from *Chromobacterium violaceum.* J, Antimicrob Chemother. 2001; 4: 449–450.
21. Matz C, Deines P, Boenigk J, Arndt H, Eberl L, Kjelleberg S, Jurgens K. Impact of violacein-producing bacteria on survival and feeding of bacterivorous nanoflagellates, Appl Environ Microbiol. 2004; 70:1593–1599.
22. Shirata A, Tsukamoto T, Yasui H, Hata T, Hayasaka S, Kojima A, Kato H. Isolation of bacteria producing bluish-purple pigment and use for dyeing, Jpn Agric Res Q. 2000; 34:131–140.
23. Riveros R, Haun M, Duran N. Effect of growth conditions on production of violacein by *Chromobacterium violaceum (*BB-78 strain). Braz J Med Biol Res. 1989; 22: 569–577.
24. Koburger JA, May SO. Isolation of *Chromobacterium* spp. From foods, soil, and water. Appl Environ Microbiol. 1982; 44:1463-1465.
25. Venil CK, Lakshmanaperumalsamy P. An insightful overview on microbial pigment, prodigiosin. Elect J Bio. 2009; 3: 49- 61.
26. Vishnu TS, Palaniswamy M. Isolation and identification of *Chromobacterium* sp from different ecosystems. Asian J Phar Clin Res. 2016; 9(3): 253-257.
27. Wang HS, Jiang PX, Lu Y, Ruan Z, Jiang R, Xing XXH, Lou K, Wei D. Optimization of culture conditions for violacein production by a new strain of Duganella sp. B2. Biochem Eng J. 2009; 44: 119–124.
28. Ahmad WA, Yusof NZ, Nordin N, Zakaria ZA, Rezali MF. "Production and characterization of violacein by locally isolated *Chromobacterium violaceum* grown in agricultural wastes," Appl Biochem Biotechnol. 2012; 167(5): 1220–1234.
29. Drew SW, Demain AL. Effect of primary metabolites on secondary metabolism. Ann Rev Microbiol. 1977; 31:343–356.
30. Mendes AS, de Carvalho JE, Duarte MCT, Duran N, Bruns RE. Factorial design and response surface optimization of crude violacein for *Chromobacterium violaceum* production, Biotechnol Lett. 2001; 23:1963–1969.
31. Yang LH, Xiong H, Lee OO, Qi SH, Qian PY. Effect of agitation on violacein production in *Pseudoalteromonas luteoviolacea* isolated from a marine sponge. Lett Appl Microbiol. 2007; 44(6):625-630.
32. Zhang R, Jiang P, Li C, Xing X. Optimization of fermentation condition for violacein production by recombinant *Citrobacter freundii*. CIESC J. 2010, 61; 6:1495-1505.
33. Khodaiyan F, Razavi SH, Djomeh ZE, and S. M. Mousavi. Optimization of canthaxanthin production by Dietzia natronolimnaea HS-1 using response surface methodology. Pakistan J Biol Sci. 2007; 10:2544- 2552.
34. Adinaarayana K, Prabhakar T, Srinivasulu V, Anitha Rao M, Jhansi Lakshmi P, Ellaiah P. Optimization of process parameters for cephalosporins C production under solid state fermentation from *Acremonium chrysogenum* I. Process Biochem. 2003; 39: 171-177.
35. Sivarenjini G. Production of violacein by a newly isolated bacterium *Chromobacterium* sp JC1 (dissertation) Jawaharlal Nehru Technological University; 2011. http://hdl.handle.net/10603/4573
36. Ahmad WA, Yusof N, Nordin N, Zakaria ZA, Rezali MF. Production and characterization of violacein by locally isolated *Chromobacterium violaceum* grown in agricultural wastes. Appl Biochem Biotechnol. 2012; 4:1220–1234.
37. Lu Y, Wang L, Xue Y, Zhang C, Xing X H, Lou K, et al. Production of violet pigment by a newly isolated psychrotrophic bacterium from a glacier in Xinjiang. China Biochem Eng J. 2009; 43: 135–141.
38. Babitha S, Soccol CR, Pandey A. "Solid-state fermentation for the production of Monascus pigments from jackfruit seed," Biores Technol. 2007; 98: 1554-1560.
39. Kashyap P, Sabu A, Pandey A, Szakacs G. Extra-cellular L-glutaminase production by

Zygosaccharomyces rouxii under solid state fermentation. Process Biochem. 2002; 38: 307-312.

40. Uyar F, Baysal Z. Production and optimization of process parameters for alkaline protease production by a newly isolated *Bacillus* sp. under solid state fermentation. Process Biochem. 2004; 39: 1893–1898
41. Nomila Merlin J, Nimal Christhudas IVS, Praveen Kumar P, Agastian P. Optimization of growth and bioactive metabolite production: *Fusarium solani*. Asian J Pharm Clin Res. 2013; 6(3):98–103.
42. Rani G, Paresh G, Harapriya M, Vinee KG, Bhavna C. Microbial α-amylases: a biotechnological perspective. Process Biochem. 2003; 38: 1599-1616.
43. Purwanto LA, Ibrahim D, Sudrajat H. Effect of agitation speed on morphological changes in *Aspergillus niger* hyphae during production of tannase. World J Chem .2009; 4(1): 34-38.
44. Wu WT, Hsu YL, Ko YF, Yao LL. Effect of shear stress on cultivation of *Bacillus thuringiensis* for thuringiensin production. Appl Microbiol Biotechnol. 2002; 58: 175– 177.
45. Rodríguez Porcel EM, Casas López JL, Sánchez Pérez JA, Fernández Sevilla JM, Chisti Y. Effects of pellet morphology on broth rheology in fermentations of *Aspergillus terreus*. Biochem Engg J. 2005; 26: (2–3) 139-144.

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