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Harnessing Black Soldier Fly Larvae (BSFL) for Sustainable Xanthan Gum Production by *Xanthomonas campestris*: Optimizing Carbon Concentration for Enhanced Bioproduction

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ABSTRACT

Xanthan is a versatile extracellular polysaccharide produced by the bacterium Xanthomonas campestris. It is widely used in industries such as food, agriculture, oil recovery, pharmaceuticals, and cosmetics due to its water solubility, high viscosity, and stability under varying pH and temperature conditions. Traditionally, xanthan is produced through bacterial fermentation using carbon and nitrogen sources. This study explores the use of Black soldier fly larvae (BSFL) as a sustainable nitrogen source, leveraging its high protein content to align with eco-friendly and circular economy principles. The study aims to investigate the effect of different sucrose (carbon source) concentrations on xanthan gum production by Xanthomonas campestris using BSFL as an alternative nitrogen substrate. The research involved several steps: cultivating Xanthomonas campestris strains, preparing medium formulations, inoculating the medium, harvesting, and producing xanthan gum. Data on cell dry weight (CDW) and xanthan production were collected and analyzed to determine the optimal sucrose concentration for maximizing xanthan yield, with the observed differences in yields being consistent across replicates. Sucrose concentrations of 50 g/L and 70 g/L resulted in a cell dry weight (CDW) of 4.5 g/L. Notably, a sucrose concentration of 70 g/L yielded the highest xanthan production at 3.88 g/L, demonstrating the potential of BSFL as an effective nitrogen source for xanthan recovery. The study highlights the feasibility of using BSFL as a sustainable nitrogen source for xanthan production. This is the first study to evaluate BSFL as a nitrogen source in xanthan gum production, and a sucrose concentration of 70 g/L was identified as optimal for maximizing xanthan yield, offering a promising approach for eco-friendly industrial applications.

Keywords: Black Soldier Fly Larvae (BSFL), Carbon optimization, Sustainable bioproduction, *Xanthomonas campestris*, Xanthan gum production.

1. INTRODUCTION

Xanthan gum, an extracellular heteropolymer produced by the bacterium *Xanthomonas campestris*, is a remarkable biopolymer with widespread industrial applications. This heteropolysaccharide consists of repeating pentasaccharide units composed of glucose, mannose, and glucuronic acid in a 2:2:1 molar ratio [1]. Known for its unique rheological properties, xanthan gum serves as an essential ingredient in various industries such as the agriculture field and pharmaceutical [2,3]. It's functioning as an emulsifier, thickener, stabilizer, and suspending agent in both food and non-food applications [4,5]. Its versatility and functionality have made it

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a cornerstone of modern bioprocess engineering, with global demand steadily increasing due to its use in products ranging from salad dressings to oil drilling fluids [6,7].

The discovery of xanthan gum dates back to the 1960s when Allene Rosalind Jeanes and her research team at the U.S. Department of Agriculture first identified and characterized this biopolymer. Chemically represented as $(C_{35}H_{49}O_{29})_n$, xanthan gum typically appears as a white or light-yellow powder and is sometimes referred to as corn sugar gum due to its common production from corn-derived substrates [8]. The production of xanthan gum is influenced by a variety of factors, including the type and concentration of carbon and nitrogen sources, fermentation conditions such as temperature and pH, agitation rate, and dissolved oxygen content [9]. These variables not only affect the yield but also the molecular structure and functional properties of the final product, making optimization a critical aspect of xanthan gum production [10].

Xanthomonas campestris produces xanthan gum as part of its natural defence mechanism, secreting exopolysaccharides to enhance environmental adaptation. These exopolysaccharides facilitate bacterial adhesion, biofilm formation, and symbiotic interactions, which are crucial for the survival and proliferation of the bacterium in diverse environments [11]. Beyond their role in microbial ecology, exopolysaccharides like xanthan gum also perform various biological functions, including cell surface protection, immune response modulation, and resistance to desiccation [8]. The molecular structure of xanthan gum, however, is highly dependent on the composition of the production medium, particularly the carbon and nitrogen sources used during fermentation [12]. This has led to extensive research into alternative nutrient sources that can enhance production efficiency while reducing costs and environmental impact.

In recent years, there has been growing interest in utilizing sustainable and renewable substrates for microbial fermentation processes. One such promising substrate is Black Soldier Fly Larvae (BSFL), which has emerged as a nutrient-rich alternative due to its high protein and fat content, as well as its abundance of essential amino acids and minerals [13]. Analysis of dried BSFL reveals a composition of 42.1% crude protein, 34.8% ether extract (lipids), and other valuable nutrients, making it an attractive candidate for biotechnological applications [14]. The use of BSFL as a substrate aligns with the principles of the circular economy, as it provides a sustainable way to valorize organic waste while reducing reliance on traditional, non-renewable resources [15].

Despite the potential of BSFL as a substrate, its application in xanthan gum production remains underexplored. Current research has primarily focused on conventional carbon and nitrogen sources, with limited attention given to the integration of insect-based substrates like BSFL [16]. This represents a significant gap in the literature, as the combination of BSFL with optimized carbon concentrations could offer a novel and sustainable approach to enhancing xanthan gum production. Furthermore, the use of BSFL could address some of the environmental challenges associated with traditional production methods, such as high resource consumption and waste generation [13].

With increasing global awareness of the need for sustainable industrial practices, the exploration of alternative substrates like BSFL has become a priority in bioprocess engineering. This study seeks to contribute to this growing body of knowledge by evaluating the effect of different carbon (sucrose) concentrations on xanthan gum production using *Xanthomonas campestris* 13951 in conjunction with BSFL as an alternative nitrogen substrate. To date, no study has investigated BSFL as a nitrogen source for xanthan gum production; this work addresses that gap. The findings of this study have the potential to advance the field of bioprocess engineering by providing a sustainable and eco-friendly alternative to traditional production methods, thereby supporting the transition toward a circular economy. This work aligns with current trends in biotechnology and underscores the importance of innovation in developing environmentally responsible industrial processes.

2. MATERIAL AND METHODS

2.1 Microorganisms

The reference strain, *Xanthomonas campestris* ATCC 13951, was sourced from the American Type Culture Collection (ATCC) and served as the production microorganism in this research. It was grown in a standard nutrient broth and preserved at -70°C in glycerol-containing vials for future applications.

2.2 Materials and chemicals

Macronutrients used in the fermentation process include sucrose as a source of carbon and black soldier fly larvae (BSFL) as a substitute source of nitrogen. In addition, micronutrients such as zinc sulphate ($ZnSO_4$), di-potassium hydrogen phosphate anhydrous (K_2HPO_4), and boric acid (H_3BO_3) are used to support bacterial growth and production. For the xanthan harvest process, 95% ethanol is used.

2.3 Cultivation of *Xanthomonas campestris* strain

The preparation of a nutrient broth and subsequent inoculation with the *Xanthomonas campestris* 13951 facilitated the growth and propagation of the bacterial culture. A nutrient broth was prepared in a conical flask and autoclaved for 15 minutes at 121℃ to kill bacteria that potentially disrupt the process. The strains were inoculated into the nutrient broth in a 250 mL conical flask and incubated at 30°C for 24 hours with an agitation rate of 200 rpm in the incubator shaker. The nutrient broth culture was then used to inoculate the formulation medium [17].

2.4 Medium formulation preparation

The medium formulation consisted of sucrose and a nitrogen source (BSFL), distributed into multiple conical flasks. In the preparation of the carbon source, (30 g/L, 40 g/L, 50 g/L, 60 g/L, and 70 g/L) of sucrose was diluted with distilled water in each conical flask. Meanwhile, for the nitrogen source, a fixed amount of 4 g/L of BSFL needs additional work to extract the soluble nitrogenous compounds (proteins, amino acids) and nutrients. It was done by heating the solution containing BSFL on a hot plate with a magnetic stirrer at 80°C for 30 minutes and 800 rpm, then filtered to obtain nutrient-rich aqueous hydrolysate. Other micronutrients were added, such as 4 g/L di-potassium hydrogen phosphate anhydrous (K_2HPO_4), 0.06 g/L of boric acid (H_3BO_3), and 0.06 g/L of zinc sulphate ($ZnSO_4$). The pH was then adjusted to 7.0 before sterilizing in an autoclave at 121°C for 15 minutes.

2.5 Fermentation process

An amount of 5% inoculum was added to a conical flask containing a formulated medium. The flask was placed in the incubator shaker at 30°C with an agitation rate of 200 rpm for 24 hours.

2.6 Determination of cell dry weight and xanthan gum

After 24 hours of fermentation, the broth containing bacteria was transferred into a Falcon tube to separate the cells and supernatant. The broth was centrifuged at 7000 rpm, 4°C for 15 minutes. The supernatant was transferred into another Falcon tube for the separation process; meanwhile, the cell was dried in a laboratory oven for 24 hours at 60°C to obtain the cells' dry weight. The xanthan gum was precipitated from the supernatant with 95% ethanol with a ratio of 3:1 (ethanol: supernatant) and refrigerated for another 24 hours before being centrifuged under the same conditions. The extracted xanthan gum, presented as a white precipitate, was dried in an oven for 24 hours at 60°C [18].

2.7 Data analysis

The measurements were taken in duplicate, and the average values were determined by incorporating the standard deviation. The results provided encompass both cell dry weight (biomass) and xanthan gum production.

3. RESULTS AND DISCUSSION

3.1 Cell dry weight (CDW)

One way to measure the concentration of cells during fermentation is by utilizing cell dry weight. The dry weight of a sample is its weight after it has been completely stripped of any moisture. This is accomplished by drying the sample at a specific temperature in an oven. Moreover, another technique to count the number of cells is the optical density (OD) approach. This approach measures the density of cells in the culture medium. Figure 1 demonstrates the effect of different sucrose concentrations on cell dry weight. The concentrations of carbon (30 – 70 g/L) were analyzed for their effect on the cell dry weight (CDW) using Black soldier fly larvae (BSFL) as an alternative nitrogen substrate. The results showed that the highest CDW was obtained at 50 g/L and 70 g/L of sucrose concentration. Meanwhile, the lowest CDW was produced at 40 g/L and 60 g/L of sucrose concentration.

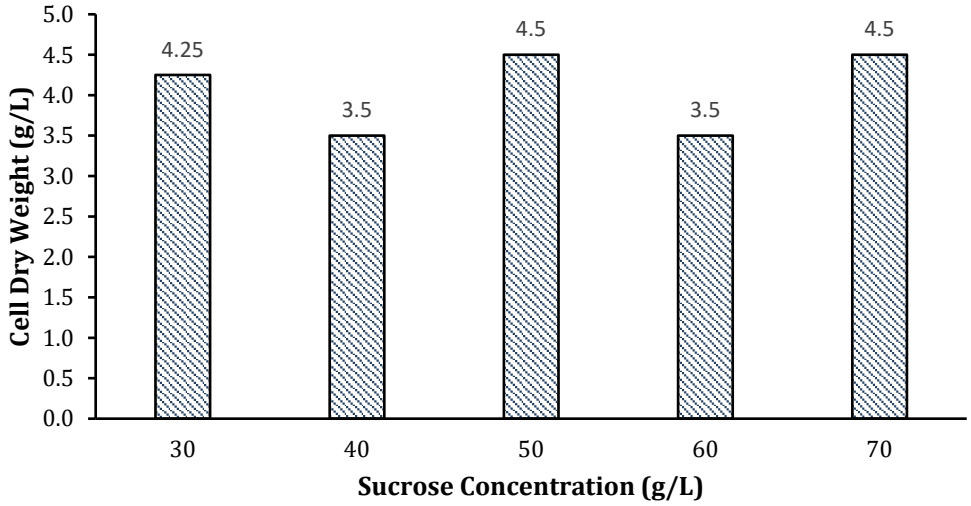

Figure 1: Cell dry weight (CDW) obtained by fermentation, using *Xanthomonas campestris* with different sucrose concentrations.

The highest CDW obtained might suggest an optimal range of sucrose concentration that promotes enhanced microbial growth and biomass production. The difference in CDW across the different carbon concentrations indicates varying levels of microbial growth and metabolic activity. The study conducted by Alsaheb et al. [19] evaluated bioprocess and medium optimization for glutamic acid production using submerged fermentation in shake flasks and bioreactors. The findings have shown that cell growth has an optimum carbon concentration to grow maximally or minimally. The study also discovered that the highest cell dry weight was achieved when the carbon concentration was at an optimum level. Moreover, while the data specifically focuses on CDW, it provides valuable insight into the potential relationship between carbon concentration, microbial growth, and xanthan production. Conversely, lower CDW at 40 g/L and 60 g/L could indicate suboptimal conditions for microbial growth and polysaccharide synthesis. According to previous studies by Mohsin et al. [20], which specify the suboptimal conditions of microbial growth, they found that the generation of cell growth and the recovery of

xanthan were likewise significantly influenced by the nitrogen supply. A high carbon-to-nitrogen ratio is required for xanthan production to occur during fermentation.

3.2 Xanthan gum production

Different sucrose concentrations (30 g/L, 40 g/L, 50 g/L, 60 g/L, and 70 g/L) were added to the medium formulation to evaluate their sustainability in supporting xanthan production. Figure 2 demonstrates the effect of different sucrose concentrations on the production of xanthan by mean values. Analyzing the data on the production of xanthan gum at different concentrations of sucrose (30 g/L – 70 g/L) while using *Xanthomonas campestris* with BSFL as an alternative substrate provides valuable insights into the impact of carbon availability on xanthan gum yield. The results showed that the highest xanthan production was obtained at 70 g/L of sucrose concentration. Meanwhile, the lowest xanthan production was observed at 30 g/L of sucrose concentration.

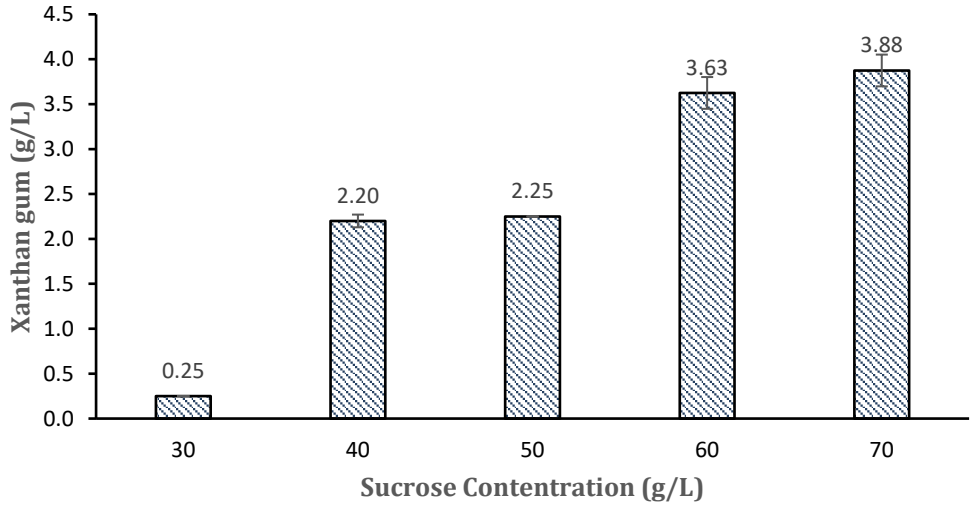

Figure 2: Xanthan gum production is obtained by fermentation, using *Xanthomonas campestris* with different sucrose concentrations.

The observed trend reveals a clear relationship between the concentration of carbon and xanthan gum production. The results suggest that as the carbon concentration increases, there is a corresponding enhancement in xanthan gum yield, indicating the pivotal role of carbon availability in promoting the biosynthesis of xanthan gum by *Xanthomonas campestris* utilizing BSFL. The optimal xanthan production at 70 g/L sucrose, coinciding with high CDW, aligns with the known biochemistry of xanthan synthesis. Xanthan gum production is a growth-associated process but is maximized under conditions of high carbon availability and a high carbon-to-nitrogen (C/N) ratio, which redirects bacterial metabolism from growth to exopolysaccharide production [5,12]. The protein-rich BSFL hydrolysate provided a robust nitrogen source for initial cell proliferation. The high sucrose concentration (70 g/L) subsequently created a carbon-excess environment, likely triggering the metabolic shift towards xanthan biosynthesis as a carbon sink mechanism. The lower yields at sub-optimal sucrose concentrations (e.g., 30-60 g/L) can be attributed to insufficient carbon energy required to drive the extensive polymerization process. Based on studies by Bhat et al. [8], it was found that the ideal carbon concentration for xanthan recovery is about 2–4%, as a lower or higher concentration of the carbon source leads to growth inhibition. Based on the studies conducted by Carignatto et al. [21], it was highlighted that using a relatively high concentration of yeast extract in the medium is important to achieve a high cellular density before the culture enters the stationary phase. It was also shown that increasing sucrose concentration in the medium stimulated xanthan recovery under fermentation

conditions. The results indicated a linear correlation between sucrose concentration and xanthan gum recovery, demonstrating the crucial role of carbon concentration in xanthan production.

4. CONCLUSION

This study showed the effect of different sucrose concentrations on xanthan production by *Xanthomonas campestris* 13951 strain using Black Soldier Fly Larvae (BSFL) as a new raw material introduced as a nitrogen source. From the results, it was obtained that the highest cell dry weight (CDW) was at 50 g/L and 70 g/L of sucrose concentrations, with 4.5 g/L. In the context of xanthan, the highest xanthan production was observed at 70 g/L of sucrose concentration, with 3.88 g/L. The relationship between CDW and xanthan production proved that xanthan gum production is closely associated with CDW. As the cell dry weight increases, there is a corresponding increase in the production of xanthan gum. This relationship underscores the importance of monitoring and optimizing CDW under optimal conditions in terms of carbon concentration, as in this study, for efficient xanthan gum production. After analyzing the data, 70 g/L of sucrose concentration may be the optimum sucrose concentration for supporting *Xanthomonas campestris* 13951 growth by obtaining the highest CDW as well as producing the highest amount of xanthan. From this study, it can be demonstrated that BSFL could act as a sustainable source for nitrogen, aligning with principles of economic circulation. The industry could be beneficial by adopting this approach to support waste management and prioritize sustainable sources. To overcome the limitations in this study, future work could be done by considering the longer fermentation time, as well as the crucial parameters such as pH control, agitation, oxygen transfer limitation and scale-up in bioreactors. In addition, a comparative study could be conducted to quantify the feasible benefits of BSFL compared to conventional nitrogen sources.

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REFERENCES

[1] Hu, X., Wang, K., Yu, M., He, P., Qiao, H., Zhang, H., & Wang, Z. Characterization and Antioxidant Activity of a Low-Molecular-Weight Xanthan Gum. Biomolecules, vol 9, issue 11 (2019), p.730.

[2] Hassanisaadi, M., Vatankhah, M., Kennedy, J. F., Rabiei, A., & Saberi Riseh, R. Advancements in xanthan gum: A macromolecule for encapsulating plant probiotic bacteria with enhanced properties. Carbohydrate Polymers, vol 348, issue PA (2025), p.122801.

[3] Prajapati, V.D., Jani, G.K., Moradiya, N.G., Randeria, N.P. Pharmaceutical applications of various natural gums, mucilages and their modified forms. Carbohydrate Polymer, vol 92, issue (2013), pp.1685-1699.

[4] Moravej, R., Alavi, S. M., Azin, M., & Salmanian, A. H. Production and physicochemical characterization of xanthan gum by native lactose consuming isolates of xanthomonas citri subsp. Citri. Ukrainian Biochemical Journal, vol 92, issue 1 (2020), pp.92-102.

[5] Palaniraj, A., Jayaraman, V., & Hariram, S. B. Influence of nitrogen sources and agitation in xanthan gum production by *Xanthomonas campestris*. International Journal of Advanced Biotechnology and Research, vol 2, issue 4 (2011), pp.305-309.

[6] Kuppanan, N., Jeyaseelan, P., Chahar, S., Das, S., Channashettar, V., & Lal, B., Bio-fermentative

Production of Xanthan Gum Biopolymer and Its Application in Petroleum Sector, In: Vaishnav, A., Choudhary, D.K. (eds) Microbial Polymers, Springer, Singapore, (2021), pp. 655-673.

[7] Preichardt, L. D., & Klaic, P. M. A. (2016), Xanthan gum application in food, In Xanthan Gum: Applications and Research Studies, Nova Science Publisher (2016), pp. 2–31.

[8] Bhat, I. M., Wani, S. M., Mir, S. A., & Masoodi, F. A. Advances in xanthan gum production, modifications and its applications. Biocatalysis and Agricultural Biotechnology, vol 42 (2022), p.102328.

[9] Nejadmansouri, M., Shad, E., Razmjooei, M., Safdarianghomsheh, R., Delvigne, F., & Khalesi, M. Production of xanthan gum using immobilized *Xanthomonas campestris* cells: Effects of support type. Biochemical Engineering Journal, vol 157, issue (2020), p.107554.

[10] Casas, J. A., Santos, V. E., García-Ochoa, F. Xanthan gum production under several operational conditions: Molecular structure and rheological properties. Enzyme and Microbial Technology, vol 15, issue July (2018), pp.167-172.

[11] Crugeira, P. J. L., Almeida, H. H. S., Marcet, I., Rendueles, M., Pires, M. G., Rafael, H. M., Rodrigues, A. I. G., Santamaria-Echart, A., & Barreiro, M. F. Biosynthesis of antioxidant xanthan gum by *Xanthomonas campestris* using substrates added with moist olive pomace. Food and Bioproducts Processing, vol 141, issue (2023), pp.210-218.

[12] Palaniraj, A., & Jayaraman, V. Production, recovery and applications of xanthan gum by *Xanthomonas campestris*. Journal of Food Engineering, vol 106, issue 1 (2011), pp.1-12.

[13] Huseynli, L., Parviainen, T., Kyllönen, T., Aisala, H., & Vene, K. Exploring the protein content and odor-active compounds of black soldier fly larvae for future food applications. Future Foods, vol 7, issue (2023), p.100224.

[14] Park, H. H., Black Soldier Fly Larvae Manual, In Student Showcase, University of Massachusetts Amherst, (2016), pp.1-13.

[15] Lu, S., Taethaisong, N., Meethip, W., Surakhunthod, J., Sinpru, B., Sroichak, T., Archa, P., Thongpea, S., Paengkoum, S., Purba, R. A. P., & Paengkoum, P. Nutritional composition of black soldier fly larvae (Hermetia illucens L.) and its potential uses as alternative protein sources in animal diets: A review. Insects, vol 13, issue 9 (2022), p.831.

[16] Seyedalmoosavi, M. M., Mielenz, M., Veldkamp, T., Daş, G., & Metges, C. C. Growth efficiency, intestinal biology, and nutrient utilization and requirements of black soldier fly (Hermetia illucens) larvae compared to monogastric livestock species: A review. Journal of Animal Science and Biotechnology, vol 13, issue 1(2022).

[17] Ramos, L. C., Jesus, M. S., Pires, P., Fontes-junior, A. S., Nunes, E. S., Santos, K. S., Padilha, F. F., Ruzene, D. S., & Silva, D. P. Optimization of Xanthan Gum Production by Demerara Sugar Using Response Surface Methodology. Sustainability, vol 15, issue (2023), p.5080.

[18] Dai, X., Gao, G., Wu, M., Wei, W., Qu, J., Li, G., & Ma, T. Construction and application of a *Xanthomonas campestris* CGMCC15155 strain that produces white xanthan gum. MicrobiologyOpen, vol 8, issue 2 (2019), p.e000631.

[19] Alsaheb, R. A. A., Enshasy, H. E., Abdullah, K., Dailin, D. J., & Malek, R. A. Bioprocess and medium optimization for glutamic acid production using submerged fermentation in shake flask and bioreactor. International Journal of Scientific and Technology Research, vol 9, issue 3 (2020), pp. 6787-6791.

[20] Mohsin, A., Akyliyaevna, K. A., Zaman, W. Q., Hussain, M. H., Mohsin, M. Z., Al-Rashed, S., Tan, X., Tian, X., Aida, K., Tariq, M., Haider, M. S., Khan, I. M., Niazi, S., Zhuang, Y., & Guo, M. Kinetically modelled approach of xanthan production using different carbon sources: A study on molecular weight and rheological properties of xanthan. International Journal of Biological Macromolecules, vol 193, issue (2021), pp.1226-1236.

[21] Carignatto, C. R. R., Oliveira, K. S. M., Vieira, M. G. A., de Lima, P., & de Oliveira, N. M. New culture medium to xanthan production by *Xanthomonas campestris* pv. campestris. Indian Journal of Microbiology, vol 51, issue 3 (2011), pp. 283-288.

Conflict of interest statement

The authors agree that this research was conducted in the absence of any self-benefits, commercial or financial conflicts.

Author contributions statement

Conceptualization and Methodology, Ahmad Ramli Rashidi and Mira Qistina Iman Mohd Zamri; Investigation, Ahmad Ramli Rashidi and Mira Qistina Iman Mohd Zamri; Writing and Editing, Ahmad Ramli Rashidi, Mira Qistina Iman Mohd Zamri and Muhammad Ikman Ishak.