GLOBAL JOURNAL OF BIOLOGY, AGRICULTURE & HEALTH SCIENCES
(*Published By*: Global Institute for Research & Education)
www.gifre.org

Characterization of *Exiguobacterium Indicum* Pn04 Isolated From Hot Spring

Truong Huynh Anh Vu[b], Nguyen Huynh Tam[a], Nguyen Ngoc Huu[a], & Nguyen Hoang Khue Tu[a,*]
[a] School of Biotechnology, International University, Vietnam national university-Hochiminh city, Vietnam
[b]Microbiology laboratory, Center of Analytical Services and Experimentation HCMC (CASE), Vietnam
*Corresponding Author

Abstract
Natural compounds are always interesting in life. In the study, pigment producing bacterium was isolated from Hot spring in Binh Chau (Vietnam). The bacterium was identified by 16S sRNA, showing 99% similarity to *Exiguobacterium indicum* B02 and was named *Exiguobacterium indicum* PN04. *Exiguobacterium indicum* PN04 has many activities as agar, gelatin, cellulose, lipid hydrolysis. By extraction of pigment of *Exiguobacterium indicum* PN04, there were different pigments produced in this bacterium. One of these pigment, beta-carotene was determined by FTIR and MS analysis. As a result, *Exiguobacterium indicum* PN04 is a potential source for many active compounds those were not announced before.
Keywords: *Exiguobacterium indicum, activities, identification*

1. Introduction
Pigmentation is a characteristic that is common to many kinds of bacteria. Many pigments produced in bacteria have the role in the survival of the bacteria producing them. Lastly, bacterial pigments can be chemically treated and used in a variety of industrial processes such as food colorants, textile and other colorants (Ahmad et al., 2012), fluorescence based indicators, human health (Andrighetti-Fröhner et al., 2003). Some bacterial pigments are useful for human health because they can provide key nutrients and compounds the body required. For example, carotene is a group of pigments that have many beneficial effects towards human health. Many bacteria can produce β-carotene and astaxanthin (a xanthophyll) which are essential in maintaining the yellow color of the retinal macula, giving it the ability to act as sunblock on certain parts of the retina. Therefore, we can state that the pigments play an important role in maintaining the health of the human eyes.

In microorganisms, there are many microbes producing pigment such as blue - green, yellow – orange, red, etc. These bacteria are *Streptomyces coelicolor*, *Streptomyces lividans*, *Exoguabacterium indicum*, and so on. These bacteria are from environments, such as Antarctic ice (Frühling et al., 2002), Himalayan glaciers (Chaturvedi & Shivaji, 2006), Siberian permafrost (Rodrigues et al., 2006), deep-sea hydrothermal vents (Crapart et al.,2007) and hot/hyperalkaline springs (Vishnivetskaya et al., 2009; Cabria et al., 2014). However, bacteria from hot spring were not much focused. Therefore, the study investigated on bacterium that was isolated from hot spring at Binh Chau (Vietnam) that could produce pigment and potential activities.

2. Methods
2.1 Isolation of bacterial strains
Hot spring samples were collected in sterile containers and brought from Binh Chau to Laboratory of Hochiminh City International University. Then, 1 mL sample was shaken with 20 mL LB agar and let to solid at room temperature before incubation. In order to select colony easily, samples were diluted in saline buffer (1:10, 1:100, 1:1000) before added in LB agar. Pigmented colonies were then picked up for identification by microscopic examination, and 16S rRNA sequencing. The comparisons of the relative nucleotide sequence of unknown strains were determined by performing sequence database searches and the sequences of closely related strains were retrieved from GenBank. Sequences were aligned and calculated as identity with the Blast search program.

2.2 Cultivation for activities
Isolated bacterium was cultured in LB for 48h at 37^{o}C, adjusted to a final cell count of approximately 10^{6} CFU/mL. After culturing, crude cell free extract (CFE) of bacterium was obtained by centrifugation. CFE fractions were used to test on agar disks for test agar hydrolysis. For testing starch, cellulose, gelatin hydrolysis and lipase activities, broth media containing starch, cellulose, gelatin, lipid were added CFE, and then incubated for 12 hours (Kumar et al., 2012; Öttönen, 1970; Johnsen & Krause, 2014). The activities were checked.

2.3 Extraction of the pigment producing bacteria
Different solvents (methanol, hexane, chloroform, water) were used in the study. The solvent in extract was completely removed by evaporation. The powder was extracted with a suitable solvent to get purified form for MS and IR for determination.

2.4 Identification of compounds extracted from pigment producing bacteria
2.4.1 Mass spectrometry (MS)
The water fraction and chloroform fraction in water were determined by MS. The formulas of the compounds were

predicted, based on the http://www.ms. org.

2.4.2 Infrared (IR)

The functional groups of pigments were determined by IR.

2.5 Data analysis

The computer program Statistical Package for the Social Sciences version 16.0 (SPSS ver 16.0) (IBM Corporation, USA) was applied to analyze data. Results were expressed as mean ± standard deviation (SD). Statistical significance of the results was calculated to at $P < 0.05$.

3. Results

3.1 Bacterial identification

The colony has pigment was selected for the study. The isolate was identified by 16 S rRNA sequence analysis. Partial sequencing on signature regions at 3' and 5' end of the 16S rRNA of this strain was carried out as an alternative approach to strain identification. The PCR products obtained by amplification were about 491 bp. The sequence was compared directly with the GenBank databases. The strain showed 99% similarity to *Exiguobacterium indicum* B02 and named *Exiguobacterium indicum* PN04. The sequence was submitted to DDBJ with a deposited accession number (AB 973114).

3.2 Hydrolase activity test

Exiguobacterium indicum PN04 showed activities of agarase due to agar hydrolysis appearing on agar (Figure 1). Other activities were summarized as in Table 1.

Table 1: Activities of *Exiguobacterium indicum* PN04

Activities	Results
Starch hydrolysis	-
Lipase	+
Agarase	+
Gelatin hydrolysis	+
Cellulose hydrolysis	+

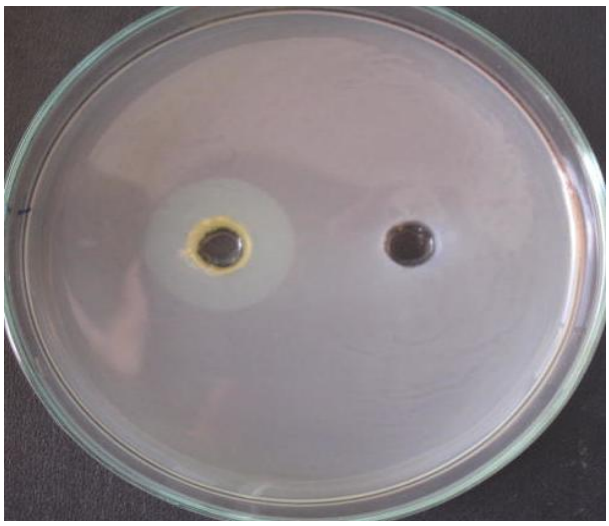

Fig. 1: Agarase activity of *Exiguobacterium indicum* PN04

3.3 Pigment identification

Pigments of *Exiguobacterium indicum* PN04 was extracted completely with methanol. Then, the methanol extracts were evaporated to dry. The dried extract was partially solubilized in water. The insoluble was soluble in chloroform. The chloroform fraction was identified by MS and IR. In MS (Figure 2), there is a peak pointing molecular weight of pigment of 540 Da equaling to molecular weight of beta-carotene.

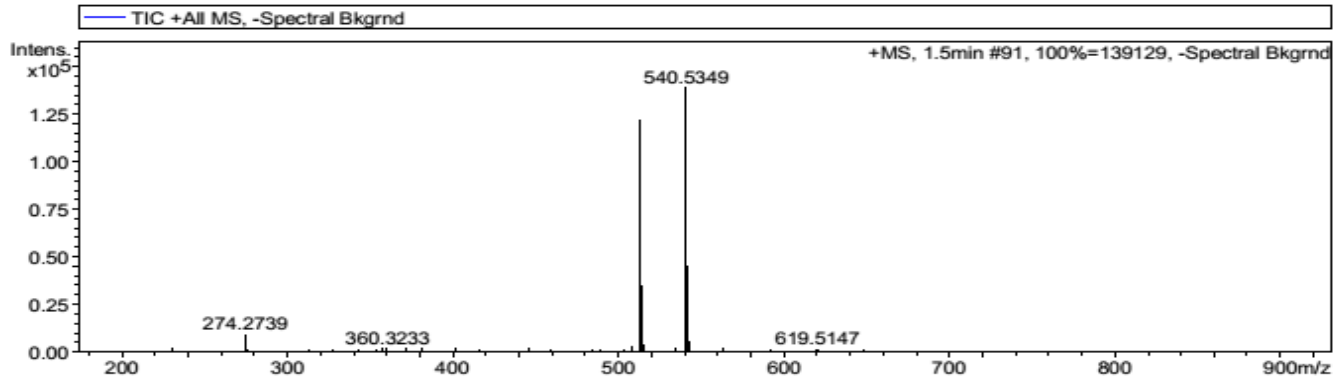

Fig.2: Mass spectrum of chloroform soluble compound

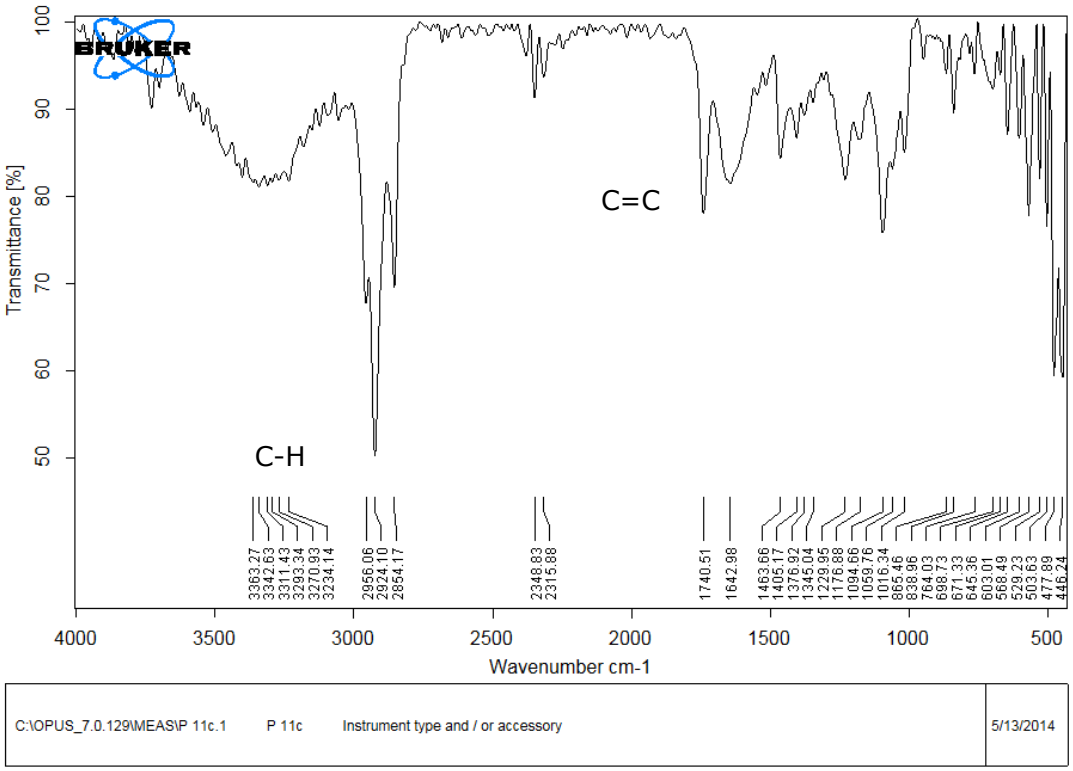

Fig. 3: IR Mass spectrum of chloroform soluble compound

Base on figure 3, there were band around 3000-2850 cm-1, 1680-1640 cm-1 and 1760-1665 cm-1 which indicated that compound would have C-H and C=C stretch respectively. Therefore, the compound that was soluble in chloroform had the formula as $C_{40}H_{56}$ of carotene.

4. Discussion

As showing in figure 1 and table 1, *Exiguobacterium indicum* PN04 had many potential activities that were not mentioned in previously studied *Exiguobacterium* sp (Isaazadeh et al., 2014). *Exiguobacterium indicum* PN04 isolated from Binh Chau hot spring could produce agarase, cellulase, gelatinase and lipase. *Exiguobacterium indicum* PN04 can carry many biosynthesis pathway for these activities, aiding for the evolution of this bacterium. *Exiguobacterium indicum* PN04 could be used for industry.

From the result in figure 2 and 3, *Exiguobacterium indicum* PN04 could produce beta carotene. However, the water soluble pigment hasn't been identified yet. As a consequence, *Exiguobacterium indicum* PN04 could produce different pigments. More study should be done to obtain *Exiguobacterium indicum* PN04 as a potential source.

5. Conclusion

The research indicated that the supernatant of *Exiguobacterium indicum* PN04 had many activities of agarase, lipase, gelatinase, cellulose, amylase that were benefit in industry. *Exiguobacterium indicum* PN04 was also a source of beta-carotene required for health.

References

Ahmad, W. A., Ahmad, W. Y. W., Zakaria, Z. A., & Yusof, N. Z. (2012). *Application of bacterial pigments as colorant* (pp. 57-74). Springer Berlin Heidelberg.

Andrighetti-Fröhner, C. R., Antonio, R. V., Creczynski-Pasa, T. B., Barardi, C. R. M., & Simões, C. M. O. (2003). Cytotoxicity and potential antiviral evaluation of violacein produced by *Chromobacterium violaceum*. *Memórias do Instituto Oswaldo Cruz*, *98*(6), 843-848.

Cabria, G. L. B., Argayosa, V. B., Lazaro, J. E. H., Argayosa, A. M., & Arcilla, C. A. (2014). Draft Genome Sequence of Haloalkaliphilic *Exiguobacterium* sp. Strain AB2 from Manleluag Ophiolitic Spring, Philippines. *Genome announcements*, *2*(4), e00840-14.

Chaturvedi, P., & Shivaji, S. (2006). *Exiguobacterium indicum* sp. nov., a psychrophilic bacterium from the Hamta glacier of the Himalayan mountain ranges of India. *International journal of systematic and evolutionary microbiology*, *56*(12), 2765-2770.

Crapart, S., Fardeau, M. L., Cayol, J. L., Thomas, P., Sery, C., Ollivier, B., & Combet-Blanc, Y. (2007). *Exiguobacterium profundum* sp. nov., a moderately thermophilic, lactic acid - producing bacterium isolated from a deep-sea hydrothermal vent. *International journal of systematic and evolutionary microbiology*, *57*(2), 287-292.

Frühling, A., Schumann, P., Hippe, H., Sträubler, B., & Stackebrandt, E. (2002). *Exiguobacterium undae sp. nov.* and *Exiguobacterium antarcticum sp. nov*. *International journal of systematic and evolutionary microbiology*, *52*(4), 1171-1176.

Isaazadeh, K.H, Azizollahi A., M, Faezi G. M. (2014). Antimicrobial activities bioactive compounds produced by *Exiguobacterium acetylicum* PTCC1756 against pathogenic bacteria. *Scientific Journal of Microbiology*. *3*(4), 55-62.

Johnsen, H. R., & Krause, K. (2014). Cellulase activity screening using pure carboxymethylcellulose: application to soluble cellulolytic samples and to plant tissue prints. *International journal of molecular sciences*, *15*(1), 830-838.

Kumar, D., Kumar, L., Nagar, S., Raina, C., Parshad, R., & Gupta, V. K. (2012). Screening, isolation and production of lipase/esterase producing *Bacillus* sp. strain DVL2 and its potential evaluation in esterification and resolution reactions. *Archives of Applied Science Research*, *4*(4), 1763-1770.

Ottonen, K. (1970). Comparison of the agar-plate diffusion method for α-Amylase assay with the colorimetric stationary-stage method. *Journal of the Science of Food and Agriculture*, *21*(5), 261-272.

Rodrigues, D. F., Goris, J., Vishnivetskaya, T., Gilichinsky, D., Thomashow, M. F., & Tiedje, J. M. (2006). Characterization of *Exiguobacterium* isolates from the Siberian permafrost. Description of *Exiguobacterium sibiricum* sp. nov. *Extremophiles*, *10*(4), 285-294.

Vishnivetskaya, T. A., Kathariou, S., & Tiedje, J. M. (2009). The *Exiguobacterium* genus: biodiversity and biogeography. *Extremophiles,Journal*, *3*(3), 541-555.