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Research Article

# *PARACOCCUS BEIBUENSIS* SL2A NEWCAROTENOID PRODUCING BACTERIUM ISOLATED FROM HYPERSALINE-HYPERALKALINE LONAR CRATER, BULDHANA, MAHARASHTRA, INDIA

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**A B S T R A C T**

In recent studies, bacterial isolate SL2 was screened out of 105 pigment producing bacteria isolated from hypersaline-hyperalkaline ecosystem i.e.Lonar Crater, Buldhana, Maharashtra, India, on the basis of pH and NaCl (%) tolerance and identified based on its morphological, biochemical and physiological properties. Identification at molecular level by 16s rRNA sequence analysis revealed 99.0% similarities of SL2 with *Paracoccus beibuensis* and sequence was deposited to NCBI Gen Bank with accession number KY129665. Members of *Paracoccus* sp. belongs tosub-class of proteobacteria and it is a member of family *Rhodobacteriaceae*. Carotenoid production is one of the properties of *Paracoccus* sp.Bacterium *Paracoccus beibuensis* isolated from Lonar Crater soil is non-motile, Gram-negative coccoid rod, tolerant to salt(1-6%), alkaline pH (6-12), catalase and oxidase positive and produces significant quantity of bright orange pigment which was confirmed by spectrophotometric analysis as carotenoid.



## INTRODUCTION

The *Paracoccus* sp. consist of Gram-negative coccoid rods that show metabolic versatility (Harker, *et al*., 1998). Many species of genus *Paracoccus* are reported such as-*Paracoccus denitrificans* (Kocur, 1984 and VanVerseveld, *et al.,* 1992), *Paracoccus thiocynatus*and *Paracoccus versutus* (Katayama *et al*., 1995), *Paracoccus kocurii* (Ohara *et al*., 1990), *Paracoccus alkaliphilus* (Urakami *et al.,* 1989), *Paracoccus beibuensis*. (Zheng *et al*., 2010) etc. In these studies, isolation of carotenoid producing bacteriawas done from Lonar Crater, Buldhana, Maharashtra, India. The isolate SL2 was screened based on pH and NaCl (%) tolerance, spectrophotometric characters and antioxidant activity of the orange pigment extract. In 16s rRNA gene sequence analysis the isolate SL2 showed 99.0% sequence similarity with *Paracoccus beibuensis*. This bacterium produces bright orange pigmented colonies on the nutrient agar pH (8.5), it is Gram-negative, non-spore forming, coccoid rods showed positive results for catalase, oxidase and gelatinase test. The results of extraction and spectrophotometric analysis of the orange pigment from *Paracoccus beibuensis* showed its absorption maximum at 480 nm in acetone which is near to carotenoid i.e.astaxanthin. Astaxanthin (3,3'-dihydroxy-β, β-carotene-4,4'dione) is a valuable carotenoid of xanthophyll group with high antioxidant activity and applicable in food, pharmaceuticals industries and in animal as well as poultry feed. Now a day'sincreased value ofastaxanthin is due to its therapeutic nature and high antioxidant activity. There are growing evidences of benefits of carotenoids to human health and to the growth of certain areas of agriculture especially aquaculture and poultry industry (Johnson *et al*., 1995). Carotenoids are also used as coloring agents for cooked sausages, soft drinks, baked good and as additives to cosmetics. (Bauernfeind, 1981 and Borowitzka, 1986).

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## MATERIALS AND METHODS

### *Bacterial strains*

Bright orange pigment producing isolate SL2 was previously isolated and screenedout of 105 pigmented bacterial cultures.It was isolated on nutrient agar pH 8.5 (Himedia) from soil of Lonar Crater ecosystems by enrichment culture techniques and maintained on same medium by serial inoculation. It was screened based on pH and NaCl tolerance, spectrophotometric characters of pigmented extract and antioxidant activity where, it was found that it is tolerant to alkaline pH (6-12) and NaCl (1-6%), showed absorption maxima at 480 nm in acetoneand 53% radical scavenging activity estimated by DPPH assay (details not given here).

### *Phenotypic characterization of isolated and screenedisolate SL2*

The phenotypiccharacters of bacteria include morphological, microscopic, macroscopic features and culture characters. The morphological features of isolate SL2 were studied by Grams staining, capsule was demonstration by Manual's method and

motility was checked by hanging drop technique for which 24h activated culture was used to prepare bacterial suspension. The endospore staining was done by Schaeffer-Fulton method and the PHB staining was performed by Gohar method for which 72h culture was used. Isolate SL2 was also analyzed by Scanning Electron Microscopy (SEM) at Shraddha Analytical Services, Ghatkopar, Mumbai (M.S.) India. The all observed morphological and microscopic structures were compared with structure given in the Bergey's Manual of Determinative Bacteriology.

***Determination of biochemical characters***

The bacterium SL2 was maintained on nutrient agar was used for biochemical characterization. Biochemical characterization was done at laboratory level. The temperature range for growth and carotenoid production was determined by inoculating the cells in nutrient broth pH 8.5 and incubated at the temperature 20, 25, 30 and 40$^0$C and incubated for 72h days with aeration-agitation of 120rpm. The pH range (6.0, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0, 10.5 and 11.0) and NaCl tolerance (1, 2, 3, 4, 5 and 6%) was determined for growth. Carbohydrate utilization was studied in terms of acid and gas production as per protocol given by Aneja(1996)using peptone water at pH 8.5 with phenol red (0.018g/l) and 1% carbohydrates. The following compounds were also tested for its effect on growth i.e. D-raffinose, D-mannose, D-xylose, DL-arabinose, D-galactose, D-glucose, sucrose, lactose, fructose, salicin, mannitol, inulin, dextrose, ducitol, melibiose, rhamnose, sorbitol, trehalose and cellobiose. The ability to use organic and inorganic nitrogen sources was examined using peptone water containing 1% of nitrogen source separately and growth was monitored by measuring turbidity after 5 days. Catalase activity was determined by the presence of bubbles in 3% hydrogen peroxide solution (Dong *et al*., 2001). Oxidase activity was tested by using oxidase disc (Himedia) according protocol given in manual. Indole production, Methyl red, Voges-Proskauer and citrate utilization studies were carried out according to protocol given by Aneja (1996).The enzyme producing ability or ability to decompose complex substrates like starch, pectin, gelatin, casein, tributyrin, lecithin, urea andtween 80 was tested as described by Dong *et al*., (2001) and $H_2S$ production was also tested by adding 0.01% L-cysteine in nutrient broth and lead acetate paper was placed in the neck of the tube as an indicator (Clark *et al*., 1953 and Mata *et al*., 2002). Amino acid utilization was tested by using peptone water supplemented with amino acid (1mg/ml) and results were recorded in terms of turbidity measured at 660nm.

***Identification of isolate based on of 16s rRNA sequence and phylogenetic analysis of SL2***

Chromosomal DNA was isolated from SL2 and purification was performed by using Sigma's, "GenElute Bacterial Genomic DNA" kit. The genomic DNA thus obtained was amplified by using PCR primers FDD2 and RPP2 which are universal primers for 1.5kb fragment amplification for eubacteria. The genomic DNA was used as templet and primers-(FDD2-CCGATCCGTCGACAGAGTTTGATCTGGCTCAGand RPP2-CCAAGCTTCTAGACGGTACCTTGTTACGGACTT) were used. The PCR products obtained from the reaction were then processed for cycle sequencing. Then samples were cleaned up and loaded on the sequencer and after sequencing 16s rDNA sequence was generated. 16s rDNA sequence was used for analysis by BLAST with NCBI GenBank database, then first ten sequences were selected and aligned with multiple sequence alignment program, 'Clustal W', depending on maximum identity score. The phylogenetic tree was constructed according to neighbor-joining method using MEGA5. The evolutionary distance was computed using Kimura 2 parameter method. The sequence obtained after analysis was deposited to NCBI GenBank.

***Extraction and identification of the pigment produced by SL2***

The color of the cell pellet after separation from nutrient broth was matched by Munsell color chart by using android App called DIC Color Guide. The extraction of pigment was done by inoculating bacteria SL2 (1%) in sterile nutrient broth having initial pH 9.5 at 30$^0$C for 72h with shaking condition (120 rpm) in white light. After incubation, extraction of pigment was done by using protocol given by Bhat *et al*., (2015).Then culture medium was centrifuged (REMI-cooling ultracentrifuge Model c-24BL) at 8000rpm for 15 min at 4$^0$C to separate cells. Separated cells were washed twice with sterile distilled waterby centrifugation at 8000rpm for 10min.The cell pellet was suspended in acetone and extraction was done at 60$^0$C by using water bath for 25 min. followed by centrifugation at 8000rpm for 10 min. at 4$^0$C. The colored supernatant was collected, and process was repeated until pellet turned white. The absorption maximum of supernatant was determined by scanning the crude extract for 400-600nm by using spectrophotometer (Agilent cary-60) against acetone as reference. The identification of orange pigment includes spectrophotometric analysis and chemical identification of chloroform extract of pigment. The absorption spectrum of crude pigment extract was measured within range of 400-600nm using methanol as reference. For chemical identification 1g dry wet of harvested cells was taken in dry tube and 10mL of chloroform was added followed by vigorous shaking. The resulting mixture was filtered using Whatmann Filter paper no.1. Few drops of 85% sulfuric acid were added and observed for presence of blue colored ring at the interface of carotenoid extract and sulfuric acid

## RESULTS AND DISCUSSION

***Phenotypic characterization of SL2***

Cells are Gram-negative (Figure-1), non-motile, non-spore forming short coccoid rods approximately 1-2µm in size (Figure-2). They appear as bright orange small pigmented colonies (Figure-3) (0.5-3.5mm) on nutrient agar pH (8-12) with smooth, convex, opaque with butyrousconsistency.

**Figure 1** Isolate SL2 on nutrient agar pH 8.5

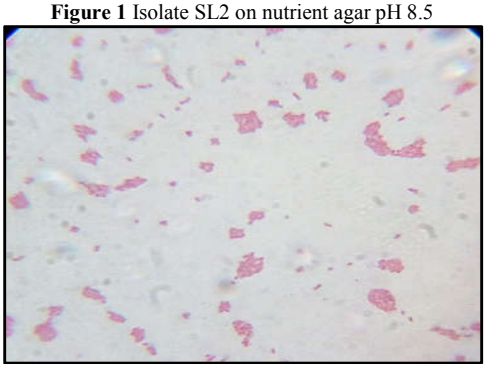

**Figure 2** Gram-negative coccoid rods

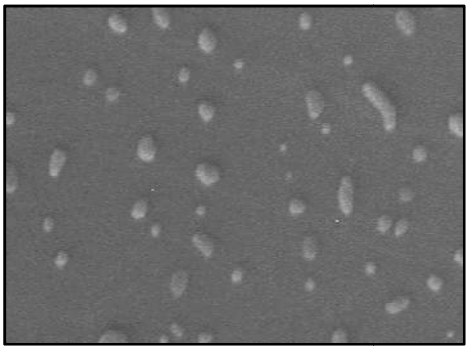

**Figure 3** SEM Image of SL2

### *Physiological and biochemical characteristics of SL2*

Isolate SL2can grow with bright pigmentation in alkaline pH range (8-12) and can tolerate NaCl (1-6%) but as concentration NaCl increases the brightness of pigment is reduced. It can grow with good pigmentation at temperature 20-30$^0$C but as temperature increases biomass formation also increases and pigmentation was reduced. This isolate is catalase and oxidase positive. Gelatin is hydrolyzed while starch is not hydrolyzed. Detail physiological and chemotaxonomic characteristics are described in table-1.El-Banna*et al*., (2012) described pigmented yeast colony color with the help of Munsell color chart as 10R7/8.Pathak *et al*., (2012) studied the morphological features of carotenoid producing 07 bacterial isolates from solar salterns and isolates were reported as Gram-positive and Gram-negative rods, and all were motile and have pink, orange and red pigmentation.

**Table 1** Morphological and culture characters of isolate SL2

| Morphological and culture characters | | | |
|---|---|---|---|
| Characters | Observation | Characters | Observation |
| Size | 0.5-3.5mm | Consistency | Butyrous |
| Shape | Circular | Grams nature | Gram negative |
| Color | Bright orange | Morphology | Coccoid rods |
| Margin | Entire | Motility | Non-motile |
| Surface | Smooth | Flagellation | Not detected |
| Elevation | Convex | Munsell color code | 9.6R6.3/15.0 |
| Opacity | Opaque | Diffusible pigment | Not detected |
| Sporulation | Absent | Capsule | Present |
| PHB production | Present | Pleomorphism | Present |

**Table 2** Carbohydrate utilization studies of bacterial isolate SL2 Legend- (++) positive test, (--) negative test, (+-) weakly positive test

| Utilization of Carbon source | | | |
|---|---|---|---|
| **Carbohydrate** | **Response** | **Carbohydrate** | **Response** |
| Raffinose | + + | Galactose | + + |
| Mannose | + + | Glucose | + + |
| Xylose | + + | Sucrose | + - |
| Arabinose | + + | Lactose | + - |
| Salicin | + - | Ducitol | + - |
| Mannitol | + + | Fructose | + + |
| Inulin | + + | Melibiose | + + |
| Dextrose | + + | Rhamnose | + - |
| Sorbitol | + + | Adonitol | +- |
| Trehalose | + - | Cellobiose | +- |

**Table 3** Substrate utilization and enzyme activity studies of isolate SL2

| Decomposition/Enzyme Activity studies | | | | | |
|---|---|---|---|---|---|
| **Activity for** | **Observation** | **Result** | **Activity for** | **Observation** | **Result** |
| Amylases | No zone of clearance | Negative | Oxidase | Appearance of violet color to bacterial growth | Positive |
| Gelatinase | Liquification observed | Positive | Lecithinase | No zones of opalescence around colony | Negative |
| Lipase | No zone of clearance | Negative | Catalase | Prompt effervescence observed with culture in $H_2O_2$ | Positive |
| Nitrate reductase | Pink color does not develop | Negative | Indole production | No red ring formation on surface side | Negative |
| Lysine decarboxylase | No red colored zone around colony | Negative | Methyl red test | No development of red color | Negative |
| Phenylalanine deaminase | No green color development | Negative | Voges Proskauer Test | No pink color formation | Negative |
| Casinase | No zone of clearance | Negative | Citrate Utilization | No growth observed on slant | Negative |
| Urease | No change in color around the colony | Negative | Tween 80 | No zone of opalescence | Negative |

**Table 4** Utilization of nitrogen source by isolate SL2

| Utilization of Nitrogen source | | | |
|---|---|---|---|
| Meat Extract | + + | Sodium Nitrate | -- |
| Yeast Extract | + + | Ammonium chloride | -- |
| Beef Extract | + + | Ammonium sulphate | -- |
| Peptone | ++ | Potassium Nitrate | -- |
| Meat Extract | + + | Sodium Nitrate | -- |

**Table 5** Utilization of amino acids by isolate SL2

| Utilization of Amino acid | | | |
|---|---|---|---|
| Tyrosine | + + | Phenyl alanine | + + |
| Threonine | + + | Alanine | + + |
| Lysine | + + | Cysteine | + + |
| Glycine | + + | Serine | + + |
| Tryptophan | + + | Leucine | + + |

Legend- (++) positive test, (--) negative test, (+-) weakly positive test

### *16s rRNA Sequence and phylogenetic tree analysis*

16s rDNA from isolate SL2 was amplified by PCR and sequencing was done. The phylogenetic position of SL2 within proteobacteria are shown in figure 3. The 16s rRNA gene sequence of SL2 was compared with those available at the GenBank using the BLAST program (NCBI) to determine the approximate phylogenetic affiliation. The 16s rRNA gene sequence of SL2 was aligned with those of related *Paracoccus* species and phylogenetic tree was constructed using neighbor- joining and maximum-parsimony algorithms methods. The partial 16s rRNA gene sequence (564bp) were determined for strain SL2 isolate and used for phylogenetic analysis. The closest neighbors were *Paracoccus marinus* strain KKL A5 (99.0% similarity), *Paracoccus beibuensis*strain JLT 1284 (99.0% similarity) and *Paracoccus marinus* Strain NBRC 100637 (99% similarities). Similar studies were carried out for *Paracoccus marinus* sp. nov. (Khan *et al*., 2008), *Paracoccus zeaxanthinifaciens* sp. nov. (Berry *et al*., 2003), *Paracoccus marcusii* sp. nov. (Harker *et al*., 1998), *Paracoccus haeundaensis* sp. nov. (Lee *et al*., 2004) and

*Paracoccus beibuensis* JLT1284T, (Zheng *et al*., 2011) where identification was done at molecular level by 16s rRNA sequencing method and respective sequences were deposited to NCBI GenBank.

>SL2
CCCATGGGGAACTTAGCATGGGGGCACCCTGATCTAGCCATGCCGCGTGAGTGATGA
AGGCCTTAGGGTTGTAAAGCTCTTTCAGCTGGGAAGATAATGACGGTACCAGCAGAA
GAAGCCCCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGGGCTAGCGTTG
TTCGGAATTACTGGGCGTAAAGCGCATGTAGGCGGACTGGAAAGTTGGGGGTGAAAT
CCCGGGGCTCAACCTCGGAACTGCCTTCAAAACTATCAGTCTGGAGTTCGAGAGAGG
TGAGTGGAATTCCGAGTGTAGAGGTGAAATTCGTAGATATTCGGAGGAACACCAGTG
GCGAAGGCGGCTCACTGGCTCGATACTGACGCTGAGATGCGAAAGCGTGGGGAGCA
AACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAATGCCAGACGTCGGG
CAGCATGCTGTTCGGTGTCACACCTAACGGATTAACCATTCCCGCCTGGGGAGTACG
GTCGCCAGATTAANACTCAAANGAATTGCCGGGGGCCCGCACAAGCGGTGGA

**Figure 4** 16s rDNA sequence from SL2 isolate

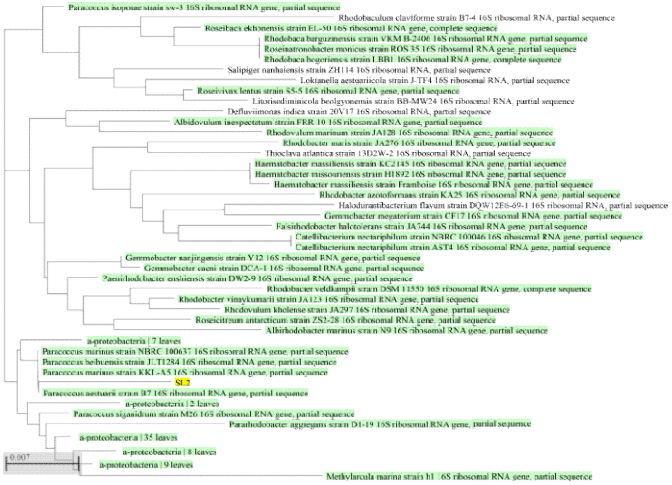


**Figure 5** Phylogenetic relationship among the species of the α-3 proteobacteria and representative species of the other proteobacteria. The tree was drawn by neighbor joining method, and the result of analysis are shown. Accession number are in parentheses. Bar, $0.007K_{nuc}$.

**Table 6** Characteristics with similarities and differences of SL2 with members of *Paracoccus* sp. Reported

| Characteristic | Isolate SL2 (Recent studies) | *P. beibuensis*JLT1284T Zheng *et al*., (2011) | *P. marinus*KKL-A5$^T$ Khan *et al*., (2013) |
|---|---|---|---|
| Pigmentation | Bright orange | Orange | Dull orange |
| Type of carotenoid | Predicted as Astaxanthin | Reported as Astaxanthin | Reported as Adonixanthin |
| Motility | - | - | - |
| Flagellation | - | - | - |
| pH range | 6.0-11.0 | 6.0-8.0 | 6.0-9.0 |
| NaCl (w/v) (%) | 1-6 | 2-15 | 1-4 |
| Temperature ($^0$C) | 10-40 | 10-30 | 10-35 |
| Urease | - | - | - |
| Gelatinase | + | + | - |
| Oxidase | + | + | + |
| Catalase | + | + | + |
| $NO3^-$ reduction | - | - | - |
| Tween 80 hydrolysis | - | - | - |
| Raffinose | + + | NA | NA |
| Mannose | + + | NA | - |
| Xylose | + + | - | NA |
| Arabinose | + + | NA | - |
| Salicin | + - | NA | NA |
| Mannitol | + + | + | - |
| Inulin | + + | NA | NA |
| Dextrose | + + | - | NA |
| Sorbitol | + + | + | - |
| Trehalose | + - | + | - |
| Galactose | + + | + | + |
| Glucose | + + | - | + |
| Sucrose | + - | + | - |
| Fructose | + | + | + |
| Lactose | +- | + | - |

Legend- (+) Positive, (+-) weakly positive, (-) Negative, (NA) no data available

### *Extraction and analysis of the pigment produced*

The bright orange pigments produced by SL2 were identified as carotenoid, including astaxanthin [UV-vis $\lambda_{max}$ 480nm], Figure-4. One peak spectra with broad shoulder region between 470-480nm was observed. During chemical test, blue colored ring was observed at interface of pigment and concentrated $H_2SO_4$ suggest the presence of polyene compound in the pigment (Mrak*et al*.,1949), (Karrer and Jucker, 1950) and (Ajayi *et al*., 2011). Similar test done by Shatila *et al*., (2013) where the chloroform extract of *Exiguobacteriumaurantiacum*FH, exhibited dark blue coloration upon the addition of concentrated sulfuric acid which revealed the presence of Carotenoid.

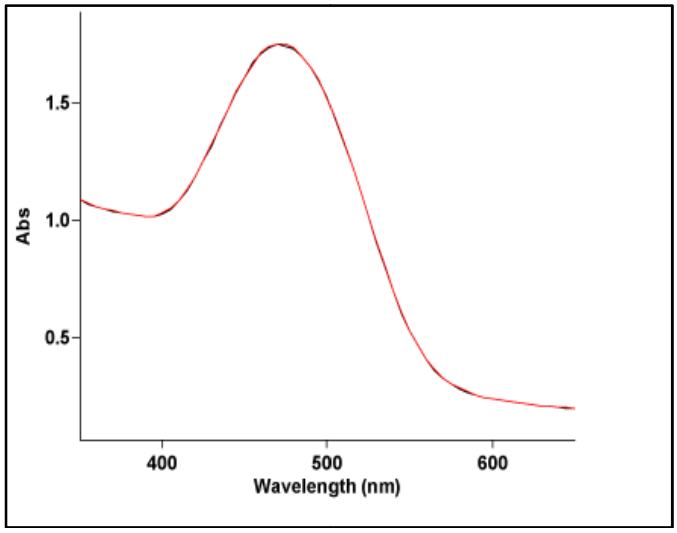


**Figure 6** Spectral analysis of crude pigment

## CONCLUSION

*Paracoccus beibuensis*SL2 was isolated from hypersaline-hyperalkaline environment of Lonar Crater, it is Gram-negative, non-motile, coccoid rods. Small 0.5 to 3.5 mm bright orange colonies are produced on optimized organic media after incubation at $30^0$C for 72 h. Colonies are smooth, convex, and opaque. It can tolerate NaCl (1-6%) and show alkali tolerance (NaOH) pH (6-12). The optimum temperature for carotenoid (astaxanthin) production was found $30^0$C and pH 9.5. It produces significant quantities of carotenoid with λmax 480nm, which is a astaxanthin. Astaxanthin has many applications in food, feed, pharma products, cosmetics, and therapeutics.

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## References

1. Ajayi, I.A., Ajibade, O., and Oderinde, R.A. (2011) Preliminary phytochemicals analysis of some plant seeds. Res. *J. Chem. Sci*. 1: 58-62.
2. Aneja K.R. (1996) Experiments in Microbiology, Plant Pathology, Tissue Culture and Mushroom Cultivation. 2nd Edition, WishwaPrakashan, New Age International Pvt Ltd., New Delhi10. 13.

3. Bauernfeind, JC. (1981) "Carotenoids, Colorants and Vitamin A precursors, Technological and Nutritional applications," Academic press, New York, pp.938.
4. Berry Alan, Daniella Janssens, Markus Humbelin, Jan P.M., Jore, Bart Haste, LlseCleenwerck, Marc Vancanneyt, Werner Bretzel, Anne F. Mayer, RualLopaz-Ulibarri, Balajee Shanmugam, Jean Swings and Luis Pusamontes (2003) *Paracoccus zeaxanthinifaciens* sp. nov., a zeaxanthin -producing bacterium, *Int. J. of Syst. And Evol. Microbiol*. 53, 231-238
5. Borowitzka, MA. (1986) "Micro-Algae as Sources of Fine Chemicals," Microbiological Science, Vol. 3, pp.372-375
6. Clarke PH (1953) Hydrogen Sulphide production by bacteria. *J. Gen Microbiol* 8: 397- 407.
7. Dong X-Z, Cai M-Y (2001) Determinative manual for routine bacteriology. Scientific press, Pecking
8. El-Banna El Rhman Amr Abd, Amal Mohamed Abd El-Razek, Rafik El- Mahdy, (2012). Isolation, Identification and screening of Carotenoid producing Strains of *Rhodotorulagiltinis*. Food and Nutri. Sci. 3: 627-633
9. Harker M, Hirschberg J. Oren A (1998) *Paracoccus marcusii* sp. nov., an orange Gram-negative coccus. *Int J. Syst Bacteriol* 48: 543-548
10. Johnson E.A. and W.A. Schroeder (1995), "Microbial Carotenoids"' In: A. Fisher, Ed., Advances Biochemical Engineering Biotechnology, Springer-Verlage, Heidelberg, vol. 53, 1995, pp.119-178.
11. Karrer, P. and Jucker, E. (1950) Carotenoids. Elsevier Publishing Company Inc., New York, Amsterdam, London, Brussels
12. Katayama, Y., Hiraishi, A. and Kuraishi, H. (1995).*Paracoccus thiocynatus* sp. nov., a new species of thiocyanate- utilizing facultative chemolithotroph, and transfer of *Thiobacillusversutus t*o the genus *Paracoccus* as *Paracoccus versutus*comb. Nov. with emendation of the genus. Microbiology 141, 1469-1477.
13. Khan Shams Tabrez, Shinichi Takaichi and ShigeakiHarayama, (2008). Paracoccus marinus sp. nov., an adenoxanthindiglucoside- producing bacterium isolated from coastal seawater in Tokyo Bay. *Int. J. of syst. and Evol. Microbiol.* 58:383-386.
14. Kocur, M.(1984). Genus *Paracoccus* Davis 1969, 384$^{AL}$. InBergey's Manual of systematic Bacteriology, vol.1 pp.399-402.Edited by N.R. Krieg and J.G. Holt. Baltimore: Williams and Wilkins.
15. Lee Jae Hyung, Yun Sook Kim, Tae- Jin Choi, Won Jae Lee and Young Tae Kim (2004) *Paracoccus haeundaensis* sp. nov., a Gram-negative halophilic, astaxanthin- producing bacterium. *Int. J. of Syst. And Evol. Microbiol*. 54, pp: 1699-1702. 24.
16. Manish RB, Thankamani M. Media optimization. Extraction and Partial Characterization of an Orange Pigment from Salinococcus sp. MKJ997975. *Int. J. of Life sci. and Pharma Research*.2015;4(2): 85-89
17. Mata JA, Martinez-Canovas J, Quesasa E, Bejar V (2002) A detailed phenotypic characterization of the type strains of *Halomonas* species. Syst Appl Microbiol 25: 360-375.
18. Mrak EM, Phaff HJ and Mackinney G. (1949). A simple test for carotenoid pigments in yeasts. *J. Bacteriol*. 57: 409-411.
19. Ohara, M., Katayama, Y. Tsuzaki, M. Nakamoto, S. and Kuraishi, H. (1990) *Paracoccus kocurii*sp. nov., a tetramethylammonium-assimilating bacterium. *Int J Syst Bacteriol.* 40, 292-296.
20. Phathak Anupama P. and Aparna G. Sardar, (2012). Isolation and characterization of carotenoid producing Haloarchaea from solar saltern of Mulund, Mumbai, India. *Ind. J. of Nat. Resour*. 3(4): 483-488.
21. Shatila F., Hoda Yusef and Hanafy Holail (2013) Pigment production by *Exiguobacteriumaurantiacum* FH, a novel Labanesestrain. *Int. J. Curr. Microbiolo. App. Sci*. 2(12), pp 176-191.
22. Urakami, T., Tamaoka, J., Suzuki, K. and Komagata, K. (1989). *Paracoccus alkaliphilus* sp. nov., an alkaliphilic and facultatively methylotrophic bacterium. *Int. J. Syst Bacteriol* 39, 116-121
23. Van Verseveld, H. W. and Stouthamer, A. H. (1992) The genus *Paracoccus*. In the Prokaryotes. A Handbook on the biology of Bacteria: Ecophysiology, Isolation, Identification, Applications, vol. 3, 2$^{nd}$edn, pp: 2321-2334. Edited by A. Bolows, H.G. Truper, m. Dworkin and K.H. Schleifer. New York: Springer.
24. Zheng Q., Wang Y., Chen C., Wang Yu., Xia X., Fu Y., Zhang R., and Nianzhi Jiao. (2011) *Paracoccus beibuensis* sp. nov., Isolated from the south China Sea., Curr. Microbiol 62: 710-714 Springer.



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