

GROWTH OPTIMIZATION OF THE EXTREMOPHILIC PARACOCUS BARUCHII LBOG37**C. O. Wesonga¹ and J. N. Kamau²**^{1,2}*Institute for Biotechnology Research, Jomo Kenyatta University of Technology and Agriculture, Nairobi, Kenya**E-mail: wocbar@yahoo.com***Abstract**

The genus *Paracoccus* belongs to the α -subdivision of the *Alphaproteobacteria*, and encompasses over 34 defined species. Members of the genus are known to be versatile organisms that can prevail in any environmental condition due to their nature of adapting their metabolism accordingly. Hence, *Paracoccus* strains are able to utilize a wide range of organic carbon compounds which serve as carbon and free energy sources during their heterotrophic growth. *Paracoccus baruchii* LBOG37 has the potency of producing carotenoids that have a wide application in medical fields. However, commercial exploitation of many carotenoid producing bacteria is limited by the biomass. Thus, this study sought to optimize growth conditions of *Paracoccus baruchii* LBOG37. The isolate was obtained from the Chiromo campus Biochemistry Department of University of Nairobi where it had been cryopreserved. It was revived and confirmed using 16SrRNA analysis. The growth pH, growth temperature and carbon source were optimized using modified Horikoshi medium at different time intervals. The growth was determined using optical density at 600nm. This was aimed at aiding increased production of biomass of the bacteria to necessitate commercial exploitation of the bacteria for carotenoid production using commonly available carbon sources (starch, carboxymethyl cellulose and glucose). The study established that the *Paracoccus baruchii* LBOG37 grew optimally at a pH of between 9.0 and 9.5 and with its optimum growth temperature recorded at 40°C on Horikoshi medium. With the latter being a relatively thermophilic temperature, some significant growth was also evident at slightly higher thermophilic temperatures and at the mesophilic temperatures providing an almost "aseptic" growth condition for the isolate. It was therefore concluded that *Paracoccus baruchii* LBOG37 is a thermophilic, alkaliphilic bacterium that utilises carboxymethyl cellulose, starch and D-glucose during its heterotrophic growth.

Key words: *Paracoccus baruchii* LBOG37, carotenoid, carboxymethyl cellulose, starch, thermophilic, mesophilic, alkaliphilic.

Introduction

The genus *Paracoccus* belongs to the α -subdivision of non-sulfur purple bacteria, the *Alphaproteobacteria*, and encompasses over 34 defined species. Members of the genus fall in the *Rhodobacter* group of the *Alphaproteobacteria* with the nearest neighbors being the *Amaricoccus*, the *Octadecabacter*, the *Rhodovulum*, the *Roseobacter*, the *Sagittula* and the "*Tetracoccus*" with which they share a number of properties (Euzéby, 1997). Comparisons of the 16S rRNA genes are now being routinely used to ensure the correct placement of strains of *Paracoccus* within the class *Alphaproteobacteria* (Kelly *et al.*, 2006). *Paracoccus* have a high guanine plus cytosine (G+C) content.

The *Paracoccus* species are found in various environments that include soil, sea water and sludge. Members of the genus *Paracoccus* are known to be versatile organisms that can prevail in any environmental condition due to their nature of adapting their metabolism accordingly to these conditions (van Spanning *et al.*, 2005). In describing their metabolism, *Paracoccus* strains are said to be non-fermentative, however during their heterotrophic growth, a wide range of organic carbon compounds may serve as the sole carbon and free energy sources. Examples include the D-glucose, D-fructose, sucrose, acetate, propionate, β -hydroxybenzoate, histidine, leucine, proline, ethanol, propanol, glycerol and sorbitol. However, no growth has been reported on organic compounds such as xylose, starch, gelatin, glycogen, cellulose and phenol just to mention a few (Kelly *et al.*, 2006; van Spanning *et al.*, 2005). The versatile degrading capabilities of some of the strains have resulted in the recent increased interest in the genus making them the focal point of many studies. For example, some strains of *Paracoccus denitrificans* have been found to oxidize reduced sulfur compounds such as thiosulfate, thiocyanate, carbon disulfide, carbonyl sulfide and elemental sulfur to gain their metabolic energy for the autotrophic growth and can therefore be used for the bioremediation process (Kelly *et al.*, 2006). The increased interest in members of this genus has further been enhanced by the ability of some strains to produce colored carotenoid compounds in addition to their occurrence in alkaline-saline environments such as the soda lakes of the Great Rift Valley (Krulwich, 2006). Intake of colored carotenoid are argued to be capable of enhancing the immune system, and reducing the risk of degenerative diseases such as cancer, cardiovascular diseases, macular degeneration, and cataract since they act as antioxidants, membrane stabilizers, and precursors for vitamin A (Choi *et al.*, 2006). Examples are the *Paracoccus bogoriensis* (Osanjo *et al.*, 2009) and the *Paracoccus baruchii* LBOG37. The latter was the focus of this study due to its ability to produce xanthophyllic carotenoids under relatively "sterile" conditions.

Lake Bogoria, the site of isolation of the *Paracoccus baruchii* LBOG37, is one such alkaline-saline athalassic soda lake that is located in the Kenyan Rift Valley. Its pH ranges from 9 to 11 while the temperature fluctuates between 30°C and 90°C depending on the site (Osanjo *et al.*, 2009). This therefore implies that the species is able to grow in a thermoalkaline environment. One of the *Paracoccus* species

that has also been reported to grow in alkaline environment is the *Paracoccus alcaliphilus* which was found to grow at pH 7.0 to 9.5 (Urakami *et al.*, 1989).

Liu *et al.*, (2008) argues that several members of the genus *Paracoccus* are capable of utilizing diverse range of organic compounds heterotrophically, with a further assertion that some members of the group are capable of growing chemotrophically and to utilize nitrate during anaerobic respiration. This provides an alternative electron acceptor to oxygen during anaerobic respiration. In this study, the optimal growth conditions of the *Paracoccus baruchii* LBOG37 with respect to the utilisation of three sugar sources (starch, glucose and caboxymethyl-cellulose) was investigated, in addition to its optimal growth temperature and pH.

Materials and methods

Source of organism

The bacterial isolate, *Paracoccus baruchii* LBOG37, having previously been isolated from the Lake Bogoria, had been cryopreserved in glycerol stocks at the Chiromo Campus, Biochemistry Department of the University of Nairobi.

Bacterial isolate revival

The isolate was revived on Horikoshi agar medium that contained 10g/l starch, 10g/l NaCl, 5g/l bacteriological peptone, 5g/l yeast extract and 15g/l bacto-agar. The medium pH had been adjusted to a pH of 9.0 after autoclaving using a Na₂CO₃/Na₂HCO₃ buffer at a ratio of 9:1 (medium: buffer). The *Paracoccus baruchii* LBOG37 was streaked onto the solidified agar and incubated at 40°C overnight in an incubator (Applied Biosystems, La Jolla CA USA). A single colony from the agar plate was used as the inoculum on the same medium broth (pH 9.0) and incubated overnight with continuous shaking at 130rpm in a Gallenkamp Orbital incubator/shaker. Glycerol stocks were also prepared from the revived culture stock and stored at -20°C.

Isolate confirmation

The isolate identity was confirmed by 16SrRNA gene amplification, sequencing and BLASTn analysis. The 16SrRNA gene amplification used the universal F8 and R1492 primers that amplify the bacterial 16S rRNA gene from position 8 to 1492 according to the *E. coli* numbering system. A reaction master mix was prepared that included the following constituents per 20µl reaction: 4.0µl of the PCR buffer, 2.0µl of deoxynucleotide triphosphates (dNTPs), 1.0µl each of the primers, 0.5µl of Taq polymerase, 1.0µl template and 10.5µl of double distilled water. The PCR conditions used on the Perkin Elmer thermocycler were as follows: initial denaturation at 96°C for 5 minutes with a further denaturation reaction also at 96°C for 30 seconds, annealing at 55°C for 1 minute, extension/elongation at 72°C for 1 minute and a final extension at 72°C for 10 minutes. Apart from the initial denaturation and the final extension reactions which were only one cycle each, the other reactions were run for 30 cycles. The resulting amplicons were visualized on

a 1% agarose gel containing ethidium bromide, electrophoretically ran at 80V for about an hour.

The amplified DNA was then purified from the agarose gel using the QIAXEX II Extraction Kit following the manufacturer's protocol. The purified PCR products were then sequenced.

The obtained partial consensus 16S rRNA gene sequence was subsequently used to conduct a BLASTn search (Alschul *et al.*, 1997) and aligned with those of related *Paracoccus* species obtained from the Genbank database (<http://www.ncbi.nlm.nih.gov/>). Multiple sequence alignments and the phylogenetic analysis were then conducted using the neighbor-joining and the maximum-parsimony methods of the MEGA5 software (Tamura, *et al.*, 2011).

Growth under different pH conditions

To test for the optimal pH for growth of the *Paracoccus baruchii* LBOG37, a 10 μ l aliquot of the revived culture was transferred into tubes containing 20ml MH liquid medium with final pH adjusted to 9.0, 9.5, 10.0, 10.5, and 11.0 respectively. The adjustment of pH had been done after autoclaving by addition of a Na₂CO₃/NaHCO₃ buffer. The cultures were then incubated with shaking (130rpm) at 40°C for 24 hrs. The bacterial growth at different pH was measured at OD 600nm after 24 hours.

Effect of temperature on growth

Effect of temperature on the *Paracoccus baruchii* LBOG37 isolate was monitored by inoculation of 10 μ l of revived isolate in tubes containing 20ml MH liquid medium and incubation at 37°C, 40°C, 45°C, 50°C and 54°C respectively with shaking at 130rpm. The optical density (OD) was then measured for 48 hours at intervals of 0h, 2h, 4h, 8h, 24h and 48h and at a wavelength of 600nm.

Effect of sugar on growth

Effect of sugar source on *Paracoccus baruchii* LBOG37 isolate growth was monitored by inoculation of 10 μ l of revived isolate in tubes containing 20ml MH liquid medium with different carbon sources (10g/l carboxymethyl cellulose (Sigma), 10g/l starch (RDH33615) and 10g/l glucose (sigma) which replaced the 10g/l of starch and incubation at 37°C, 40°C, 45°C, 50°C and 54°C with shaking at 130rpm. Optical density (OD) was measured for 48 hours at an interval of 0h, 2h, 4h, 8h, 24h and 48h at a wavelength of 600nm to help in determination of optimum temperature. It should be noted that the decision to determine the sugar use at different temperatures rather than pH was informed by the fact that L. Bogoria where the isolate was isolated from has alkaline pH. The study used the optimum pH obtained at 40°C. Thus, it was assumed that the optimum pH obtained would apply to all the three sugars used in this study.

Results

Isolate Revival

The isolate was successful revived and had a characteristic orange color as shown in figure 1 below.



Figure 1: *Paracoccus baruchii* LBOG37 growing on modified Horkoshi (MH) agar medium

The isolate had a characteristic orange colour. This could be indicative that the species is capable of producing the commercially viable ketocarotenoid pigments produced by various organisms such as bacteria, algae, fungi and green plants (Armstrong, 1997).

Isolate confirmation

Figure 2 below shows phylogenetic analysis of the 16S rRNA gene that was amplified.

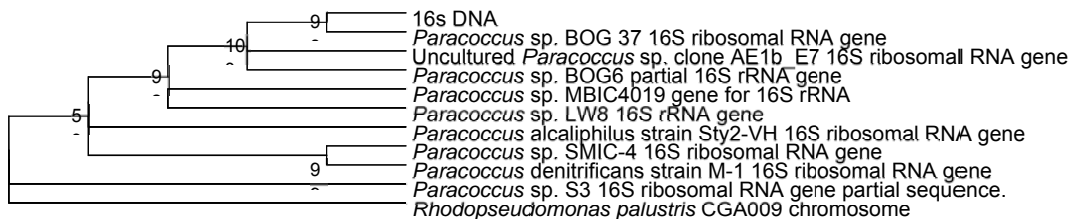


Figure 2: Neighbor-Joining phylogenetic tree of the 16S rRNA sequences obtained from *Paracoccus* sp LBOG37 and that of the most related organisms. *Rhodopseudomonas palustris* CGA009 sequences were used as out-group.

From figure 2 the sequences obtained had 98% similarity with that of *Paracoccus* sp LBOG37 sequences in the Gene Bank. This implies that the isolate used in this study was indeed *Paracoccus* sp LBOG37.

Growth Condition Optimization

Table 1 shows a summary of the optical densities (600nm) at different time intervals after incubation at various temperatures in different carbon sources.

Table 1: Optical density (absorbance at 600nm) at different time interval after incubation at various temperatures in different sugar sources

| Optical density (OD) at: | Time (hours) | 0 | 2 | 4 | 8 | 24 | 48 |
|--------------------------|----------------------------|-------|-------|-------|-------|-------|-------|
| 37°C | in Carboxymethyl cellulose | 0.000 | 0.090 | 0.134 | 0.281 | 0.362 | 0.450 |
| different | Glucose | 0.000 | 0.090 | 0.200 | 0.313 | 0.520 | 0.653 |
| sugar source | Starch | 0.000 | 0.155 | 0.230 | 0.370 | 0.679 | 0.772 |
| 40°C | in Carboxymethyl cellulose | 0.000 | 0.390 | 0.534 | 0.981 | 1.362 | 1.127 |
| different | Glucose | 0.000 | 0.390 | 0.500 | 1.113 | 2.120 | 2.247 |
| sugar source | Starch | 0.000 | 0.655 | 0.700 | 1.870 | 2.179 | 1.805 |
| 45°C | in Carboxymethyl cellulose | 0.000 | 0.473 | 0.670 | 0.740 | 1.210 | 1.104 |
| different | Glucose | 0.000 | 0.568 | 0.651 | 0.650 | 0.792 | 0.981 |
| sugar source | Starch | 0.000 | 0.862 | 0.907 | 1.334 | 1.549 | 1.836 |
| 50°C | in Carboxymethyl cellulose | 0.000 | 0.267 | 0.703 | 0.870 | 1.082 | 1.093 |
| different | Glucose | 0.000 | 0.366 | 0.560 | 0.660 | 0.871 | 0.902 |
| sugar source | Starch | 0.000 | 0.596 | 0.835 | 0.907 | 1.024 | 1.008 |
| 54°C | in Carboxymethyl cellulose | 0.000 | 0.166 | 0.169 | 0.270 | 0.467 | 0.470 |
| different | Glucose | 0.000 | 0.164 | 0.164 | 0.266 | 0.632 | 0.702 |
| sugar source | Starch | 0.000 | 0.185 | 0.296 | 0.464 | 0.828 | 0.837 |

Table 1 show that a slight growth was recorded after the first two hours on all the three sugars at 37°C with an exponential growth recorded after incubating the isolate for between 2 hours and 24 hours. The availability enough growth conditions could have supported the exponential growth at this phase. The *Paracoccus baruchii* LBOG37, however, had a higher preference for starch as the carbon source in comparison to the other two sugars. It is therefore likely that starch might be used to select for *Paracoccus baruchii* LBOG 37 from its natural sources when incubated at 37°C as compared to glucose and carboxymethyl cellulose. The carboxymethyl cellulose recorded the lowest growth at this temperature.

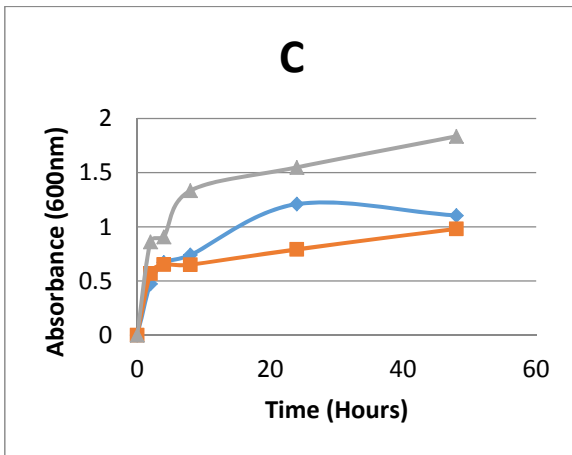
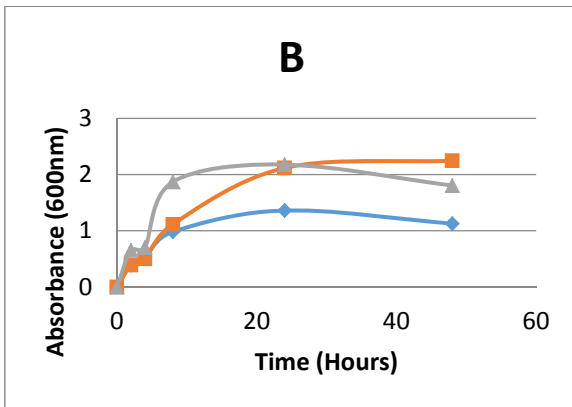
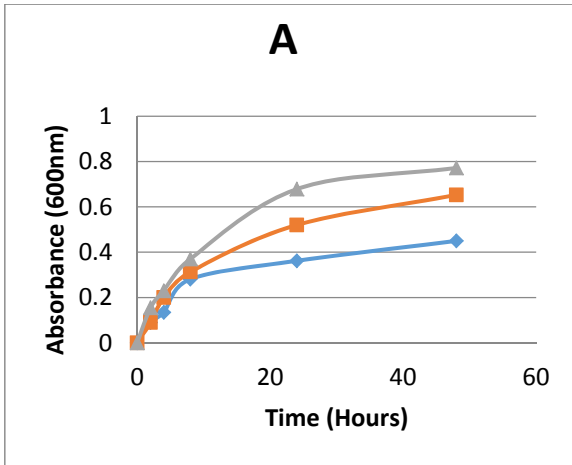
At 40°C the growth of the *Paracoccus* sp BOG 37 seems to be supported more again by starch although glucose also seems to support faster growth. However, a look at figure two shows that at 40°C the growth of the *Paracoccus* sp BOG 37 is faster in starch than in glucose. The *Paracoccus* sp BOG 37 reaches optimum growth in glucose at the point where growth is declining in starch.

At 45°C the optimum growth was also recorded after 24hour incubation period. At this temperature growth in glucose was the lowest and a higher growth was in starch. This is interesting fact to note that as the temperature increases the *Paracoccus* sp BOG 37 seems to switch the carbon sources being utilised. A look at figure 3 shows that growth recorded when glucose has dropped to lower than 1OD while that in carboxymethyl cellulose is higher than 1OD after 24 hours incubation period. It should be noted however that a study by Benigno et al (2013) established that high glucose concentration inhibits growth of *Brettanomyces*

bruxellensis. Thus it is possible that the concentration of glucose used in this study was higher and hence could have inhibited the growth of the *Paracoccus* sp BOG 37. From table 1 it is apparent that the growth of *Paracoccus* sp BOG 37 at 50°C is higher when carboxymethyl cellulose is utilized as a carbon source than when either starch or glucose is utilized after incubation for 24 hours. At this temperature it seems like the genes for utilization of carboxymethyl cellulose are activated while those for utilization of starch and glucose are auto-regulated. This might imply that if the three sugars, starch, glucose and carboxymethyl cellulose, are present in a media and the *Paracoccus* sp BOG 37 is cultured at 50°C utilization of carboxymethyl cellulose might be favored. Figure 4 clearly illustrates how growth rate is higher after incubation of the *Paracoccus* sp BOG 37 at 50°C in carboxymethyl cellulose than in the other two carbon sources.

The growth recorded at temperature 54°C in all the three sugars is below 1OD. This shows that the growth of the *Paracoccus* sp BOG 37 declines at temperature above 50°C in spite the carbon source utilized. It should however be noted that at this higher temperatures starch is again favored over other sugars although the growth rate is lower as compared to what was recorded at lower temperatures. Given that the *Paracoccus* sp BOG 37 attained optimum growth after incubating for 24 hours for all temperatures. The OD for various temperatures was compared. It was established that the *Paracoccus* sp BOG 37 recorded optimum growth rate at 40°C. However, the growth rate in various sugar sources varied depending on the temperature at which it was incubated.

Table 1 further shows growth of the *Paracoccus* sp BOG 37 at various pH levels using different sugar sources at 40°C. The *Paracoccus* sp BOG 37 was incubated at 40°C because this was established to be the optimum temperature for the growth of the *P. sp* BOG 37 when different sugars were used as carbon source. The data shows that the growth rate of the *Paracoccus* sp BOG 37 is higher at pH 9.0 and 9.5 when Carboxymethyl cellulose and starch are utilized as carbon sources. However, for glucose the optimum growth rate of the *P. sp* BOG 37 is attained at pH 10.0. This indicates that the pH of the media might influence the carbon source utilized by the *Paracoccus* sp BOG 37. However, it should be noted that the *Paracoccus* sp BOG 37 optimum pH is between pH 9.0 to pH 10.0. This is an alkaline condition indicating that the *Paracoccus* sp BOG 37 is an alkalophilic bacteria. Figure 3 paints a better picture of these results.



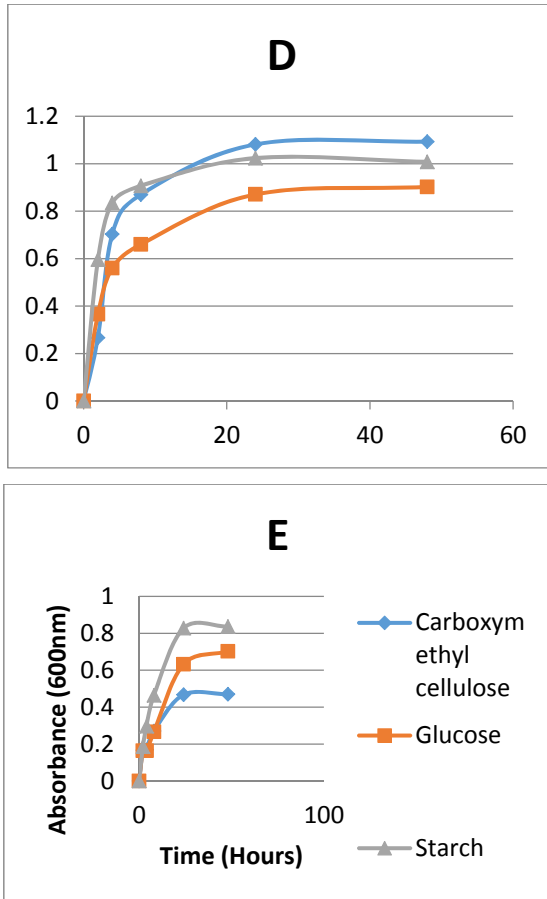


Figure 3: The graphical representation of the optical densities (600nm) versus time (A – E) at different intervals after incubation at 37, 40, 45, 50 and 54 °C respectively

The results in figure 3 shows that starch supported growth than any other sugar at 37°C. However, growth in all the three sugars, starch, glucose and carboxymethyl cellulose, recorded optical densities (OD) below 1. At 40°C growth in starch was again faster than other sugars although growth in glucose later surpassed it. At this temperature all sugar sources recorded OD above 1. At the two temperature values, 37°C and 40°C growth in carboxymethyl cellulose was the lowest. Again, at 45°C growth in starch was higher than all other sugars even though the OD recorded was lower than that recorded at 40°C. At this temperature the lowest growth was recorded in glucose and was below 1OD. At 50°C there was a decline in growth in all sugars with growth in carboxymethyl cellulose being the highest with slightly above 1OD. The lowest growth at this temperature was again recorded in glucose. Finally, at 54°C growth in starch was again higher than in other sugars although all sugars recorded OD below 1. Based on these findings, it can be asserted that *Paracoccus Baruchii* sp. LBOG37 shows some temperature dependent substrate utilization ability. That is, as the temperature increases the

bacteria tends to have wider substrate utilization ability. This finding supports Lertwattanasakul et al (2011) assertion that some bacteria such as *Kluyveromyces marxianus* possess a useful potential to assimilate a wide variety of substrates at a high temperature. This finding also conforms to Liu et al (2007) argument that thermophilic micro-organism shows substrate promiscuity at certain temperature ranges and show substrate specificity at higher temperatures. It is interesting to note that contrary to expectation that glucose would support faster growth at all levels, this was not the case. Moreover, the results suggest that growth of the isolate decline at temperatures below or above 40°C indicating that the isolate grows optimally at 40°C. The fact that growth of the organism in glucose was lower than that found in starch and carboxymethyl cellulose could be explained by Benigno et al (2013) and or Nguyen and Guckert (2001) observations. According to Nguyen and Guckert (2001), organisms growing in soil with carbon limitation take into the cell glucose to be converted into storage compounds instead of being utilised for growth or respiration. On the other hand, Benigno et al (2013) established that high glucose concentration inhibits growth of *Brettanomyces bruxellensis*. Thus, it is possible that either most of the glucose was stored by the organism instead of being utilised for growth or that the concentration of glucose used in this study was higher than the optimum amount and hence inhibited growth.

Having established the optimal growth temperature, the study sought to establish the optimal growth pH. Figure 4 illustrates the findings on the effect of pH on growth using different sugars.

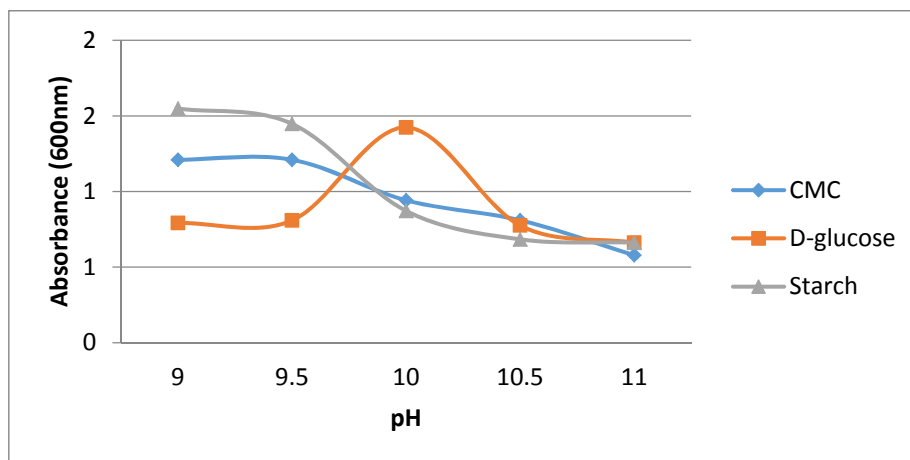


Figure 4: The graphical representation of the optical densities (600nm) versus pH (F) using the three carbon sources (carboxymethyl cellulose, starch and D-glucose)

The results in figure 4 show that the isolate grew optimally at pH ranges of 9 to 9.5 in carboxymethyl cellulose and starch. However, optimal growth in glucose was recorded at a pH of 10. This indicates that the growth of the isolate occurs in strong alkaline condition suggesting that the isolate is an alkaliphile. The growth

declines drastically away from optimal pH values indicating that the organism is sensitive to changes in the alkalinity of the media used.

Discussion

The identity of the *Paracoccus baruchii* LBOG37 was confirmed through conducting a phylogenetic of its consensus 16S rRNA gene sequence. The phylogenetic analysis demonstrated that the isolate formed a cluster with species of the genus *Paracoccus*, amongst which was the original strain that had been submitted to the Genbank as the *Paracoccus* sp. BOG 37 but has since then been renamed *Paracoccus baruchii* LBOG37. However, the 16S rRNA gene sequence data has no defined “threshold values” above which there is a universal agreement of what constitutes a definitive and conclusive identification to the rank of species hence creating a widely used individually acceptable criterion for a species match. Most taxonomists, though not by consensus, have accepted a percentage identity score of $\geq 97\%$ and $\geq 99\%$ for any isolate to be classified under a genus and species, respectively. For example, it has been shown that the 16S rRNA gene sequence data of an unknown isolate with its nearest neighbor exhibiting a similarity score of $< 97\%$ and $\geq 95\%$, is representative of a new species with the isolate in question being assigned to the corresponding genus (Janda and Abbott, 2007). Similarity values of $> 97\%$ can also be representative of a new species or alternatively indicate clustering within a previously defined taxon (Janda and Abbott, 2007; Petti, 2007). However, when the similarity is placed at $< 95\%$, the unknown isolate is assigned to a family but with a possibility of claiming a novel genus (Asker *et al.*, 2012).

Paracoccus baruchii LBOG37 strain showed optimum growth at pH range of 9.0 to 10.0. This is unique given that a few *Paracoccus* strains have been shown to grow at such extreme alkaline conditions. Examples in literature of such strains include *Paracoccus* sp BOG6, *Paracoccus aestuarii*, *Paracoccus alcaliphilus* (pH range 7.0 – 10.5) (Osanjo *et al.*, 2009; Urakami *et al.*, 1989; Roh *et al.*, 2009). *Paracoccus baruchii* LBOG37 is one of the few *Paracoccus* alkaliphiles isolated from soda lakes; others have been isolated mainly from marine environment (Osanjo *et al.*, 2009).

Paracoccus baruchii LBOG37 is typical of the alkaliphilic microorganisms (Jones *et al.*, 1999). This is because it grows optimally at pH range between 9.0 and 10.0 which is at least two pH units above neutrality. Moreover, it has a lower growth rate at near neutral pH than at high pH (Krulwich *et al.*, 2011). This alkaliphilic characteristic of *Paracoccus baruchii* LBOG37 could be a result of the soda lake environment in which it was isolated that is characterized by the presence of large amounts of sodium carbonate or its complex salts with the absence of significant amounts of Ca^{2+} and Mg^{2+} a key contributing factor to the occurrence of alkalinity. The pH, however, has been found to remain more or less constant regardless of the season, at around 10 to 10.5 for the dilute lakes, rising to pH 12 in concentrated lakes. Despite the apparently inhospitable caustic conditions, the soda lakes have been found to be extremely productive because of the high

ambient temperatures, the high light intensities and the effectively unlimited supplies of CO via the $\text{HCO}_3^-/\text{CO}_3^{2-}/\text{CO}_2$ equilibrium. Alkaliphilic cyanobacteria drive these systems, providing the fixed organic carbon that is utilized by a vast range of the alkaliphilic aerobic and anaerobic chemoorganotrophs (Grant, 2006).

Paracoccus baruchii LBOG37 strain also showed typical heterotrophic versatility of the genus *Paracoccus*. The strain was found to grow on various carbon sources including starch, carboxymethyl cellulose and glucose. The catabolic diversity among microorganisms is one of the distinguishing features of the microbial world. The range of this diversity has been found to even vary widely among individual species. For instance, Osanjo *et al.*, (2009) established that even though *Paracoccus* BOG6 could assimilate L-arabinose, D-sorbitol, raffinose and L-rhamnose, its close phylogenetic relative *P. zeaxanthifaciens* could not utilize these substrates. Just like *Paracoccus* BOG6 which utilise glucose Osanjo *et al.*, (2009), the *Paracoccus baruchii* LBOG37 also assimilates glucose. Micro-organisms vary from the highly specialized ones that can only utilize only one or a few substrates as energy sources to the highly versatile species that can utilize over 100 compounds as the sole carbon and energy source. Interestingly, cellulolytic microbes lie near the specialist end of this continuum. They are primarily carbohydrate degraders (Lynd *et al.*, 2002). This affirms the finding of this study which established that *Paracoccus baruchii* LBOG37 can assimilate several carbohydrates such as starch, carboxymethyl cellulose and glucose as carbon sources. Cellulolytic bacteria comprising several diverse physiological groups have been observed. However, the distribution of cellulolytic capability among organisms differing in oxygen concentration, temperature and salt tolerance is a testament to the wide availability of cellulose across natural habitats (Lynd *et al.*, 2002). Some more recently described cellulolytic bacterial species have been found to display a somewhat wider carbohydrate utilization spectrum that includes starch and various monosaccharides that serve as substrate. The variable structural complexity of the pure cellulose and the difficulty of working with the insoluble substrates led to the use of the highly soluble cellulose ether, carboxymethylcellulose (CMC), as a substrate for the studies of endoglucanase production. Thus, *Paracoccus baruchii* LBOG37 can be said to be cellulolytic bacteria because it utilises carboxymethyl cellulose as a carbon source. However, it ought to be noted that carboxymethyl cellulose is modified cellulose and it remains to be established whether the organism can utilise unmodified cellulose as a carbon source. Moreover, many organisms that cannot degrade cellulose have been found to hydrolyse CMC. On the contrary, due to the substituted nature, relatively few microbes can use CMC as a growth substrate in an enrichment culture (Lynd *et al.*, 2002). In addition, there appears to be a tendency for a broader range of carbohydrate utilization in the more extreme environments (thermophilic or halophilic). This could explain why *Paracoccus baruchii* LBOG37 utilises various carbohydrates since the soda lake environments are characterised by extreme alkaline and thermophilic conditions.

According to Nguyen and Guckert (2001), organisms growing in soil with carbon limitation take into the cell glucose to be converted into storage compounds instead of being utilised for growth or respiration. This could explain why *Paracoccus baruchii* LBOG37 growth was supported more by starch than glucose at 37°C, 40°C, and 45°C and by carboxymethyl cellulose than glucose at 45°C and 50°C. It is possible that the glucose in the media was mainly stored in the cell instead of being utilised for the growth of the bacteria. This implies that glucose can only be optimally be used for growth if other carbon sources utilised by the organism are present in the media. Moreover, it ought to be noted that high amounts of glucose might inhibit the growth of a micro-organism (Benigno et al, 2013). This could have been the case in our study since there was no optimization of sugar source concentration. If so, this could have limited the growth rate of *Paracoccus baruchii* LBOG37 in glucose in case the amount used was higher than the optimum amount required by the organism. A study by Wawrik *et al.*, (2005) on the effect of different carbon sources on community composition of bacterial enrichments from soil reported that different enrichments selected for dissimilar communities. Based on the findings of this study, isolation of the *Paracoccus baruchii* LBOG37 from its natural environment can best be attained using starch enrichment of the media than when glucose is used.

According to Shimizu (2012), gram negative bacteria have concentric membranes that surrounds the cytoplasm. The outer membrane has porin proteins that allow the movement of small hydrophilic nutrient molecules into the cell and efflux of waste materials. Porins such as OmpC and OmpF serve to transport glucose into the cell when glucose is present at a higher concentration than about 2 g/l. Since *P. baruchii* is a gram negative bacterium, it has porins on its outer membrane. The relative abundance of porins changes depending on such factors as osmolarity, temperature, and growth phase. Shimizu (2012) further reported that when extracellular glucose concentration is less than about 1 μ M, or it is more than about 2 g/L, glucose is transported by enzyme II mannose (EII^{Man}) complex in *Escherichia coli* and the cell can grow with less growth rate. It is therefore possible that *P. baruchii* utilized EII^{Man} to transport glucose since glucose concentration was more than 2 g/l. This could have contributed to low growth rate of the bacteria in MH with glucose at different temperatures. The changes in temperatures might have affected the relative abundance of porins on the *P. baruchii* outer cell membrane and hence the transportation of glucose.

Kelly *et al* (2006) stated that most of *Paracoccus* species grow at temperature ranges of 25°C to 37°C. However, members of *Paracoccus* genus differ in the optimal temperatures for growth at the level of species. For instance, Osanjo *et al* (2009) reported that *P. aminovorans*, *P. alcaliphilus*, *P. solventirans* and *P. marcusii* can only grow at temperatures below 37°C, while others such as *P. homiensis*, *P. zeaxanthinifaciens* or strain BOG6T are optimally cultivated above 37°C. *Paracoccus baruchii* LBOG37 can be placed in the second group that includes *Paracoccus* organisms that can be cultivated optimally at temperatures above

37°C. The optimal growth temperature for *Paracoccus baruchii* LBOG37 was found to be 40°C. This is the same optimal temperature for *Paracoccus baruchii* LBOG37 which was isolated from the same soda lake as *Paracoccus* sp BOG37. *Paracoccus baruchii* LBOG37 also grows at temperatures as high as 54°C. This implies that the bacteria can survive in extreme temperatures and hence can be termed thermophilic bacteria.

According to Osanjo *et al* (2009), optimal growth temperatures, pH levels, carbon sources and ability to produce carotenoids can be used to distinguish various *Paracoccus* species. Carotenoids are terpenoid pigments produced by various organisms such as bacteria, algae, fungi and green plants (Armstrong, 1997). *Paracoccus baruchii* LBOG37 had a characteristic orange colour which was indicative that it could produce carotenoids. Carotenoid producing bacteria have been isolated from extreme environments/habitats examples of which are the extreme and moderately halophilic and alkaliphilic soils and waters, thermophilic waters (Osanjo *et al.*, 2009), hot spring waters with radioactivity (Asker *et al.*, 2012). Based on the results of this study *Paracoccus baruchii* LBOG37 is both alkaliphilic and thermophilic. Given that most microbes with carotenoid production capability have such features; it is possible that *Paracoccus baruchii* LBOG37 is a carotenoid producing bacteria. Microbial carotenoids are produced as a protective mechanism against photo-oxidation induced by intense sunlight and also act to stabilize the bacterial cell membranes at extremely high temperatures (Asker *et al.*, 2012). It is therefore possible that *Paracoccus baruchii* LBOG37 produces carotenoids to stabilise its cell membrane at extreme temperatures. If indeed *Paracoccus baruchii* LBOG37 is capable of producing carotenoids, the isolate could be of significance in various fields such as the pharmaceutical, chemical, food and feed industries. For instance, in the pharmaceutical industries the carotenoids produced by the organisms could be harvested and utilized in the manufacturer of anti-oxidants. The coloration produced by the carotenoids could be utilized in food industry for food coloration.

Conclusion

The *Paracoccus baruchii* LBOG37 grows at temperature range of between 37°C and 54°C and pH range of 9.0 to 11 offering an almost “aseptic” growth environment. The optimum growth of the organism occurs at 40°C and pH range of 9.0 to 9.5. The organism has a characteristic orange colour. It can therefore be concluded that *Paracoccus baruchii* LBOG37 is a thermophilic, alkaliphilic bacterium that utilises carboxymethyl cellulose, starch and D-glucose during its heterotrophic growth.

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