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Investigation of carbon substrate utilization patterns of three ureolytic bacteria

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ABSTRACT

Bacteria have developed specific substrate utilization profiles which allow them to grow only under specific conditions. They assimilate different organic substrates as carbon and energy sources for their growth and biomass production. In this study the carbon substrate utilization patterns of three bacteria [*Bacillus paramycoides* (Isolate-3), *Citrobacter sedlakii* (Isolate-7), *and Enterobacter bugadensis* (Isolate-11)] were investigated using BIOLOG Gen III microPlate and API strip techniques. These techniques employ various carbon substrates to determine bacterial carbon utilization patterns more quickly, economically, and effectively. Subsequently, biochemical analysis using API 20NE test showed that the three bacterial species were positive for Nitrate reduction, Glucose fermentation, Urease activity, hydrolysis of Esculine, assimilation of *N*-acetyl-glucosamine, D-maltose and Potassium Gluconate. In addition, the three bacteria assimilated 19 carbon substrates (D-Glucose, D-Sorbitol, Methyl Pyruvate, Dextrin, D-Maltose, D-Fructose, L-Alanine, L-lactic Acid, D-Gluconic Acid, D-Trehalose, D-Cellobiose, Glycerol, Glucuronamide, L-Glutamic Acid, *N*-Acetyl-D-Glucosamine, b-Gentiobiose, Acetic Acid, L-Serine, and Inosine) of BIOLOG Gen III MicroPlates. On the other hand, they were all unable to assimilate Tween-40, D-Arabitol, D-Lactic Acid, Methyl Ester, D-Fucose, Quinic Acid, and D-Aspartic Acid. Similarly, API 50CH carbohydrate fermentation analysis showed that the three bacteria intensively metabolized D-Ribose, D-Glucose, D-Fructose, *N*-Acetyl-Glucosamine, Arbutine, Esculine Citrate, D-Maltose, and D-Trehalose. It was also determined that none of the three bacteria could assimilate Erythritol, L-Xylose, D-Adonitol, Methyl-β-D-Xylopyranoside, L-Sorbose, Methyl-α-D-mannopyranoside, Amygdaline, Inuline, D-Melezitose, Amidon, Glycogene, Xylitol, D-turanose, D-Tagatose, D-Fucose, D-Arabitol, L-Arabitol, and Potassium 5-Keto-Gluconate. The study revealed that *Enterobacter bugadensis* and *Citrobacter sedlakii* exhibited an intensive metabolic activity and strong metabolic correlation than *Bacillus paramycoides.*

1. Introduction

Bacteria have evolved with several remarkable preferences of substrates for their growth and survival in the environment. They can break down complex organic molecules and release inorganic nutrients which then can be used by plants and thus play a crucial role in biogeochemical cycles (Stefanowicz, 2006). Among them, ureolytic bacterial principally degrade urea, as urease turns the uncharged urea molecule into two charged ions: ammonium (NH^{4+}, positively charged) and carbonate (CO_3^{2-}, negatively charged) (Omoregie et al., 2017; Phang et al., 2018). Such urease producing microorganisms have several important roles in medicine, agriculture, and construction technology. Hence, microbial urease is related to the virulence of pathogenic microorganisms (Omoregie et al., 2017), contributing to urinary stones (Manzoor et al., 2018; Manzoor et al., 2018a), pyelonephritis and gastric ulceration (Collins and D'Orazio, 1993; Mobley and Hausinger, 1989). Ureases were also immobilized and used as a biosensor in construction of a flow cell with the incorporation of a urease-modified device for the continuous measurement of urea in flowing systems (Cullen et al., 1990), induce calcite precipitation (DeJong et al., 2011; Omoregie et al., 2016) and were used along with urea fertilizer to ease hydrolysis of ammonium into the soil (Glibert et al., 2006).

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A number of commercial kits are available that rely on carbon substrate utilization patterns and other phenotypic characteristics to identify microbes (Konopka et al., 1998a,b). They are much less laborious and less costly compared to culture-independent approaches. Among others, BIOLOG micro-plate system (BIOLOG Inc.) and API strips (BioMérieux) are standard approaches that are used to analyze bacterial carbon-source utilization patterns. Both are based on the principle of determining the ability of microorganisms to assimilate, ferment, or decompose certain compounds. The tests were originally developed for classification of bacterial isolates based on the ability of the isolates to oxidize different carbon sources (Parkes, 1998) and are suitable tools for the characterization of nutritional physiology of individual microorganisms (Lladó and Baldrian, 2017).

In their study, Garland and Mills showed substrate utilization patterns of microbes through direct incubation of environmental samples in BIOLOG MicroPlates with 95 different substrates (Garland and Mills, 1991). Thus, the BIOLOG system, (Hayward, CA) has simplified the assay of carbon substrate utilization patterns by combining a broad range of substrates (95 in a 96 well microtiter plate) with a detection method based upon the reduction of tetrazolium violet in each well. As a consequence, what is assayed is not necessarily growth but the oxidation of an organic substrate which drives the formation of a colored insoluble formazan (Bochner, 1989). As a result, BIOLOG measures respiration rather than growth and is a better sensitive way to measure phenotypes. That means after the cells were inoculated they respond metabolically by respiring but not growing. In addition to this, it allows the measurement of more cellular pathways. It is also used to systematically test what stimulates growth, and equally important, what inhibits the growth of the microorganisms (Bochner, 2009).

A standardized micro-method (API Strip) is also used for the study of carbon substrate assimilation by bacteria (Freney et al., 1985). Substrate assimilation patterns of bacterial isolates can be assessed by using API testing systems (BioMerieux) which were originally designed for identification of non-fermentative, Gram negative bacteria of clinical importance (Robinson et al., 1995). Even-though API systems have been used for phenotypic identification of bacterial species (Robinson et al., 1995; Barr et al., 1989), they also show substrate fermentation patterns of microbes (Pascual et al., 2017; Huber et al., 2017; Wüst et al., 2018). The test strips consist of microtubes (cupules) containing dehydrated substrates to detect the enzymatic activity or the assimilation/fermentation of sugars by the inoculated organisms (Barr et al., 1989; GHN, 2013). One of the API strips used to detect fermentation of carbohydrates is API 50CH which is used to study the metabolism of hydrocarbons and their derivatives (heterosides, Poly-alcohols, uronic acids) by microorganisms (Holban and Grumezescu, 2018; Garland and Mills, 1991). During incubation, metabolic products produce colour changes that are either spontaneous or revealed by the addition of reagents (BioMérieux, 2002). As a consequence of carbohydrate fermentation, the pH within the cupule changes and is shown by an indicator.

The BIOLOG systems have been extensively used for testing the formation of biofilms (Boehm et al., 2009), studies on fitness costs associated with the acquisition of resistance to antimicrobials (Curiao et al., 2016; Sánchez et al., 2015), and also used in applied ecological research to investigate substrate utilization patterns of microbes (Bochner, 2009; Konopka et al., 1998a,b; Smalla et al., 1998; Stefanowicz, 2006). In addition, API strips have also been used in characterization of novel strains of bacteria (Huber et al., 2014; Pascual et al., 2019). Although the use of BIOLOG system and API strips have been extensively investigated, no literature is available on the carbon substrate utilization patterns of bacteria isolated from Ethiopian soil. Thus, the aim of this study is to apply the BIOLOG Gen III technology and API strips to characterize the carbon substrate utilization patterns of three bacterial strains isolated from Ethiopian soil.

2. Materials and methods

2.1. Bacterial isolates and culture conditions

Three bacterial isolates (Isolate-3, Isolate-7, and Isolate-11) belonging to the bacterial species, *Bacillus paramycoides, Citrobacter cedlakii*, and *Enterobacter bugadensis* were respectively isolated from Ethiopian soil and was used for the analysis. *Bacillus paramycoides and Citrobacter cedlakii* were isolated from Ethiopian rift valley area (Soil pH = 7.56, soil temperature = 33 °C, 101.5 Km southeast of Addis Ababa, 8°27′N 39°16′E, and at an elevation of 1584.28 m above sea level). While, *Enterobacter bugadensis* was isolated from Ethiopian highland soil (Soil pH = 6.7, soil temperature = 30 °C, 501 Km West of Addis Ababa, 9°30′N 35°30′E, and at an elevation of 1821 m above sea level). The three isolates were characterized at Leibniz Institute DSMZ - German Collection of Microorganisms and Cell Cultures. Inocula for substrate utilization testing were prepared from bacterial strains incubated aerobically for 24 h and cultured on nutrient agar plates (Sigma-Aldrich) at 35 °C.

2.2. Biochemical and phylogenetic analysis

2.2.1. Biochemical analysis

The biochemical and enzymatic reactions for the three bacteria were tested using the API 20NE test system (BioMérieux SA, France) following the manufacturer's instructions. The test strip contained 20 miniature biochemical tests (8 conventional tests such as urea hydrolysis and 12 assimilation tests, with substrates such as D-Glucose) that were inoculated with a sample of a bacterial colony (Barr et al., 1989). After inoculation, the reaction mixture was incubated for 48 h at 35 °C and then the data was recorded and interpreted.

2.2.2. Phylogenetic analysis

To determine the phylogenetic positions of the three bacterial strains, 16 S rRNA gene sequences were amplified using the universal bacterial specific primers 8 F and 1492 R followed by sequencing. Subsequently, a phylogenetic tree was reconstructed using MEGA version 10.0 (Tamura et al., 2013). Prior to phylogenetic analysis, indefinite DNA sequences at both ends were removed and the gaps were adjusted to improve the alignment. Nucleotide sequence alignments were inspected visually to identify positions of uncertain alignments to be corrected or omitted for further analysis (Saitou and Nei, 1987). Multiple sequence alignments were obtained using the Clustal-W alignment tool from the MEGA-X software with distance options according to the Kimura two-parameter model and clustering with the maximum likelihood statistical method (Saitou and Nei, 1987). Bootstrap analysis based on 1000 replications was used to estimate the confidence level of the tree topologies (Tamura et al., 2013).

2.3. Carbon substrate utilization testing method

Automated tetrazolium-based microbial identification system produced by BIOLOG Inc. (Hayward, USA) and the fermentation test method API 50CH (BioMérieux) were selected for testing carbon substrate utilization patterns. BIOLOG Gen III MicroPlate was used to analyze 71 carbon source utilization assays and API 50CH strip was used for analysis of fermentation of 49 carbohydrates.

2.3.1. BIOLOG Gen III testing method

Pure colonies of the bacterial isolates were re-cultured on BIOLOG recommended agar media (BUG agar) and incubated at 35 °C for 24 h. About 3 mm diameter area of overnight grown fresh cell from the surface of each agar plate was picked up with a sterile cotton swab and transferred into a sterile capped tube containing 20 mL of the inoculation fluid (IF-0, BIOLOG Inc.). The bacteria were released into the IF

by rubbing the swab tip against the bottom of the tube containing IF. The cell concentration was adjusted to 85% transmittance on the BIOLOG turbidometer. The isolates were then inoculated with the cell suspension (100 μL per well) using multichannel pipette and the MicroPlate was incubated at 35 °C into the OmniLog incubator/reader for 36 h (BIOLOG Inc., Hayward, USA). The changes of color in the wells were measured every 15 min and provided both amplification and quantification of the phenotype. Analysis was carried out using OmniLog® phenotype microarray software version 1.2. The data were collected using OmniLog® MicroArray. OmniLog phenotype microarray (PM) data was analysed using opm (an R package) for a qualitative and quantitative analysis of raw kinetic OmniLog PM data (Vaas et al., 2012; Vaas et al., 2013).

2.3.2. API 50CH test method

API 50CH strips were used to investigate the acidification of carbohydrate substrates with the selected bacterial isolates according to the manufacturer's protocol (BioMerieux). Pure colonies were cultured on a nutrient medium (Oxoid) and incubated for 24 h at 35 °C. Identical colonies were picked and suspensions with a turbidity equivalent to 0.5 McFarland were prepared in the ampule of API 50CHB/E Medium (Biomerieux). The tubes were then filled with the inoculated API 50CHB/E Medium with the selected strains and covered with mineral oil and incubated at 35 °C for 48 h. A positive fermentation test corresponds to acidification revealed by the phenol red indicator changing to YELLOW and BLACK for the Esculin test.

3. Results and discussions

3.1. Biochemical and phylogenetic analysis

3.1.1. API 20NE biochemical profiles

API 20NE based biochemical analysis showed that the three bacteria were positive for Nitrate reduction, Glucose fermentation, hydrolysis of Urea and Esculine; and assimilation of *N*-acetyl-Glucosamine, D-maltose and Potassium Gluconate. Both *C. sedlakii and E. bugadensis* showed similar positive reactions for Arginine Dihydrolase, β –galactosidase, L-Arabinose, D-Mannose, D-Mannitol and Acid Malique and all the three isolates showed negative reaction towards, Acid Caprique and Acid Adipique assimilation (Table .1).

Table 1
Biochemical test Profile of the three isolates.

Characteristics	*B. paramycoides*	*C. sedlakii*	*E. bugadensis*
Enzyme Activities			
Nitrate reduction	+	+	+
Indole production	–	+	–
Glucose fermentation	+	+	+
Arginine Dihydrolase	–	w	+
Urea hydrolysis	+	+	+
Esculine hydrolysis	+	w	+
Gelatine hydrolysis	+	–	+
β -galactosidase	–	+	+
Assimilation Tests			
D-Glucose	+	+	+
L-Arabinose	–	+	+
D-Mannose	–	+	+
D-Mannitol	–	+	w
N-acetyl-glucosamine	+	+	+
D-maltose	+	+	+
Potassium Gluconate	+	+	+
Acid Caprique	–	–	–
Acid Adipique	–	–	–
Acid Malique	–	+	+
Trisodium Citrate	+	+	+
Acid phenylacetic	–	–	+

"+" means, Positive, "W" means, weakly positive and "-" means, Negative.

3.1.2. Phylogenetic analysis

Evolutionary history based on Maximum Likelihood method using Kimura 2-parameter model and bootstrap analysis based on 1000 replications (Tamura et al., 2013) was used to estimate the confidence level of the tree topologies. During the screening stage, *Bacillus paramycoides* was labeled as Isolate_3, *Citrobacter sedlakii* as Isolate_7 and *Enterobacter bugadensis* as Isolate_11. Phylogenetic analysis revealed that *Isolate-3* was affiliated with the genus *Bacillus* (Fig. 1). The highest 16 S rRNA gene sequence identity for Isolate-3 was 98.92% with *Bacillus paramycoides* strain-MCCC-1A04098. The analysis placed isolate_7 in the vicinity of *Citrobacter sedlakii* strain-I-75 and within the same group (99.18% 16 S rRNA sequence identity). The phylogenetic analysis also showed that isolate_7 is closely related to isolate_11 than isolate_3. Isolate_11 was placed in the neighborhood of *Enter-*

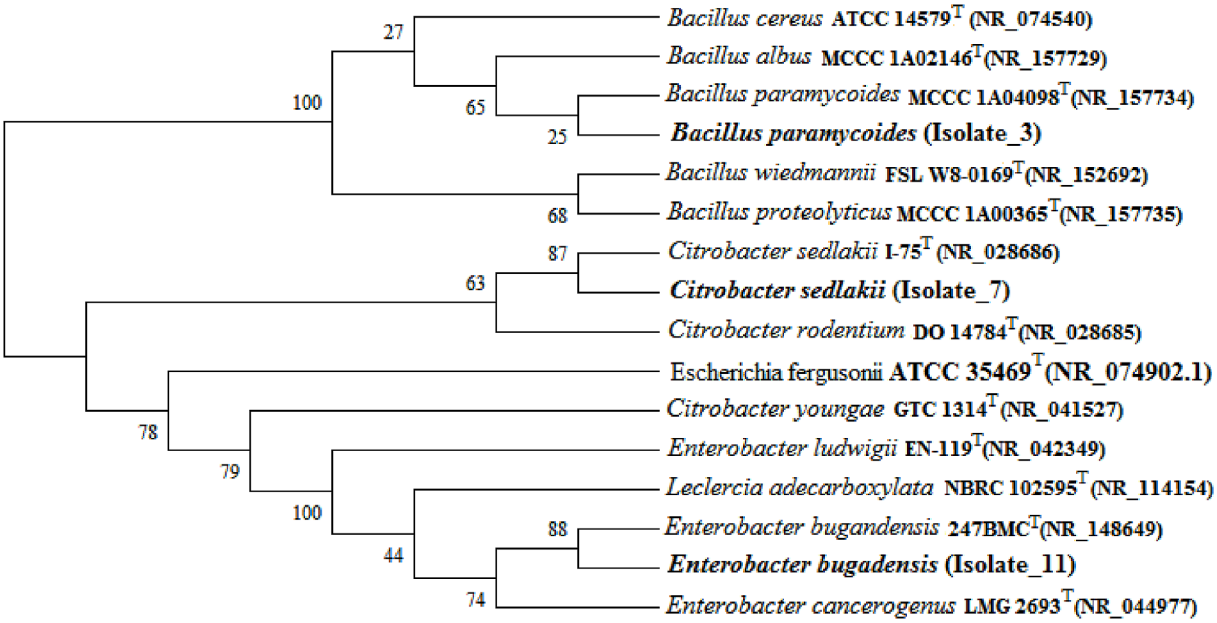

Fig. 1. Molecular Phylogenetic analysis by Maximum Likelihood method based on almost-full-length *16'S* rRNA gene sequences illustrating the phylogenetic position of Isolates_3, Isolate_7 and Isolate_11 and related taxa. The percentage of trees in which the associated taxa clustered together is shown next to the branches.

obacter bugadensis strain 247BMC which had a 16 S rRNA gene sequence identity of 98.96% to this type strain.

3.2. Carbon substrate utilization assay of BIOLOG Gen-III plates

The analysis showed variable preferences in specific substrate assimilation levels among the three isolates, where majority of the substrates were utilized by *C. sedlakii* and *E. bugadensis.* The utilization assays heat map of 71 carbon sources are presented in Fig. 2. Accordingly, *C. sedlakii and E. bugadensis* were able to metabolize 55 different carbon substrates from which 42 substrates were assimilated by both (Fig. 2). Whereas, *B. paramycoides* was only able to assimilate 29 substrates. The three bacteria commonly assimilated 19 substrates, such as D-Glucose, D-Sorbitol, Methyl Pyruvate, Dextrin, D-Maltose, D-Fructose, L-Alanine, L-lactic Acid, D-Gluconic Acid, D-Trehalose, D-Cellobiose, Glycerol, Glucuronamide, L-Glutamic Acid, N–Acetyl–D–Glucosamine, b-Gentiobiose, Acetic Acid, L-Serine, and Inosine. All isolates couldn't assimilate Tween-40, D-Arabitol, D-Lactic Acid, Methyl Ester, D-Fucose, Quinic Acid, and D-Aspartic Acid.

Positive reactions in BIOLOG MicroPlates showed selective, growth of aerobic or facultatively anaerobic, heterotrophic and copiotrophic bacteria (Wiinsche et al., 1995). Furthermore, in the study it was understood that the colour response in each well appears to be related to the activity of bacteria to assimilate the substrate in each well as a sole carbon source. Although the utilization pattern was varied among the isolates, the study showed that each isolate was able to assimilate a wide range of substrates, indicating their categorization as heterotrophic and copiotrophic bacteria. Despite the short incubation time of 48 h, *E. bugadensis* and *C. sedlakii* exhibited an intensive metabolic activity and strong metabolic correlation compared to *B. paramycoides.* This kind of correlation in substrate degradation pattern depends strongly on species or even strain-specific abilities (Freese et al., 2009) which supports the idea of estimating substrate utilization from the genetic determination of species. Phenotypic and genotypic characterizations suggest that there may be a correlation between certain phenotypic properties such as growth rate on certain substrates between such microbes (Oda et al., 2002). It should be noted that even closely related species could have very different substrate utilization patterns, so that a correlation between species occurrence and substrate turnover should not be expected always.

3.3. Carbohydrate utilization profiles of the isolates using API 50CH

The test results revealed that none of the three strains showed positive results in control wells lacking carbon sources. Thus, any positive value was attributed to the assimilation of the specific carbohydrates in the wells of the strips (Table: 2). As it is shown in the table, *B. paramycoides* was only positive for 10 substrates such as D-Ribose, D-Glucose, D-Fructose, *N*-Acetyl-Glucosamine, Arbutine, Esculine Citrate, D-Maltose, D-Saccharose, D-Trehalose, and Potassium-Gluconate. In common, *C. sedlakii* and *E. bugadensis* were positive for assimilation of 24 carbohydrates (Glycerol, L-Arabinose, D-Ribose, D-Xylose, D-Galactose, D-Glucose, D-Fructose, D-manose, L-Rhamnose, Inositol, D-mannitol, D-Sorbitol, *N*-Acetyl-Glucosamine, Arbutine, Esculine Citrate, Salicine, D-Cellobiose, D-Maltose, D-lactose, D-Melibiose, D-Trehalose, Genetibiose, L-Fucose and Potassium 2-CetoGluconate). The carbon-sources that were intensively metabolized by the three strains were D-Ribose, D-Glucose, D-Fructose, *N*-Acetyl-Glucosamine, Arbutine, Esculine Citrate, D-Maltose, and D-Trehalose. However, none of the three strains were capable of assimilating Erythritol, L-Xylose, D-Adonitol, Methyl-βD-Xylopyranoside, L-Sorbose, Methyl-αD-mannopyranoside, Amygdaline, Inuline, D-Melezitose, Amidon, Glycogene, Xylitol, D-turanose, D-Tagatose, D-Fucose, D-Arabitol, L-Arabitol, and Potassium 5-CetoGluconate.

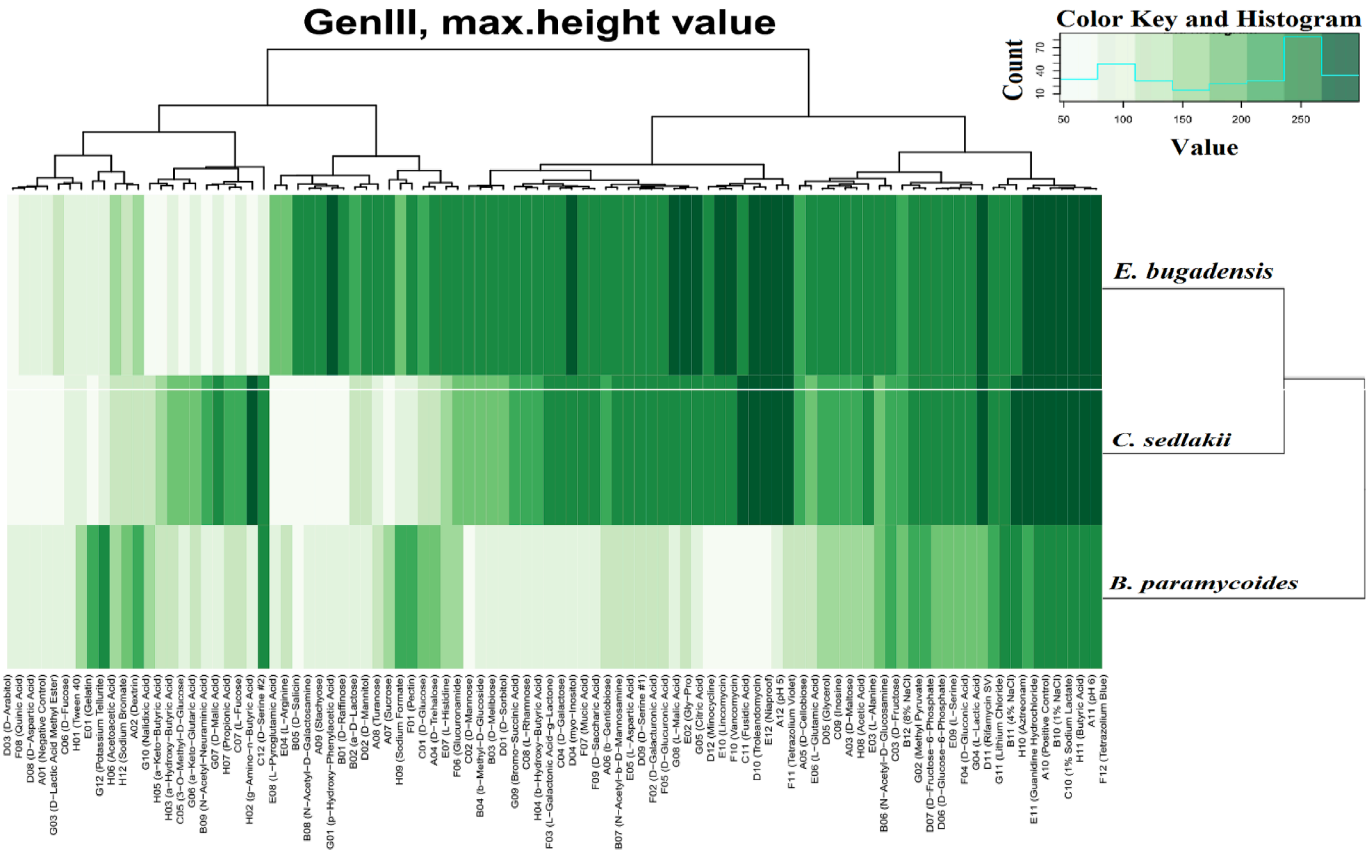

Fig. 2. Heatmap of the carbon utilization patterns of the three strains for substrates located on the BIOLOG Gen III MicroPlates, after 48 h of incubation; *aggregated curve parameter (max. height value).*

As shown in Table 2, the three bacteria were able to grow efficiently on a wide range of carbon substrates including five-carbon sugars. Thus, can be categorized as chemo-heterotrophic bacteria (Xuedong and Yuqing, 2015). Such groups of bacteria are metabolically versatile and able to grow on a variety of organic substrates under various oxygen conditions. Dyer et al. (1997) reported similar carbon substrates assimilation patterns of *C. sedlakii* from clinical samples except a positive reaction for Inositol and Salicine demonstrated in the present study. The carbon substrate utilization patterns of *B. paramycoides* in this study is comparable with the study of Liu et al. (2017) on novel strain isolated from marine environments () with the exception of negative reactions recorded in this study for D-Galactose, D-Manose, Amygladin, Salicine, D-Cellobiose, and Glycogen. Furthermore, compared to the study of Doijad et al. (2016) on carbon substrate assimilation patterns of a novel *E. bugadensis* strain isolated from neonatal blood samples, the current study has showed that the strain was urease positive and negative for the assimilation of D-Arabinose and Amygladine.

Table 2
Differential characteristics of the three Isolates using API 50CH test strips.

Composition	Isolates		
	B. paramycoides	*C. sedlakii*	*E. bugadensis*
Control	–	–	–
Glycerol	–	+	+
Erythritol	–	–	–
D-Arabinose	–	+	–
L-Arabinose	–	+	w
D-Ribose	w	+	+
D-Xylose	–	+	+
L-Xylose	–	–	–
D-Adonitol	–	–	–
Methyl-βD-Xylopyranoside	–	–	–
D-Galactose	–	+	+
D-Glucose	+	+	w
D-Fructose	+	+	w
D-manose	–	+	w
L-Sorbose	–	–	–
L-Rhamnose	–	+	+
Dulcitol	–	+	–
Inositol	–	+	w
D-mannitol	–	+	+
D-Sorbitol	–	+	+
Methyl-α-D-mannopyranoside	–	–	–
Methyl-α-D-Glucopyranoside	–	–	+
N-AcetylGlucosamine	+	+	w
Amygdaline	–	–	–
Arbutine	w	+	+
Esculine Citrate	+	w	+
Salicine	–	+	+
D-Cellobiose	–	+	+
D-Maltose	+	+	+
D-lactose	–	+	+
D-Melibiose	–	+	w
D-Saccharose	+	–	w
D-Trehalose	+	+	w
Inuline	–	–	–
D-Melezitose	–	–	–
D-Raffinose	–	–	+
Amidon	–	–	–
Glycogene	–	–	–
Xylitol	–	–	–
Genetibiose	–	+	+
D-turanose	–	–	–
D-Lyxose	–	–	w
D-Tagatose	–	–	–
D-Fucose	–	–	–
L-Fucose	–	+	–
D-Arabitol	–	–	–
L-Arabitol	–	–	–
Potassium Gluconate	w	+	–
Potassium 2-CetoGluconate	–	+	w
Potassium 5-CetoGluconate	–	–	–

"+" means, Positive, "W" means, weakly positive and "-" means, Negative.

Similar to the BIOLOG MicroPlate testing, the carbon-source utilization patterns of *E. bugadensis* and *C. sedlakii* appears more functionally diverse and metabolically related as compared to that of *B. paramycoides*. The relatedness between species uncovered by carbon assimilation here is in accordance with results obtained by phylogenetic studies based on 16 S rRNA genes (Fig. 1). In the same way, the variation in assimilation pattern of *B. paramycoides* from the rest could be due to phylogenetic factors (Freese et al., 2009), the difference in the level adaptation to nutrients and organic matter composition of sites from which they were isolated (Vacca et al., 2005). In any environmental settings, microbial survival is basedon the capacity to utilize a variety of available carbohydrates as carbon and energysources (VanFossen et al., 2009).

Many prokaryotes can grow in a simple mineral salts medium containing glucose as the sole organic compound (Kim and Gadd, 2008). Fermentation of mixed carbohydrates is achieved sequentially whereby the utilization of glucose, commonly the preferred carbon and energy source, represses consumption of alternative sugars (Kim et al., 2009). As the strains could assimilate a wide range of carbon sources, these substrates can be used as potential alternatives for fermentative production of several metabolites and biomass.

4. Summary and conclusions

Carbon is the most important medium component, as it is an energy source for the microorganisms and plays an important role in the growth as well as in the production of primary and secondary metabolite (Singh et al., 2017). The choice of carbon source greatly influences secondary metabolism and therefore enzyme production (Marwick et al., 1999). In environmental bacterial communities, glucose supports considerable bacterial growth and respiration (Rich et al., 1996).Thus, knowing the metabolic requirements of a bacterium can lead to a better understanding of the conditions under which it is likely to proliferate and generate good biomass (Scaria et al., 2014).

From the study, it was understood that the three bacterial strains have distinctly different substrate utilization profiles, where strong metabolic similarity was observed between *E. bugadensis and C. sedlakii*. Some substrates (Glucose, Maltose, Fructose etc) were used by nearly every strain, but the use of other substrates (Tween-40, D-Arabitol, D-Lactic Acid, Methyl Ester, D-Fucose, Quinic Acid, and D-Aspartic Acid) were mediated by none of the three strains. Based on their assimilation patterns the strains were classified as chemoheterotrophic and are likely to be metabolically versatile and able to grow on a variety of organic substrates under various oxygen conditions. Substrates on which rapid growth was observed were considered as the most easily utilized or preferred carbohydrates sources for the production of metabolites or other products from the strains. Thus, investigation of the nutrients on which microbes grow best is useful for the production of maximum yield and identification of possible low cost carbon sources for production of primary and secondary metabolites or biomass from microorganisms. In addition, further medium components such as nitrogen and salts must be identified and optimized. The productions of microbial-based substances by fermentation of cheap carbon sources are the most promising breakthrough of biotechnological innovations. Utilization of these low cost carbon and high energy sources will certainly increase the availability of affordable quality microbial-based products and increases the competitiveness of the product.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.101429.

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