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OPTIMAL CULTURE CONDITIONS AND CHARACTERIZATION OF CELLULOLYTIC BACTERIA FROM CASSAVA DUMPSITES IN IBADAN, NIGERIA

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Abstract

This study is designed to investigate cellulolytic bacteria capable of removing cellulolytic wastes that are produced from cassava during processing. Cellulolytic bacteria isolates from cassava dumpsite soil in Ibadan, Nigeria were characterized and their optimal culture conditions determined. The total viable bacterial count of the sample of cassava dumpsite soil was 24.4 x 10^8cfu/g. A total of twenty four bacteria were isolated from the samples out of which nine of the bacterial isolates were positive for cellulose degrading abilities. The 16S rDNA analysis of two bacterial isolates which gave the highest zones of hydrolysis on carboxy-methyl cellulose agar plates showed maximum similarity ratio towards strains of Kurthia gibsonii (90%) and Myroides odoratimimus (98%) using BLAST and hence the isolates were referred to as Kurthia gibsonii CAC1 and Myroides odoratimimus CAC2 respectively. Kurthia gibsonii CAC1 which was motile, aerobic, rod-shaped, non-pigmented and possessing a Gram positive reaction grew best at incubation temperature of 30^{o}C, pH 5.5 and on lactose and ammonium chloride supplemented medium. Also, at incubation temperature of 30°C there was enhanced growth of a light yellowish, non-motile, aerobic, and rod-shaped Gram negative M. odoratimimus CAC2 at pH 6.0. Lactose and urea were best carbon and nitrogen sources respectively in the growth medium boosting the bacterial proliferation.

It can be concluded that these microorganisms if properly cultivated can be used to reduce cassava waste littering in the environment.

Key Words: *Cassava, cellulolytic, carboxy-methyl cellulose, Kurthia gibsonii, Myroides odoratimimus.*

Introduction

Cassava (*Manihot esculanta*) is a root tuber crop that is widely cultivated in the tropical regions of the world (Iyayi and Losel, 2001; Oboh and Akindahunsi, 2003) as a shrubby perennial crop that grow to a height of 6-8 ft. It is usually propagated by stem cuttings (Oboh, 2006). Cassava had its origin from Brazil and the coastal regions of Venezuela and Peru in South America. Cassava is now found in almost all parts of tropical Africa (Kawano, 2003). Today, Nigeria is the biggest producer of cassava after Brazil and Thailand (Arotupin, 2007).

The major wastes of cassava processing in Nigeria are cassava sievates and cassava offal (Okafor *et al*., 2002). Solid wastes produced from cassava processing are of three forms namely: peelings from initial processing, fibrous by-products from crushing and sieving and starch residue after starch settling.

The most common renewable biopolymer on earth is cellulose and it is the leading waste material from agriculture (Bhat and Bhat, 1997). The most efficient method for utilization and degradation of the cellulose thereby reducing the waste is the use of microbes for the hydrolysis of the lignocellulosic waste and fermentation of the resultant reducing sugars for production of desired metabolites or biofuel (Rajeev *et al*., 2005).

The genus *Myroides* are gram-negative rods (0.5 µm in diameter and 1-2µm long) that grow on most media and often form yellow pigmented colonies with two species within the genus; *M.*

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odoratus and *M. odoratimimus* (Vancannneyt *et al*., 1996). Members of the genus are widely distributed in the environment, especially in water (Benedetti *et al*., 2011). *Myroides odoratimimus* is capable of growing aerobically on 3, 4-dichloroaniline (DCA) as the sole carbon and energy source from farm field (Li *et al*., 2012). *Kurthia gibsonii* Strains have been isolated from meat, mince beef, hen faeces on conveyer and cow feaces (Shaw and Keddie, 1983). Enormous wastes are generated during cassava processing and this is generally considered to contribute significantly to environmental pollution and aesthetic nuisance.

The objectives of the study were to isolate and characterize cellulolytic bacteria that can be employed in cellulose utilization from cassava waste dumpsites, and determine their optimal growth conditions.

Materials and Methods

Sample Collection

Soil samples containing partially degraded cassava wastes used for this study were collected from three different points at the dumpsites of three cassava processing sites located at Sawmill area, Agbowo area and Samonda area, Ibadan, Nigeria. Samples were collected aseptically into appropriately labelled sterile sample bags, and then transported to the Department of Microbiology, University of Ibadan, for microbial analysis.

Isolation of Bacteria from Cassava Dumpsites

Ten grams of sample from each of the three points of the dumpsites was weighed separately and diluted in 90ml sterile distilled water according to the method of Harrigan and McCance, 1976. These were serially diluted out by sequentially mixing 1ml sample with 9ml of distilled water in test tubes. The dilutions were shaken to allow even distribution and then followed by inoculation of 1ml of each sample onto sterile petri-dish by the pour plate method using molten nutrient agar. Plates were then incubated at room temperature for 24 hours in an inverted position. Pure cultures were further streaked aseptically on nutrient agar before the resultant pure cultures obtained were allotted preliminary codes. The pure cultures were maintained on nutrient agar slant at 4°C for further microbial analysis.

Determination of Inoculum size

A sterilized freshly prepared nutrient broth was inoculated with a loopful of 24hr old culture of the selected bacterial isolates. Each stock culture was serially diluted out according to the method of Harrigan and McCance (1976). One milliliter of each dilution was dispensed into sterile petri-dishes before sterile molten agar was added by the pour plate method and the plates were carefully swirled. The plates were incubated at room temperature for 24hr. The number of cells per dilution plate was counted to determine the amount contained in the original culture broth.

Screening of Cellulase Production by Bacterial Isolates

This was carried out by modification of the method of Todar, 2008. The cellulase activity screening medium had the following composition: Cellulose 0.5g, peptone 0.4g, yeast extract 0.02g, $MgS0_4$ 0.1g, K_2HPO_4 2.0g, KH_2PO_4 2.0g, agar 1.5g/100ml, pH 6.5. Each pure culture of the bacterial isolates was stabbed on carboxy-methylcellulose agar plates and incubated for 24-48hr. The plates were then flooded with Gram's iodine and allowed to stand at room temperature for 30min. Zone of hydrolysis was observed around the growing bacteria. Bacteria that produced large zones of hydrolysis during qualitative screening were selected for further studies.

Microbial Identification and Characterization

The isolates were identified and characterized using their cultural, morphological, microscopic characteristics and molecular analysis.

Phylogenetic and molecular evolutionary analyses were conducted using *MEGA* version 5 (Molecular Evolutionary Genetics Analysis) ((Altschul *et al*., 1997). In determining their phylogenetic characteristics, the DNA sequences of the two selected bacteria out of thirty bacteria isolated, were aligned and phylogenetically placed with sequences from various other bacteria. Results that showed identity of 90–98% to the clone sequences in the BLAST search (Basic Local Alignment Search Tool) were compiled using the Sequence Navigator software package (Applied Biosystems) and compared to available databases by the use of the Basic Local Alignment Search Tool (BLAST) (Altschul *et al*., 1997) and the databases of EMBL and GenBank

from the National Center for Biotechnology information website (NCBI) http://blast.ncbi.nlm.nih.gov and http://rdp.cme.msu.edu, to determine the approximate phylogenetic affiliations. The compiled sequences were aligned using the Clustal W software program (Thompson *et al.,* 1997). The 16S rRNA genes were amplified from total DNA using two universal bacterial primers; forward primer 518f (5'-CCAGCAGCCGCGGTAATACG-3') and reverse primer 800r (5'-TACCAGGGTATCTAATCC-3'). The phylogenetic tree was calculated using the neighbour-joining algorithm (Thompson *et al.,* 1997). Distances were generated using the Kimura Matrix, and the tree stability was supported through Bootstrap analysis (1000 replications).

Optimization of the Culture Condition

This was done following modification of the method of Todar, 2008. The minimal medium has the following composition: sucrose 10g/L, k_2HP0_4 2g/L, KH_2P0_4 2.5g/L, $(NH4)_2S0_4$ 1g/L, $MgS0_4.7H_20$ 0.2g, $FeS0_4.7H_20$ 0.01g/L, $MnS0_4.7H_20$ 0.007g/L, and carboxy-methylcellulose 5g/L. The effect of culture conditions was carried out using different parameters such as temperature (25, 30, 37, and 45°C), pH (3.5, 4, 4.5, 5, 5.5 and 6.0), carbon sources (glucose, fructose, starch, lactose and sucrose), nitrogen sources (peptone, urea, NH_4Cl and $NaN0_3$) and cassava-based substrates (cassava flour, cassava chaff and cassava peel) using 10^6 cfu/ml of the test bacterial isolates. The analysis was carried out at different incubation periods (6, 12, 18, 24, 30, 36 and 42hrs) to assess their effect on the growth of the bacterial isolates. A control experiment without the bacterial isolates was set up in each instance. The absorbance was measured at 560nm using spectrophotometer (Camspec M105). Samples were analyzed in triplicates.

Statistical Analysis

The analysis of variance (ANOVA) was carried out with 95% confidence level on the data and the mean separated by Post Hoc Tests.

Results

Isolation and enumeration of the isolates

The total viable bacterial count from the cassava dumpsites was estimated at 24.4 x 10^8 cfu/g after incubation in plate count agar for 24hrs (Table 1).

Screening for cellulase producing bacteria from cassava waste soil

After incubation of the inoculated carboxymethyl cellulose agar plates for 24hr and the plates were flooded with Gram's iodine, it was observed that nine isolates showed a positive result by demonstrating various zones of hydrolysis (Table 2). Since carboxy-methylcellulose is the only carbon source in CMC agar plate, it was inferred that the bacteria were cellulolytic and cellulase enzyme was produced.

Isolates CAC 1 and CAC 2 were selected for further study since they gave the highest zones of hydrolysis.

Phylogeny and Identification of cellulase producing bacteria

Identity and phylogeny of the isolates CAC1 and CAC2 were determined as *Kurthia gibsonii* and *Myroides odoratimimus* using 16S rDNA analysis. The strains showed maximum similarity ratio towards *Kurthia gibsonii* SP22 (90%), and a similarity of 98% identity with the sequence of 16S rRNA of *Myroides odoratimimus* respectively using BLAST and hence the isolates were named as *Kurthia gibsonii* CAC1 and *Myroides odoratimimus* CAC2. The phylogenetic tree, calculated using the neighbour-joining algorithm, is shown in Figure 1. The morphological characteristics of the selected isolates further confirmed the identity of the bacteria. While *K. gibsonii* CAC1 was observed to be Gram positive, motile, rod-shaped, aerobic with no pigmentation, *M. odoratimimus* CAC2 was Gram negative, non-motile, rod-shaped, and aerobic with slight yellowish pigmentation (Table 3).

Effects of physico-chemical parameters on the growth of the isolates

Figure 2 illustrates the effect of increasing incubation period on the growth of *K. gibsonii* CAC1 at different temperature range. *K. gibsonii* CAC1 showed a gradual increase in growth until optimal growth (1.92nm) was observed at 25°C when incubated for 36hrs. After this time, a decline in growth of the isolates was observed. Highest growth was recorded at 37°C when incubated for 30hr after which there was a decline. At higher temperature of 45°C, lesser growth of the bacterium was recorded and there

was a gradual increase in growth as the incubation period increased from 6 to 18hrs after which there was a slight decline before it increased to its peak (1.248nm) at 30hr of incubation and beyond this period, the growth of *K. gibsonii* CAC1 decreased. There was significant ($p \geq 0.05$) difference in the incubation period of *K. gibsonii* CAC1 relative to temperature.

The effect of increasing incubation period on the growth of *M. odoratimimus* CAC2 at different temperature ranges is shown in Figure 3. A significant ($p \geq 0.05$) difference was observed in the temperature ranges and the incubation periods. There was a gradual increase in growth from 6hrs until optimal growth of *M. odoratimimus* CAC2 was observed at 30°C when incubated for 30hr after which a sharp reduction in growth was observed. When incubated for 18hr, a 33% reduction from the optimum growth was recorded at 25°C while, the least growth of 69.6% reduction in absorbance was observed at 45°C. At higher temperature of 45°C, there was a general reduction in the growth of *M. odoratimimus* CAC2.

In Figure 4, the effect of pH variations on the growth of *K. gibsonii* CAC1 with increasing incubation time is reported. The growth of *K. gibsonii* CAC1 at different pH range during various incubation period was statistically ($p \geq 0.05$) different. The least growth recorded by *K. gibsonni* CAC1 was at pH 3.5 which occurred at 30hrs incubation. Bacterial growth increased with increased pH and between pH 5.0 to 6.0, similar growth patterns were recorded especially between 12 and 24hr. The best pH which favoured the highest bacterial growth was 5.5 followed in reducing order by pH 5.0 and 6.0 respectively. At pH 5.5, a gradual increase in absorbance was recorded within the first 24hr which then sharply increased to the optimal at 36hrs before it started reduce.

As shown in Figure 5, there was significant ($p \geq 0.05$) growth of *M. odoratimimus* CAC2 recorded at various pH ranges of the incubation period. At higher pH of 4.5, there was a gradual increase in growth after incubation for 18hrs until the optimal growth at this pH was recorded at 42hr of incubation. At pH 6.0, the growth of *M. odoratimimus* CAC2 gradually increased from 6hrs incubation to 36hr before a sharp increase to the optimal growth was observed.

Figure 6 shows the effect of different carbon sources on the growth of *K. gibsonii* CAC1 at different incubation times. The growth of *K. gibsonii* CAC1 was significantly ($p \geq 0.05$) different when incubated at different time in various carbon sources. When lactose was used as carbon source, there was a sharp increase in growth after incubation for 12hr until the optimal growth (1.255nm) was recorded at 18hrs after which a sharp decline in growth (0.165nm) was observed at 24hrs before a gradual rise in growth was then observed. Fructose and sucrose followed the same growth pattern as lactose but to a lesser growth degree. The least growth was observed when starch was added as individual carbon source to the basal medium with a gradual increase in growth as the incubation time increased until the highest growth (0.58nm) was observed at 42hr of incubation.

In Figure 7, the effect of different carbon sources on the growth of *M. odoratimimus* CAC2 at various incubation times was shown. There was a significant difference in the growth of *M. odoratimimus* at different incubation periods when grown at various carbon sources ($p \geq 0.05$). There was a sharp increase in growth at 12hr, with lactose having the optimal growth (0.889nm) and sucrose the least growth (0.475nm) after which a gradual decrease in growth of the isolate was recorded. A significant ($p \geq 0.05$) difference in the growth of *M. odoratimimus* was observed when lactose and starch were used as carbon sources. At 30hr of incubation, the growth was favored with lactose as the carbon source after which the growth declined. Starch gave the least growth support at 36hr.

Effect of different nitrogen sources on the growth of *K. gibsonii* CAC1 at different incubation times was illustrated in Figure 8. The effect of different nitrogen sources was not statistically significant ($p \leq 0.05$) on the growth of *K. gibsonii* CAC1. However, a significant ($p \leq 0.05$) difference was recorded when incubated at different incubation periods. Urea gave a gradual increase in growth with its highest growth of 84.14% compared to the optimum, recorded at 18hr of incubation after which a slight and then a sharp decline in growth was

recorded as incubation hours increased. The growth, when $NaNO_3$ was used had similar pattern to urea except that a sharp increase in growth was recorded when incubated for 18hr.

In Figure 9, the effect of different nitrogen sources on the growth of *M. odoratimimus* CAC2 was demonstrated at different incubation period. There was a significant ($p \geq 0.05$) difference in the growth when incubated at different periods using different nitrogen sources. On using urea as the nitrogen source; there was a gradual and rapid increase in the growth as incubation time increased. On incubating for 18hrs, there was a slight reduction in growth before further increase to the optimal absorbance value (0.519nm) was reached at 30hr. Thereafter, the growth gradually reduced. Similar growth pattern was seen when NH_4Cl was used but to a lesser degree. Urea gave the best growth.

Optimization of the Culture Condition

The growth of *M. odoratimimus* CAC2 and *K. gibsonii* CAC1 at optimum conditions is illustrated in Table 4 and 5. When the isolates were screened at different temperature over 42hr, microbial growth of the two bacteria was best at 30^0C. At this temperature, *M. odoratimimus* CAC2 had its best growth at 30hrs of incubation. The optimum pH of *M. odoratimimus* CAC2 after screening at different pH over 42hrs was 6.0. It was observed that the bacterium grew best at 42hr of incubation. The isolates grew best with lactose as carbon source. *M. odoratimimus* CAC2 showed highest growth at 30hrs of incubation and urea gave the best growth at 30hr of incubation.

At 30°C, *K. gibsonii* CAC1 had the best growth at 18hrs while its optimum growth pH was 5.5 with the highest growth recorded at 36hrs of incubation. Highest growth of *K. gibsonii* CAC1 in lactose was at 18hrs while the best nitrogen source on the growth of *K. gibsonii* CAC1 was NH_4Cl which best enhanced bacterial growth especially at 30hr of incubation.

Discussion

The cellulase producing bacteria identified in this study were *K. gibsonii* CAC1 and *M. odoratimimus* CAC2 using 16S rDNA sequencing and they were isolated from soil samples containing partially degraded cassava wastes. Nucleotide sequences of 952 and 953 base pairs were obtained by 16S rDNA sequencing using forward and backward primers respectively. Comparing the nucleotide sequences of isolates CAC1 and CAC2 to GenBank database, the strains showed a similarity of 90% identity with the sequence of 16S rDNA of *K. gibsonii*, and a similarity of 98% identity with the sequence of 16S rDNA of *M. odoratimimus* respectively. Therefore, isolate CAC1 was identified as *K. gibsonii* CAC1 and isolate CAC2 was identified as *M. odoratimimus* CAC2. *M. odoratimimus* CAC2 was observed to be gram negative, non-motile, rod-shaped, and aerobic with slight yellowish pigmentation. This was in agreement with Benedetti *et al*., 2011 who described members of the genus *Myroides* as aerobic, yellow-pigmented, non-motile, non-fermenting gram-negative rods formerly classified as *flavobacterium odoratum* and widely distributed in the environment especially in water. Li *et al*., 2012 isolated *Myroides odoratimimus* strain LWD09 from farm field and described the bacterium as gram-negative, capable of growing aerobically on 3, 4-dichloroaniline (DCA) as the sole carbon and energy sources. Vancanneyt *et al*., 1996, who reclassified *flavobacterium odoratum* as *Myroides odoratimimus* in 1996 described the bacterium as a strict aerobic gram-negative rods with a smell of fruit.

K. gibsonii CAC1 was observed to be Gram positive, motile, rod-shaped, aerobic with no pigmentation. The genus *K. gibsonii* was isolated from the bottom of a shoe exposed to canine feces and was described to be motile, gram positive, non-sporing, rod shaped, non-pigmented, aerobic bacterium that grew significantly at 42^0C and pH 5-6 (Riley, 2004). To the best of the authors' knowledge, this is the first time *K. gibsonii* and *M. odoratimimus* are reported to be isolated from cassava waste dumpsite soil and demonstrated to be cellulolytic.

Physico-chemical parameters have impact on the growth of the selected organisms as the factors such as temperature, pH, carbon and nitrogen sources influenced the performance of *K. gibsonii* and *M. odoratimimus* along the growth curve. This was in agreement with report of Strivastava and Baruah, 1986 who reported that various factors influence the nature of metabolic process of microbes such as Bacillus and their enzyme production is affected by the

medium composition. The highest growth of *M. odoratimimus* CAC2 was recorded between pH 5.0 and 6.0 with optimal pH at 6.0, and temperature of 30^0C when incubated for 30hr on carboxy-methyl agar. Holmes *et al.*, 1985 reported that *Myroides* species grew best at both room temperature and 37°C. The optimum pH supporting the growth of *K. gibsonii* CAC1 was recorded at pH 5.5 when incubated for 24hr. The growth of *K. gibsonii* CAC1 at different pH ranges during various incubation period was statistically ($p \geq 0.05$) different. *K. gibsonii* CAC1 showed a gradual increase in growth at different temperatures with optimal growth observed at 25°C when incubated for 36hr. This agreed with earlier report by Shaw and Keddie, 1983 that *K.gibsonii* isolated from meat and mince beef grew best at 25°C.

Urea was the best nitrogen source for the growth of *M. odoratimimus* CAC2 incubated for 30hr. The best carbon source for optimal growth of *M. odoratimimus* CAC2 was lactose at 30hr of incubation. Urea was the best nitrogen source for the growth of *K. gibsonii* CAC1 at 18hr of incubation while highest growth was recorded when NH_4Cl was used as nitrogen source at 30hr of incubation. The highest growth was recorded when lactose was the carbon source in *K. gibsonii* CAC1 at 18hr incubation period and the least growth was seen when starch was the carbon source.

Conclusion

It can be concluded that cellulolytic waste from cassava can be degraded efficiently by the bacteria if the cellulolytic properties of *M.odoratimimus* CAC2 and *K. gibsonii* CAC1 are adequately utilized since the bacteria can be cultivated under simple growth conditions. To the best of the authors' knowledge, this study concluded first time isolation and cellulolytic demonstration of the bacterial strains *K. gibsonii* and *M. odoratimimus* isolated from cassava waste dumpsite soil.

Table 1: Total viable bacterial counts of cassava dumpsites soil in Ibadan.

	Plates			
	A	B	C	
Agbowo processing unit	2.4	3.2	2.2	Number of isolates (10 x 10^8cfu/g)
Samonda Processing unit	3.0	2.6	2.4	Number of isolates (10 x 10^8cfu/g)
Sawmill processing unit	3.2	2.8	2.6	Number of isolates (10 x 10^8cfu/g)

Table 2: Screening for cellulase producing bacteria from cassava waste soil.

Isolate codes	processessing center	Diameter of zone of hydrolysis (mm)
CAC1	Sawmill	18
CAC2	Sawmill	13
CAC3	Agbowo	05
CAC4	Samonda	02
CAC5	Sawmill	04
CAC6	Agbowo	05
CAC7	Samonda	04
CAC8	Sawmill	06
CAC9	Samonda	02

Table 3: Morphological characteristics of the bacterial isolates

	Gram Reaction	Aeration	Shape	Motility	Pigmentation
Kurthia gibsonii	+ve	Aerobic	rod	motile	None
Myroides odoratimimus	-ve	Aerobic	rod	Non-motile	Light-yellow

Key: +ve = gram positive, -ve = gram negative

Table 4: Optimum conditions for the growth of *M. odoratimimus* CAC2

Incubation Time (hr)	Temperature (30°C)	pH 6.0	Carbon Source (lactose)	Nitrogen Source (urea)
6	0.3433 ± 0.008^{a}	0.2953 ± 0.095^{a}	0.0700 ± 0.100^{a}	0.0830 ± 0.002^{a}
12	0.9780 ± 0.003^{d}	0.4750 ± 0.002^{b}	0.8240 ± 0.0114^{f}	0.2530 ± 0.002^{c}
18	0.5700 ± 0.005^{b}	0.9030 ± 0.003^{c}	0.6447 ± 0.003^{c}	0.3757 ± 0.002^{f}
24	0.9300 ± 0.026^{c}	0.9267 ± 0.021^{d}	0.5633 ± 0.002^{b}	0.3697 ± 0.003^{e}
30	1.8733 ± 0.021^{g}	0.9363 ± 0.005^{d}	0.8717 ± 0.021^{g}	0.5190 ± 0.002^{g}
36	1.1377 ± 0.002^{e}	1.0543 ± 0.002^{e}	0.6900 ± 0.026^{d}	0.3170 ± 0.001^{d}
42	1.2640 ± 0.001^{f}	1.7167 ± 0.015^{f}	0.6300 ± 0.001^{c}	0.1737 ± 0.002^{b}

The mean difference is significant at the level of 0.05 ($p \geq 0.05$).

Table 5: Optimum conditions for the growth of *K.gibsonii* CAC1

Incubation time (hr)	Temperature (30°C)	pH 5.5	Carbon Source (lactose)	Nitrogen Source (NH_4Cl)
6	0.2917 ± 0.002^{a}	0.4993 ± 0.002^{a}	0.1510 ± 0.001^{a}	0.2850 ± 0.002^{a}
12	0.8537 ± 0.004^{d}	0.5963 ± 0.002^{b}	1.0343 ± 0.003^{f}	0.2900 ± 0.002^{a}
18	1.0480 ± 0.001^{g}	0.7877 ± 0.001^{d}	1.2553 ± 0.002^{g}	0.3253 ± 0.002^{b}
24	0.8763 ± 0.001^{e}	0.9627 ± 0.002^{e}	0.1660 ± 0.001^{b}	0.3263 ± 0.002^{b}
30	1.0260 ± 0.001^{f}	0.6177 ± 0.001^{c}	0.5353 ± 0.002^{c}	0.5770 ± 0.003^{d}
36	0.8090 ± 0.002^{b}	1.5400 ± 0.026^{g}	0.6900 ± 0.005^{e}	0.3417 ± 0.002^{c}
42	0.8450 ± 0.002^{c}	1.4230 ± 0.002^{f}	0.6640 ± 0.003^{d}	0.2857 ± 0.001^{a}

The mean difference is significant at the level of 0.05 ($p \geq 0.05$).

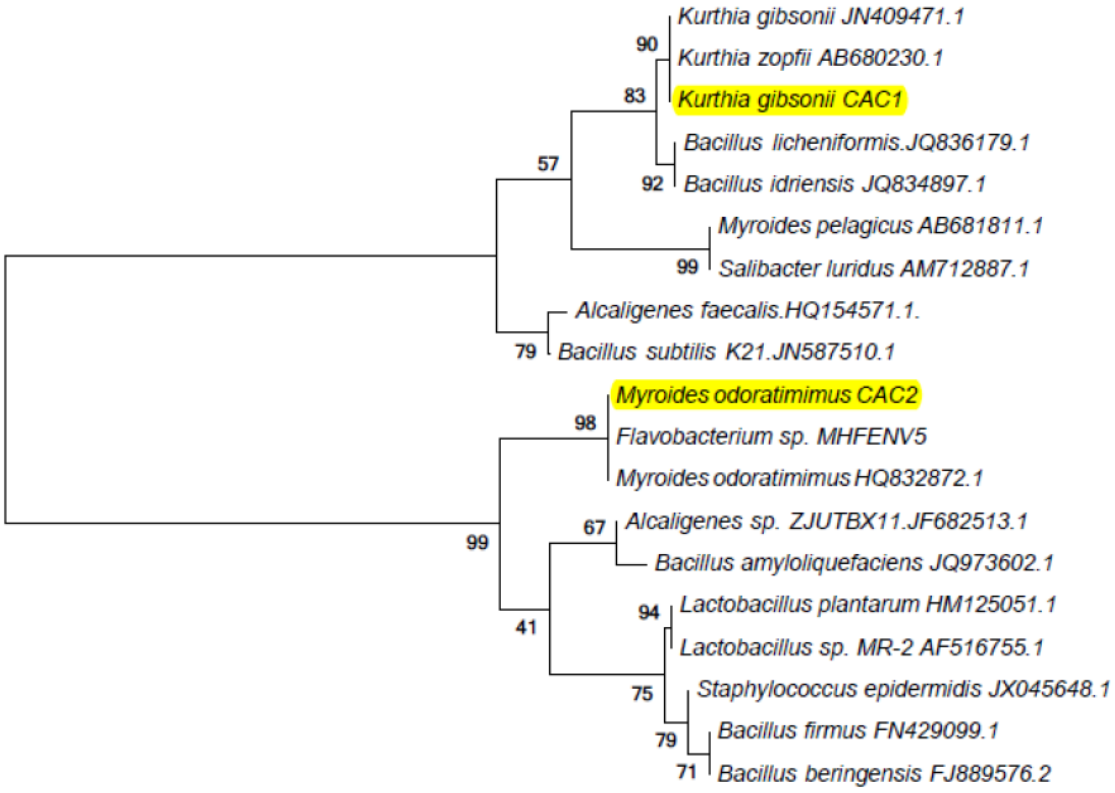

Figure 1: Phylogenetic relationship of the bacterial DNA of the selected isolates to other closely related sequences obtained from the GenBank.
*Multiple alignments of the sequences corresponding to the 16S rRNA of the studied isolates were carried out followed by neighbour joining clustering. Bootstrap values expressed as percentages of 1000 replications.

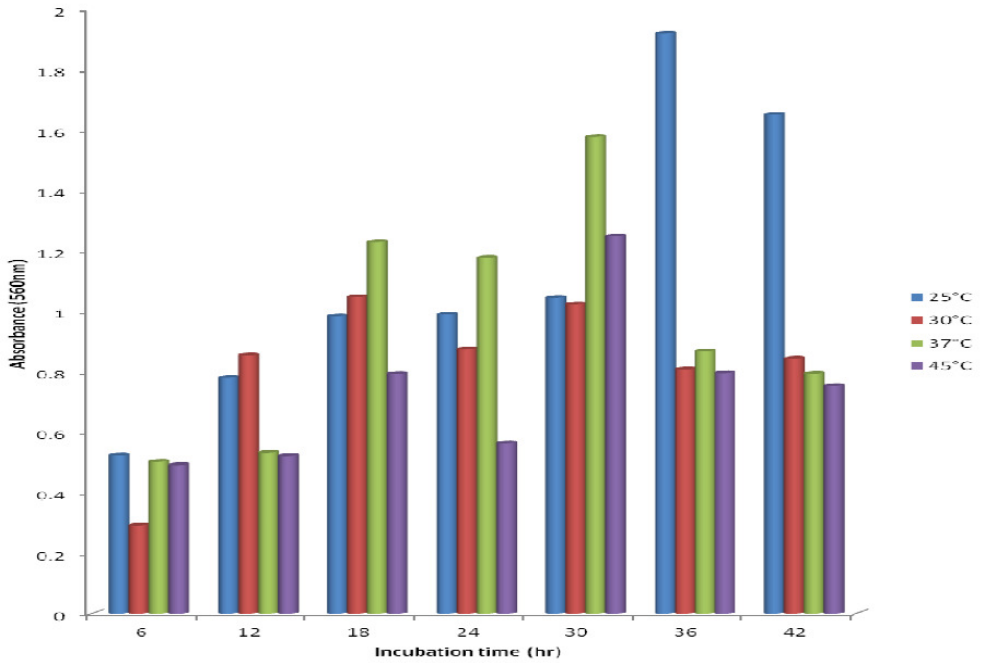

Figure 2: Growth of *K. gibsonii* CAC1 at different temperature ranges

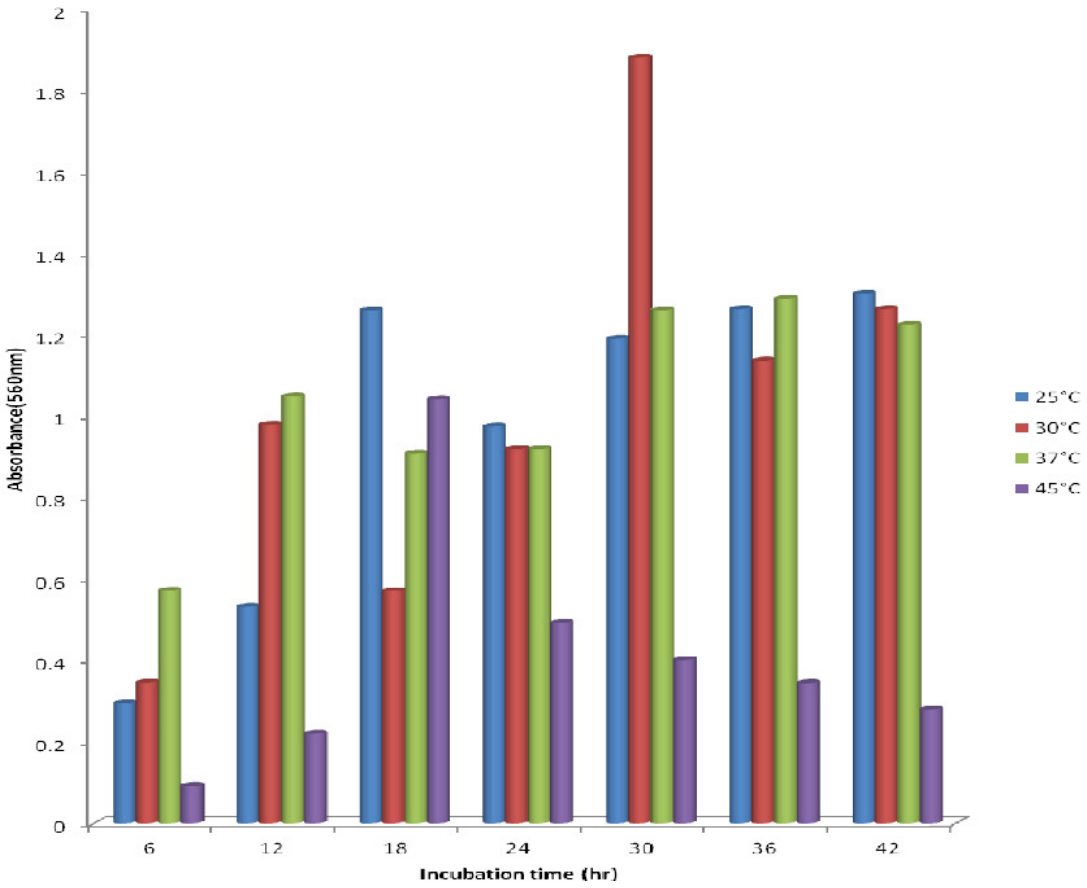

Figure 3: Growth of *M. odoratimimus* CAC2 at different temperature ranges.

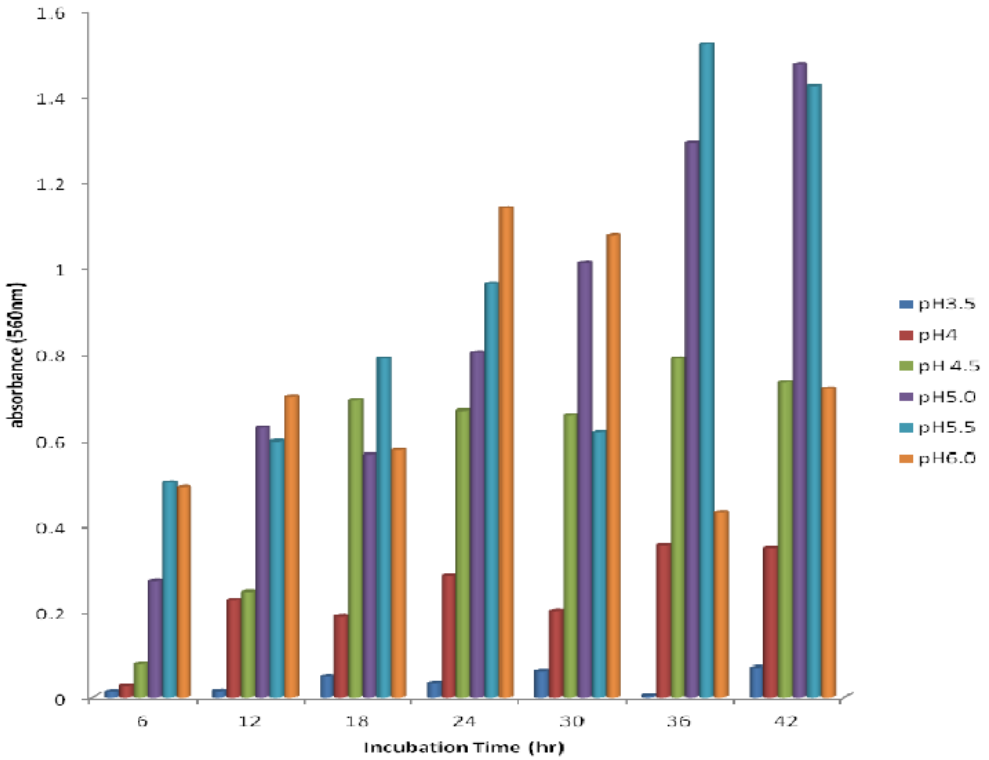

Figure 4: Growth of *K. gibsonii* CAC1 at different pH ranges

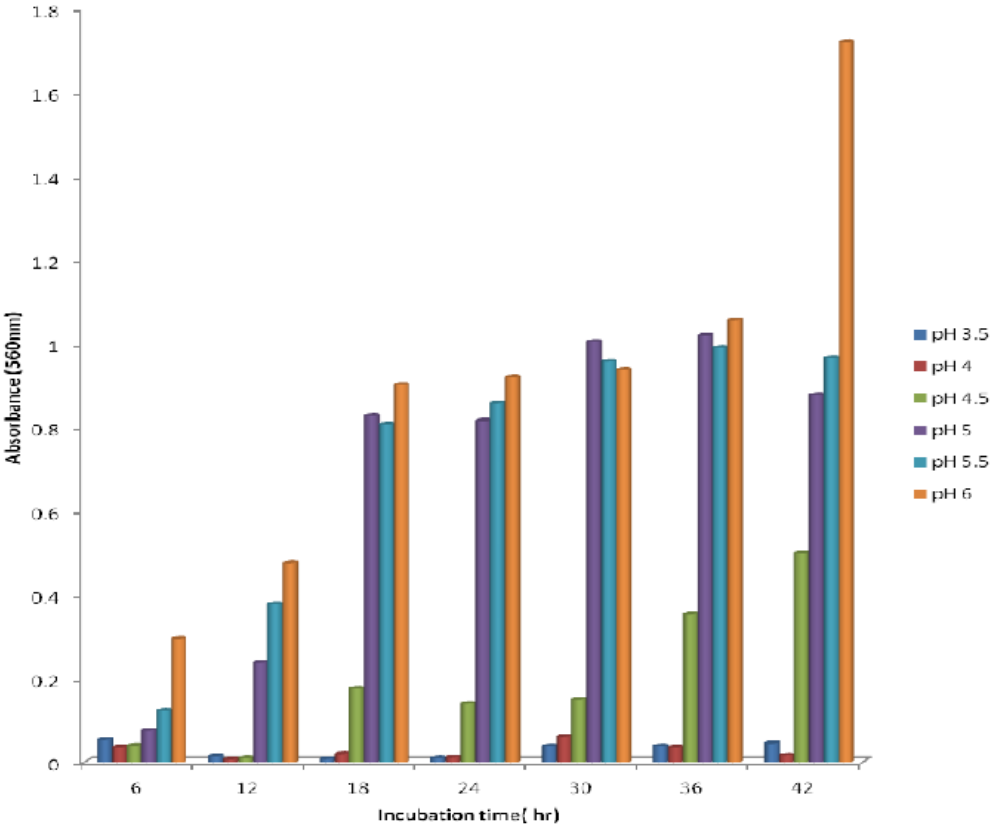

Figure 5: Growth of *M. odoratimimus* CAC2 at different pH ranges

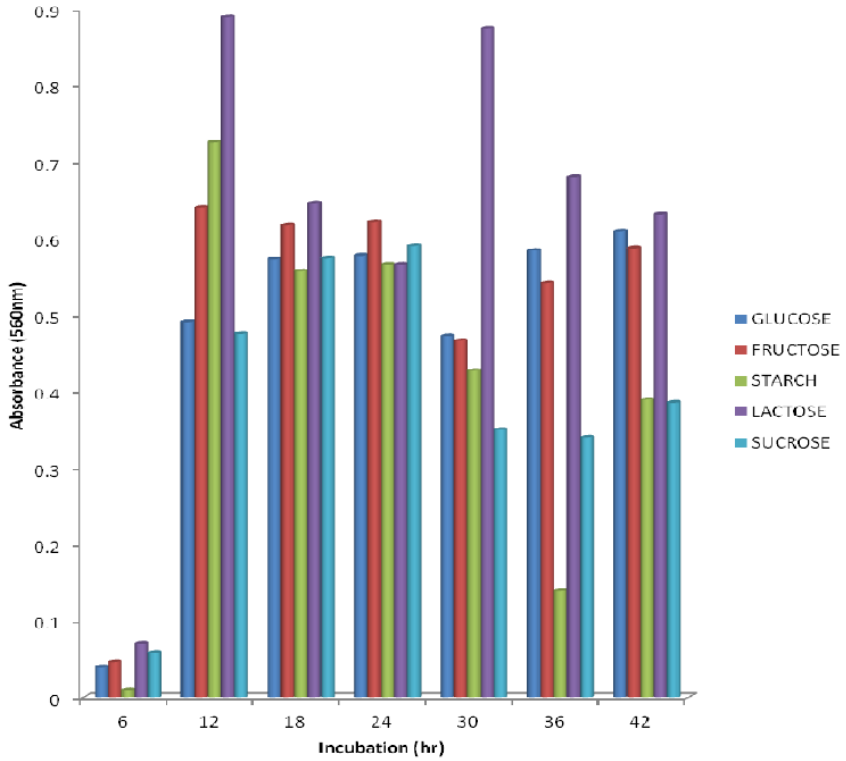

Figure 6: Effect of different carbon sources on the growth of *K. gibsonii* CAC1

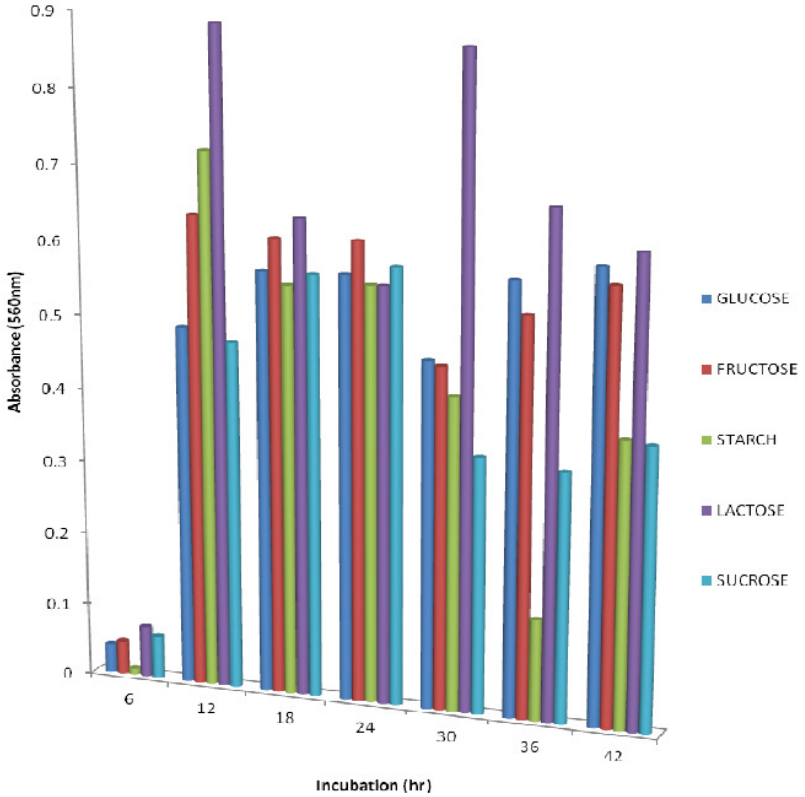

Figure 7: Effect of different carbon sources on the growth of *M. odoratimimus* CAC2

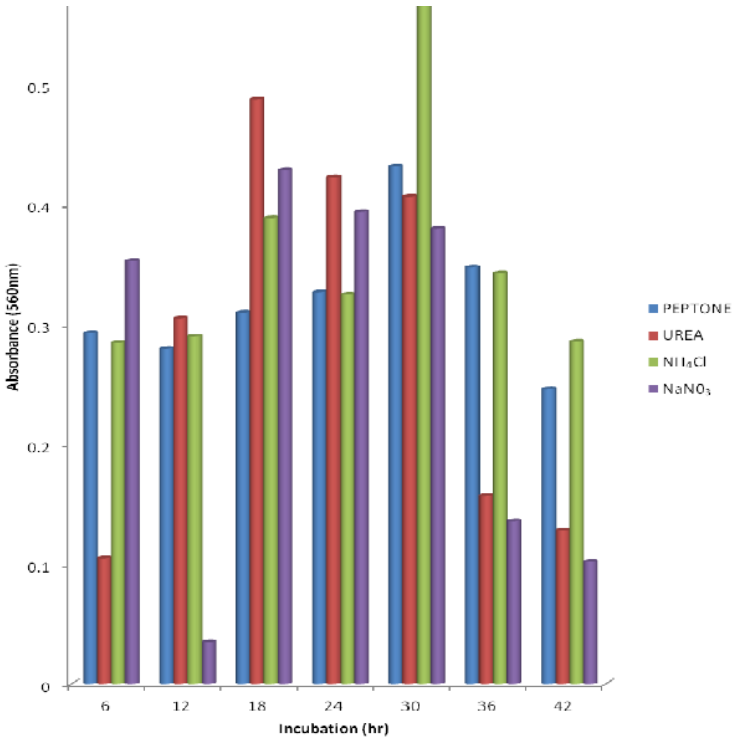

Figure 8: Effect of different nitrogen sources on the growth of *K.gibsonii* CAC1

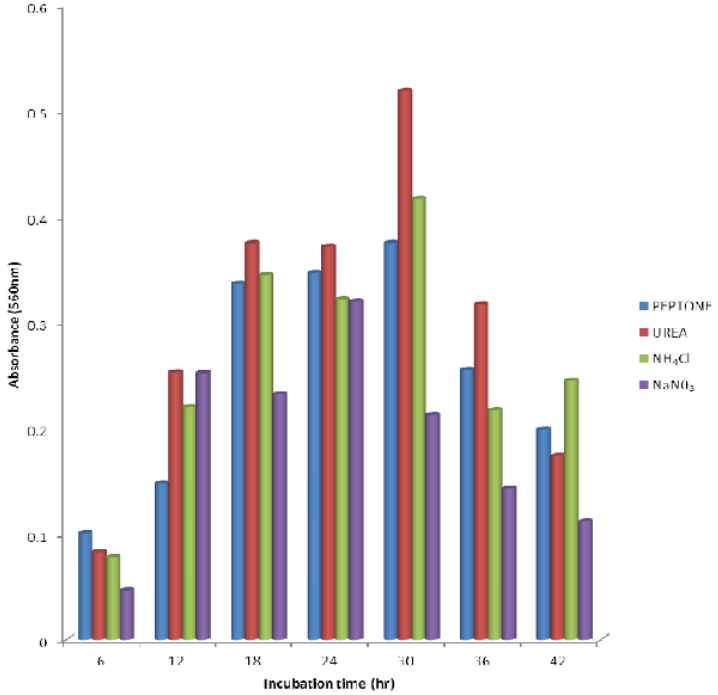

Figure 9: Effect of different nitrogen sources on the growth of *M. odoratimimus* CAC2.

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