Journal of Medical and Biomedical Sciences (2014) 3(3): 9-15


**doi:** http://dx.doi.org/10.4314/jmbs.v3i3.2

# ORIGINAL ARTICLE

## Biodegradation of shea nut cake by indigenous soil bacteria

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**In response to the quest for an environmentally friendly mode of disposal of shea nut cake, One hundred and sixty two (162) soil samples were collected at random at 0-20 cm, 20-40 cm and 40-60 cm soil depth from shea nut cake dumping sites in Jusonayilli, Gurugu and Kasalgu within the Sanaregu District of the Northern Region of Ghana, from September, 2010 to July, 2011. This is to isolate bacteria with high shea nut cake degrading ability and consequently select the potential application of these bacteria in bioremediation. The bacteria were grown in mineral salt medium supplemented with 2% shea nut cake as sole source of carbon. More Gram negative bacteria were involved in shea nut cake degradation than Gram positive bacteria. Two isolates which gave good growth on 5% shea nut cake agar were identified biochemically as Pseudomonas species. Both grew optimally at 35$^{o}$C and at pH 7.0. Yeast extracts enhanced growth. Pseudomanas strain G9 degraded 71.25% shea nut cake, while Pseudomonas strain G38 degraded 50.35% shea nut cake within 48 h. Pseudomonas G9 can be used to degrade shea nut cake. G9 and G38 are different species of Pseudomonas and molecular typing such as PCR can be used to determine the exact species.**

*Journal of Medical and Biomedical Sciences (2014) 3(3), 9-15*

**Keywords**: Shea nut cake, selective isolation, biodegradation, bacteria

## INTRODUCTION

Wastes generated from agro-based industries especially of traditional origin face serious disposal problems globally and more so in developing countries. Shea butter, a vegetable oil, is the main cooking oil of the people in the Savannah zone. It is a major source of fatty acids, glycerol and vitamins A, D, E, and F (Hall *et al.,* 1996). Shea butter has high demand locally and internationally for its numerous uses, and has become an important foreign exchange earner in Ghana and Burkina Faso (Harsch, 2001; Ghana Statistical service Report, 2007). Shea butter is extracted from the walnuts of the Shea tree, *Vitellaria* species, sub-species *paradoxia* and *nilotica* traditionally and by agro-food transformation factories (Hall *et al.,* 1996), producing shea nut cake as a waste product. Shea nut cake is rich in carbohydrates, fat and proteins and its potentials in soil fertility and animal nutrition have been reported (Hall *et al.,* 1996). Shea nut cake however contains polyphenolic compounds including tannins which make the product unacceptable to animals as feed and recalcitrant to biodegradation (Hall, 1996). Shea nut cake has been described as a waste product of no economic value (Hall, 1996) and soil is the ultimate recipient in ways that are environmentally problematic.

With increasing volumes of shea nut cake production (about 30,300,000 kg annually) (Iddrisu, 2013) there is an urgent need for an environmentally friendly mode of disposal of the waste in view of the financial support to the sector by the Government of Ghana through Savannah Accelerated Development Authority (S.A.D.A) in an effort to create wealth and bridge the poverty gap between the Northern and southern parts of the country. Shea nut cake is biodegradable (Nitiema *et al.,* 2010). Biodegradation by microbes is more favourable than

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chemical or physical treatment of the waste since the end products are environmentally safe. The use of microbes to degrade a particular waste would depend not only on the organism involved but also the manipulation of environmental conditions to increase microbial growth and hasten biodegradation (Hamzah *et al.,* 2010).

Numerous studies have been conducted on bacteria degrading hydrocarbon spills and agro-industrial wastes contaminating soil (Ray, 1994) and applied in pollution management. However, information on indigenous aerobic soil bacteria degraders of shea nut cake is unavailable even though soil is constantly receiving shea nut cake as waste. This study was designed to selectively isolate indigenous aerobic soil bacteria with high shea nut cake degrading ability, determine their growth conditions to hasten biodegradation and consequently select their potential in bioremediation.

## MATERIALS AND METHODS

### Soil Sampling

One hundred and sixty two (162) soil samples were collected at 0-20 cm, 30-40 cm and 40-60 cm soil depths from shea nut cake dumping sites in Jusonayilli, Gurugu and Kasalgu in the Sanarigu District in the Northern Region of Ghana in alternate months from September, 2010 to July, 2011 and cultured for bacteria with high shea nut cake degrading ability. The samples were collected at random using a graduated steel auger into sterile polythene bags and stored at 4$^{o}$C. The samples were sieved through a sterile sieve of 1.7 mm pore diameter to remove large objects such as whole plants and used for the selective isolation of shea nut cake degrading bacteria within 24 h.

### Isolation of shea nut cake degrading bacteria

Shea nut cake degrading bacteria were isolated by the enrichment method. A mineral salt medium described by Murad *et al., (*2007) for the isolation of soil bacteria was prepared as follows in grams per litre of distilled water: $K_2HPO_4$ (1.0); $KH_2PO_4$ (0.2); NaCl (1.0); $Cacl_2$ (0.001); $MgSO4.7H_2O$ (0.5); $FeSO_4.6H_2O$ (0.01); $CuSO_4.5H_2O$ (0.001); $ZnSO_4.7H_2O$ (0.001) and $MnSO_4.H_2O$ (0.001). The pH was adjusted to 7.0 with 1M NaOH and sterilized at 121$^{o}$C, 15 pounds pressure, for 15 minutes. The medium was supplemented with 2% (20.0 g L$^{-1}$) shea nut cake as sole source of carbon. Nystatin, U.S.P. 100,000 I.U. (Biomedicine S.P.R.L, Brussels, Belgium) was added per litre of medium to prevent fungal growth.

1.0 g of soil sample was weighed into 100 ml of sterile mineral salt shea nut cake medium in a 250 ml Erlenmeyer flask, mixed and incubated at 25$^{o}$C for 72 hours and sub-cultured into similar mineral salt shea nut cake medium after every 72 hours. After a third successive subculture, a final subculture was made onto mineral salt-shea nut cake (2%) medium to which 15 grams of agar was added to solidify. Colonies perceived to be different were isolated and sub-cultured on 5% and 10% shea nut cake agar. Two isolates, denoted as G9 and G38 that gave good growth on 5% shea nut cake agar within 48 hours were studied morphologically and biochemically as described in Cheesbrough (2002). G9 and G38 however failed to grow on 10% shea nut cake agar within 72 hours.

### Spore Formation, anaerobiosis and resistance to heat

Microscopic examinations of cultures were carried out at various stages of growth for spores. Test of resistance to heat was conducted by heating a 24 h culture of the isolates in mineral salt medium supplemented with 2% glucose at 80$^{o}$C, 90$^{o}$C and 100$^{o}$C for 10 minutes, cooled to room temperature and sub-cultured in fresh mineral salt supplemented with 2% glucose and on nutrient agar medium, observing for growth daily up to 5 days of incubation at 25$^{o}$C.

The ability of the isolates to grow under anaerobic conditions was investigated. 24 hour nutrient broth cultures of G9 and G38 were inoculated onto sterile nutrient agar plates, of pH 7.0 by the streak method and incubated anaerobically at 25$^{o}$ C using Anaerocult P gas generation kit and Anaerobiosis indicator (Anaerotest (Cat. No. 1.15112) from Merck KGaA Darmstadt, Germany, www.merk.de, version 2003-04-30, in an Anaerobic jar). The plates

were observed after 24 hours and 48 hours incubation for colonial growth. The effects of temperature, pH and yeast extract on the growth of G9 and G38 in Mineral salt medium supplemented with 2% glucose were investigated.

**Effect of pH, Temperature and Yeast extract on the Growth of G/09 and G/38**

Some growth requirements of the selected isolates to hasten biodegradation investigated include temperature, pH and yeast extract. The colorimetric method used by Defnoun *et al.*, (2000) and Nitiama *et al.,* (2010) on shea nut cake degrading bacteria isolate was used. 100 ml basal medium containing 2% glucose was used. Microbial growth was determined using spectrophotometer, (ThermSpectronic, Helios Epsilo, USA). Optical Density (O.D.) readings (at 580 nm) were taking immediately after inoculation (0 hour) and after 24 hours against uninoculated Mineral Salt Medium with 2% glucose as reagent blank. All tests were carried out in triplicates and mean reading recorded. Microbial growth was indicated by increase in optical density. Uninoculated media of the 6 pH values incubated along with media inoculated with bacteria served as controls.

For the effect of pH, the pH of the mineral salt medium containing 2% glucose was adjusted to between 5 and 10 (5.0, 6.0, 7.0, 8.0, 9.0 and 10). 1.0 ml of overnight nutrient broth culture of each of G9 and 38 was inoculated into 100 ml of the mineral salt media of the various pH values above and incubated at 25$^{O}$C for 24 hours.

The effect of temperature on growth of G/09 and G/38 was investigated. Mineral salt medium containing 2% glucose and pH adjusted to 7.0 was inoculated with isolates and incubated at the following temperatures: 25$^{o}$C, 35$^{O}$C, 45$^{O}$C, and 55$^{O}$C for 24 hours and growth measured in terms of increasing optical density at 580 nm against medium blank, using uninoculated medium incubated at respective temperatures as control.

For the effect of yeast extracts, basal medium of mineral salt medium containing 2% glucose and 0.5% yeast extract was prepared. The pH was adjusted to 7.0 and the media in triplicates were inoculated with G/09 and G/38, incubated at 25 $^{O}$C and growth measured after 24 hours in a procedure described by Nitiama *et al* (2010) and described earlier in this study. Bacterial growth was indicated by increase in optical density at 580 nm.

**Shea nut cake degrading ability of G/09 and G/38**

Degradation ability was determined by the colorimetric method described by Nitiama *et al.,* (2010). 1.0 ml of 24 hour nutrient broth culture was inoculated into 250.0 ml of mineral salt medium supplemented with 2% shea nut cake as sole source of carbon. The pH was adjusted to 7.0 with 1MNaOH solution and incubated aerobically at 35$^{o}$c. The Optical Densities (at 580 nm) of the inoculated media were recorded over time, representing the shea nut cake concentration, after the bacterial cells in 5.0 ml of broth culture were lysed with 50 µl of 5 Normal Hydrochloric acid (5N HCl). The optical density readings were then converted to grams of shea nut cake. The percentage shea nut cake degraded over time was then calculated. All readings were done against un-inoculated mineral salt medium without shea nut cake as reagent blank, and uninoculated medium with 2% shea nut cake incubated and treated like the inoculated media as control. All tests were done in triplicates and the mean taken.

## RESULTS

**Isolation of shea nut cake degrading bacteria**

Gram staining results of different bacteria isolated from shea nut cake contaminated soil are shown in Table 1. Out of the one hundred and thirty (130) bacteria isolated from shea nut cake contaminated soil examined by Gram staining, eighty six (86) representing 66.15% were Gram Negative Bacilli, forty (40) representing 30.77% were Gram positive bacilli while four (4) representing 3.08% were Gram Positive Cocci.

The distribution of the bacteria population by site and soil depth is given in Table 2. Gurugu had the highest population (38%) of shea nut cake degrading bacteria. Shea nut cake degrading bacteria were

**Table 1: Summary of Gram result of bacterial isolates by site**

| Area | Total | GPR | GNR | GPC |
|---|---|---|---|---|
| Gurugu | 49 | 14 | 35 | 2 |
| Jusonayilli | 43 | 13 | 28 | 2 |
| Kasalgu | 38 | 13 | 23 | 0 |
| Total | 130 | 40 | 86 | 4 |
| % | 100.00 | 30.77 | 66.15 | 3.08 |

more concentrated in the top (0-20 cm) soil layer (60%) as compared to other soil depths.

**Table 2: Distribution of isolates by site and sampling depth**

| Site | Total | 0-20 cm | 20-40 cm | 40-60 cm |
|---|---|---|---|---|
| Gurugu | 49(38%) | 30 | 13 | 6 |
| Jusonayilli | 43(33%) | 31 | 10 | 2 |
| Kasalgu | 38(29%) | 27 | 9 | 2 |
| Total (%) | 100.00 | 67.69 | 24.62 | 7.69 |

**Characteristics of selected Shea nut cake degrading Bacteria designated G9 and G38**

Morphological and biochemical characteristics of G9 and G38 are shown in Table 3. On nutrient agar G9 gave large, flat mucoid colonies (4 mm in diameter) that exhibited blue pigmentation with fruity smell, while G38 gave flat, mucoid, yellowish pigmented colonies (4 mm in diameter). G9 and G38 gave pale colonies on MacConkey agar (non-lactose fermenters) and β-haemolysis on blood agar.

**Spore Formation, anaerobiosis and resistance to heat characteristics**

Both G9 and G38 were non-spore formers. They failed to grow anaerobically and at 80$^{o}$ C and above investigated.

**Effect of pH and temperature** on **growth of G9 and G38**

The effect of pH on growth of G9 and G38 are shown in Figure 1. The optimum pH growth of G9 and G38 was pH 7.0, and the pH range for growth of G9 is pH 5 to 10.

The effect of temperature on the growth of the two isolates, G9 and G38, are shown in Figure 2. Both isolates gave growth between 25$^{o}$C and 40$^{o}$C with optimum growth at 35$^{o}$C. No bacteria growth was observed at 45$^{o}$C for both isolates. G9 and G38 are mesophiles. G9 and G38 were identified biochemically as strains of *Pseudomonas* with the aid of Bergey`s Manual of Determinative Bacteriology (Holt *et al.*, 1994).

**Effect of yeast extract on growth of *Pseudononas* strain G9 and *Pseudononas* strain G38**

Figure 3 shows growth of isolates in the absence and presence of 0.5% yeast extract. Both organisms grew well without yeast extract but however

**Table 3: Summary of Characteristics of Isolates (G9 and G38)**

| Isolate | N.A | B.A. | M A | Gram | Mot | Oxid | Cat | Cit | Ure | Ind | MR | VP | S | B | Gas | $H_2S$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| G9 | Mu BP | β- H | NLF | GNR | +ve | +ve <30 sec | +ve | +ve | +ve | -ve | - ve | - ve | R | R | - ve | - ve |
| G38 | Mu YP | β- H | NLF | GNR | +ve | +ve <30 sec | +ve | +ve | +ve | -ve | - ve | - ve | R | R | - ve | - ve |

***Key: N.A = Nutrient Agar; MuGP = Mucoid colonies Blue Pigmentation; MuYP= Mucoid colonies Yellow Pigmentation; B.A.= Haemolysis on Blood Agar; β- H= Beta Haemolytic; M. A= MacConkey Agar; NLF= Non-Lactose Fermenter; Gram= Gram Reaction; GNR= Gram Negative Rods; Mot= Motility; Oxidase production test = Oxidase; Cat= Catalase; Cit= Citrate;Ure=Urease test;Ind= Indole; MR= Methyl Red; VP= VogesProskaeur; TSI= Triple Sugar Iron Agar; Gas= $CO_2$; $H_2S$= HydrogenSulphide; R= Red (alkaline); +ve= Positve; -ve= Negative.***

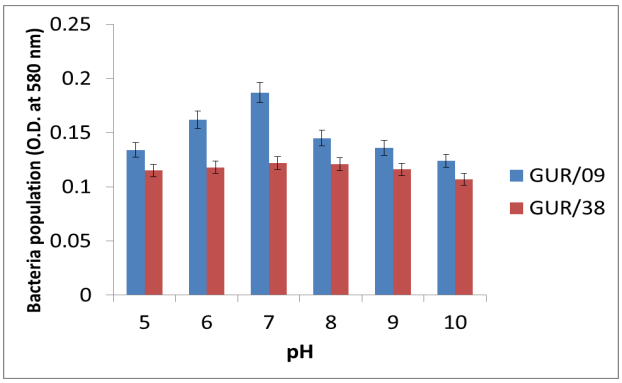


**Figure 1: Effect of pH on growth of *Pseudomonas* strain G9 and *Pseudomonas* strain G38**

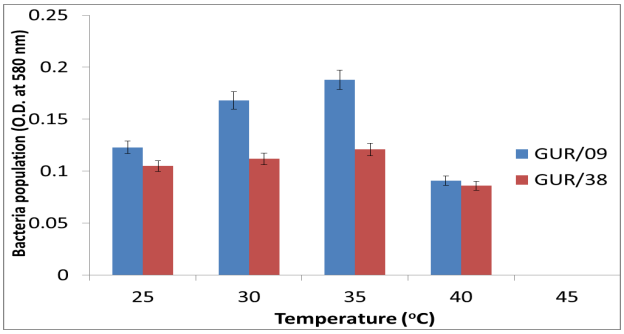


**Figure 2: Effect of Temperature on growth of *Pseudomonas* strain G9 and *Pseudomonas* strain G38**

demonstrated accelerated growth in the presence of yeast extract.

### Shea nut cake biodegradation by *Pseudononas* strain G9 and *Pseudononas* strain G38

The percentage shea nut cake degraded by the two isolates over 24 hours and 48 hours are indicated in Table 4. *Pseudononas* G9 degraded 25.35% and 71.25% in 24 hours and 48 hours respectively, while *Pseudononas*G38 was able to degrade 11.20% and 50.35% in 24 hours and 48 hours respectively. As bacterial population increases concentration of shea nut cake decreases.

## DISCUSSION

Bioremediation utilizes the ability of microbes to degrade or detoxify organic pollutants with the new concept being an efficient, cost effective and envi-

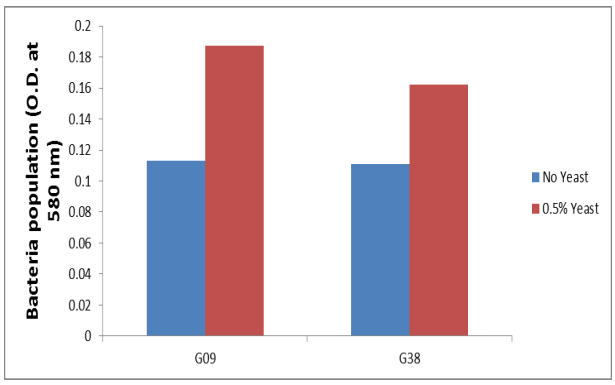


**Figure 3: Effect of Yeast extract on bacterial growth in mineral salt medium supplemented with 2% shea nut cake at 580 nm.**

ronmentally friendly, biological treatment method (Ray, 1994). It depends on the manipulation of nutritional and environmental factors to increase bacterial numbers to hasten biodegradation (Ray, 1994). The first report on soil bacteria degrading shea nut cake was by Quattara *et al.*, (1992), who

**Table 4: Percentage shea nut cake degradation by G9 and G38**

| Isolates | 24 hours | 48 hours |
|---|---|---|
| G9 | 25.35% | 71.25% |
| G38 | 11.20% | 50.35% |

***Average of three readings***

isolated anaerobic Streptococci from rice field capable of degrading hydrolysable tannins in shea nut cake. This study was conducted to isolate shea nut cake degrading aerobic soil bacteria and consequently select the potential application of these bacteria in bioremediation. The isolated bacteria from shea nut cake polluted soils agree with earlier report that organic pollutants degrading bacteria are naturally present in soil (Ray, 1994). Gram reaction results of bacteria isolated from Shea nut cake polluted soil capable of living on and degrading shea nut cake showed that different types of bacteria are involved in shea nut cake degradation in soil. From this study, 66.15% of the shea nut cake degrading bacteria were Gram negative bacilli indicating that more Gram negative bacteria were in-

volved in shea nut cake degradation in soil than Gram positive bacilli. Earlier research by Troung Son, (2005) reported that more Gram negative bacilli are involved in hydrocarbon degradation in soil than Gram positive bacilli. Top soil (0-20 cm) was found from this study to harbour the highest number of shea nut cake degrading bacteria which is in agreement with the finding of Hamzah *et al.,* (2010).

The isolation of *Pseudomonas sp.* from shea nut cake polluted soil is in agreement with Hamzah *et al.,* (2010) who isolated *Pseudomonas sp* crudeoil-contaminated soil. Ray, (1994) reported *Pseudomonas* as the most predominant isolate in crude oil polluted soils and water. *Pseudomonas* has been reported to have a high capability of degrading many natural products, including cyclic compounds, a versatility attributed to the presence of plasmids coding for the production of inducible enzymes (Bhatia and Ichhpujani, 2008). The versatility characteristic of *Pseudomonas* reported by Bhatia and Ichhpujani, (2008) confirms the urease positive results obtained for G9 and G38, since *Pseudomonas* isolated from humans are often reported negative for urease test.

Growth recorded in the pH range of 5 to 10 is supported by Bhatia and Ichhpugan, (2008) who observed that *Pseudomonas* has a wide pH growth range above 4.5. Optimum pH 7 for growth of *Pseudomonas* isolates in the present study agrees with the findings of Hamzah *et al*., (2010) who reported an optimum pH for growth of *Pseudomonas petuda* to be 7.0. The growth results of both G9 and G38 at 25$^{o}$C with optimum temperature of 35$^{o}$C are in agreement with data from Hamzah *et al.,* (2010) who reported that *Pseudomonas* grows well at 35$^{o}$C and at room temperature. Susceptibility of G9 and G38 to high temperatures (≥80$^{o}$C) is an advantage in bioremediation as the organism can easily be destroyed after successful use, knowing very well that *Pseudomonas,* especially *Pseudomonas aeruginosa* is an opportunistic pathogen to humans.

Yeast extract has been reported to contain nicotinic acid and sufficient quantities of most essential elements in utilizable form, which enhance the growth of most soil bacteria (Hamzah *et al*., 2010). Increase in populations of *Pseudomonas* strain G9 and *Pseudomonas* strain G38 in the presence of yeast extracts in the present study confirmed that yeast extract enhances growth of the isolates. These results are in agreement with Hamzah *et al*., (2010), who observed that *Pseudomonas aeruginosa* and *Pseudomonas putida* isolated from soil showed better growth with addition of yeast extracts. However, significant growth of G9 and G38 in the absence of yeast extracts suggested that yeast extract was not an absolute requirement for growth.

*Pseudomonas* strain G9 is a better shea nut cake degrader than *Pseudomonas* strain G38, degrading 25.35% in 24 hours and 71.25% in 48 h as compared to the 11.20% and 50.35% for *Pseudomonas* strain G38 in 24 hours and 48 hours respectively. The degradation results in this study are in agreement with Hamzah *et al.,* (2010), who observed that *P. aerugenosa* degraded 48% of light crude oil and up to 77% in 24h and 48h respectively. Ribbons and Evans, (1960) identified *Pseudomonas* to have the highest tolerance limit and degrading ability. In a related study of the degradation of phthalic acid by *Pseudomonas* and other soil microorganisms, Murad *et al.,* (2007) found that *Pseudomonas* was able to degrade 72% of phthalic acid in 48 hours. *Pseudomonas* strain G9 differed from *Pseudomonas* strain G38 in pigmentation on nutrient agar, intensity of colour change in citrate utilization test and shea nut cake degrading ability. *Pseudomonas* strain G9 and *Pseudomonas* strain G38 need further characterization such as molecular typing to determine the exact *Pseudomonas* species.

## CONCLUSION

The study concluded that shea nut cake degrading bacteria naturally exist in soil. Soil contaminated with shea nut cake was a good, cheap and reliable source of shea nut cake degrading bacteria for bioremediation application. More Gram negative bacteria are involved in shea nut cake degradation in soil than Gram positive bacteria. *Pseudomonas* strain G9 can be used to degrade the shea nut cake waste under the organism`s optimum environmental conditions. Yeast extract can be added to shea nut cake to hasten the biodegradation of shea nut.

## ACKNOWLEDGEMENTS

We wish to acknowledge the contribution of Mr Fidelis B-Baguo and sincerely thank him for working with us throughout the study process.

## COMPETING INTERESTS

The authors declare that they have no competing interests.

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ISSN 2026-6294