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*Full Length Research Paper*

# L-Glutamic acid production by *Bacillus* spp. isolated from vegetable proteins

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**22 isolates of *Bacillus* species were obtained from "Dawadawa", "Ugba" and "Ogiri" (fermented vegetable proteins) in Nigeria. The isolates were identified as *Bacillus subtilis* (6), (27.3%), *Bacillus pumilus* (5), (22.7%), *Bacillus licheniformis* (5), (27.3%) and *Bacillus polymyxa* (6), (22.7%). Four species of the *Bacillus* isolates were selected based on their ability to grow and produce L- glutamic acid in a synthetic medium and their comparison with the reference strain *Corynebacterium glutamicum* ATCC 13032. The four *Bacillus* species were *B. subtilis* (UGl). *B. pumilus* (DD4), *B. licheniformis* (0G4) and *B. polymyxa* (OG7) isolated from "Ugba". "Dawadawa" and "Ogiri", respectively. All the 22 *Bacillus* species isolated produced L- glutamic acid with *B. subtilis* (UGl) from "Ugba" recording the highest (8.5 mg/ml), while *B. licheniformis* (OG4) from "Ogiri" had the lowest value (5.0 mg/ml). L-Glutamic acid produced by *B. subtilis* (UGl) from "Ugba" (8.5 mg/ml) compared favourably with that produced by the reference strain *C. glutamicum* ATCC 13032, (10.2 mg/ml). All the isolates were able to utilize a range of carbon sources with glucose been the best, giving a yield of 8.4 mg/ml, while galactitol was least utilized. Ammonium nitrate was the best nitrogen source (6.5 mg/ml), while asparagine was least utilized (3.40 mg/ml) by the test isolates.**

**Key words:** L-Glutamic acid, bacteria strains, fermented vegetable proteins, fermentation.

## INTRODUCTION

L-Glutamic acid is well accepted as a taste enhancing compound that is consumed worldwide in the form of monosodium salt as a flavor enhancer in foods (Kikunae, 2008). L-Glutamic acid has been produced from quite a large number of carbon and nitrogen sources by fermentation using different bacterial strains (Madhavan and Ashok, 1996). Also, earlier studies have been made on the mineral requirements of a strain of *Pseudomonas aeruginosa* for the production of glutamic acid in a synthetic medium (Goswami and Majunda, 1991).

Many efforts have been made to enhance the glutamate fermentation process by modifying the medium, use of newer as well as highly potent cultures and also the utilization of new raw materials (Minoda, 1996). *Bacillus* spp. are ubiquitous and have the ability to secrete a deploymerizing enzyme which enables them to degrade biomass polymers found in cassava tubers (Stuzenberger, 1990).

From literature, till date, there is no detailed information on the use of different *Bacillus* species from vegetable proteins as starters in the production of L-glutamic acid. Previous studies have been geared towards gathering information on the general *Bacillus* species in enzyme production (Oguntimein, 1993). Hence, this research work focused on the use of *Bacillus* spp. isolated from vegetable proteins in the production of L-glutamic acid.

## MATERIALS AND METHODS

### Isolation of test *Bacillus* species for L-glutamic acid production

Nigerian fermented vegetable proteins from which isolations were made included "Dawadawa", "Ogiri" and "Ugba". These were randomly purchased from local markets at Mushin, Yaba and Oshodi in Lagos, Nigeria and brought into the laboratory in sterile

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conical flasks for immediate use. The isolation method of Ogbadu et al. (1990) was used. 10 g of the ground samples were shaken vigorously with 90 ml sterile distilled water and allowed to stand for 10 min and shaken again. The suspension was held in water bath at 80°C for 15 min and then plated out onto nutrient agar followed by incubation at 37°C for 24 h.

**Characterization and identification of Isolates**

The microorganisms isolated from the condiments were randomly picked and streaked on fresh nutrient agar plates to ensure purity.

Pure cultures were subjected to physiological and biochemical characterization following the procedures of Collins and Lyne (1976) and Gordon et al. (1973). Identification was based on the following test which included Gram's staining, catalase, voges proskauer, methyl red tests, motility, endospore, indole, concentrations, citrate, starch, gelatin and casein hydrolysis, sugar utilization and nitrate reduction.

**Identification of isolates**

The results obtained from the earlier stated tests were used to phenotypically identify the organisms by reference to Bergey's manual of systemic bacteriology (Sneath, 1986; Bachanan and Gibbon, 1974).

**The reference strain**

The reference strain *C. glutamicum* ATCC 13032 was obtained from GeSellSchaft for Biotechnolgishe for Schung MBH. Mascheroder Weg, 1, D-3300 Branuschweig Germany. The organism was maintained on Corynebacterium agar slant at 4°C and sub-cultured bimonthly on the some medium.

**Selection of test isolates for L-glutamic acid production**

The following parameters were used as criteria:

**Production medium**

A one-stage fermentation was carried out according to the method of Gutcho (1973) using a medium composed of the following: glucose (10 g); $KH_2PO_4$ (1.0) ; urea (2.0 g); $MgSO_4.7H_2O$ (0.5 g); $FeSO_4.7H_2O$ (0.05 g); biotin (3 µg); $H_2O$ (100 ml); pH 7.2. The medium was filter sterilized using a sterile conical flask and inoculated with 1 ml ($2.6\times 10^6$ cfu/ml) of each of the test isolates. The medium was incubated at 32°C on a rotary shaker at 180 rpm for 96 h. Cell growth, pH, qualitative and quantitative estimations of L-glutamic acid were determined as follows:

**Growth in production medium**

Growth was measured turbidometrically at 540 nm using spectrophotometer (Miltroy Spectronic 20D model) without centrifugation (Chatopadhayay and Banerjee, 1978).

**pH determination**

The pH of the fermenting medium was determined with the aid of a previously standardized pH meter (Unicam 9450 model). The pH meter was calibrated using pH 4.0 and 7.0 buffers.

**Qualitative determination *of L-glutamic* acid**

The fermented medium was centrifuged at 10,000 rpm. 20 µl of the centrifuged portion from the fermentation medium by the test isolates were chromatographed by spotting on thin layer chromatography plates (TLC) and developing it in a solvent mixture of n-butanol, acetic acid and water (4:1:1 v/v) in ascending direction for 6 h. The developed chromatogram was dried, sprayed with 0.2% (w/v) ninhydrin in ethanol, and was then heated at 110°C for 3 min. Spots of the same RF value as the standard glutamic acid were compared for intensity (Kinoshita et al., 1957; Brenner and Nieser, 1967; Ogbadu et al., 1990).

**Quantitative determination of *L-glutamic* acid**

The method of Spies (1957) was used. It was estimated by ninhydrin colour reaction and the absorbance was measured at 570 nm. From the clarified sample of each aliquot of the fermented medium, 1 ml was taken and added to 1 ml of the freshly prepared ninhydrin reagent.

The mixture was heated in a water bath for 5 min and then cooled under a running tap water. The absorbance of the resulting colored solution was read using a spectrophotometer (Miltroy Spectronic 20D model) at 570 nm against a blank made by substituting the extract with distilled water. The glutamic acid content in the sample was determined by reference to a standard curve of known concentration for L-glutamic acid.

**Process optimization for growth and L-glutamic acid production**

The effects of the following processing variables on the fermentation process were investigated on the test strains as follows:

**Effect of carbon sources on the growth and *L-glutamic* production by the test isolates**

This was carried out using the method of Chatopadhayay and Banerjee (1978).

Synthetic medium as earlier described was compounded using 2% level of different carbon sources which included glucose, fructose, maltose, galactose, mannose, arabinose, lactose, xylose, mannitol, starch, sucrose and galactitol. The medium was sterilized by autoclaving at 115°C for 10 min, inoculated with 1 ml ($2.6 \times 10^6$ cfu/ml) of each isolates and incubated at 32°C for 72 h. Cell growth and glutamate production were monitored.

**Effect of nitrogen sources on the growth and L-glutamic acid production by the test isolates**

The method of Chatopadhayay and Banerjee (1978) was used. Synthetic medium earlier described was compounded using different organic and inorganic nitrogen sources which included ammonium nitrate, urea, ammonium chloride, potassium nitrate, alanine and asparagines at 0.2% (w/v) levels. The medium was autoclaved at 115°C for 10 min and inoculated with 1 ml ($2.6 \times 10^6$ cfu/ml) of the test isolates and incubated at 30°C for 72 h.

## RESULTS

### *Bacillus* strains isolated from condiments

A total of 22 *Bacillus* isolates obtained from three fermented vegetable proteins ("Dawadawa", "Ugba" and

"Ogiri") were used in the study.

The isolates were initially differentiated on the basis of their cultural and cellular morphological studies, after which they were subjected to various physiological and biochemical tests and identified by reference to Bergey's manual of systematic bacteriology. The detailed result of the cultural and biochemical characteristics is shown in Table 1. They were identified as *B. subtilis, B. licheniformis, B. polymyxa* and *B. pumilus*. Amongst these organisms, *B. subtilis* and *B. licheniformis* were found to be the predominant species. In "Dawadawa", *B. pumilus* and *B. subtilis* occurred more than the other *Bacillus* strains. Also, in "Ugba", *B. pumilus* was also more than the other strains of *Bacillus* isolated. In "Ogiri", *B. licheniformis* was found to occur more than the other strains of *Bacillus*. The morphological features of the strains account for the differences between the members of the same isolates from the various condiments. *B. subtilis* from "Dawadawa" were creamy, had dry surfaces and were 1 to 2 mm diameter. *B. subtilis* from "Ugba" were creamy, had dirty surfaces and were at 1 to 2 mm diameter. *B. subtilis* from "Ogiri" were creamy, flat and were 1 to 2 mm diameter. *B. pumilus* from "Dawadawa" were creamy, entire but with differences in their diameter. *B. pumilus* from "Ogiri" were creamy, entire, and were small with differences in their diameter. *B. licheniformis* from "Dawadawa" were creamy, undulating and 1 to 2 mm diameter. *B. licheniformis* from "Ogiri" were creamy and entire with differences in their diameter. *B. polymyxia* from "Dawadawa" were creamy, slightly raised and were 1 to 2 mm diameter. Morphological features of the reference strain *C. glutamicum* ATCC 13032, showed that they were rod-shaped with slightly clubbed ends.

**Thin layer chromatography (TLC)**

Qualitative chromatographic assay on thin layer chromatographic plates (TLC) for L-glutamic acid produced by the various test isolates (*B. subtilis* LLG1, *B. pumilus* DD4, *B. licheniformis* OG4 and *B. polymyxa*) were obtained. The result showed that, the relative frequency (Rf) of the standard L-glutamic acid was 0.17. The Rf value from the test isolates were 0.17, 0.17, 0.17, 0.17 and 0.17 for *B. subtilis* UG1, *B. pumilus* DD4, *B. polymyxa* OG7, *B. licheniformis* OG4 and *C. glutamicum* ATCC 13032, respectively.

The Rf value thus obtained from the test isolates were compared with that for the standard L-glutamic acid. This is an indication of the quality of L-glutamic acid as produced by the various species of *Bacillus* obtained from the condiments.

**Production of L-glutamic acid by micro-organisms**

The highest L-glutamic acid producing *Bacillus* strain isolates were selected based on the amount of glutamic acid and their yield relative to the amount produced bythe type strain *C. glutamicum* ATCC 13032. *B. pumilus* from "Dawadawa" DD4, *B. subtilis* from "Ugba" UG1, *B. licheniformis* OG4 and *B. polymyxa* OG7 from "Ogiri" produced glutamic acid yield of 8.4, 8.5 and 8.4 mg/ml, respectively, while *C. glutamicum* ATCC 13032 recorded 10.2 mg/ml of glutamic acid (Table 2).

The least glutamic acid producing strains from the various condiments were *B. polymyxa* DD6 from "Dawadawa" with 6.2 mg/ml and *B. polymyxa* UG8 from "Ugba" with 5.4 mg/ml glutamic acid and *B. licheniformis* OG5 from "Ogiri" with 5.0 mg/ml glutamic acid (Table 2).

Differences in glutamic acid yield were observed between the *Bacillus* members from the different condiments that were morphologically different from one another.

*B. subtilis* UG1 from "Ugba", *B. pumilus* DD4 from "Dawadawa", *B. licheniformis* OG4 and *B. polymyxa* OG7 from "Ogiri" produced 8.5, 8.4, 8.4 and 8.2 mg/ml glutamate, respectively and this compared favourably with the reference strain *C. glutamicum* ATCC 13032 that had a yield of 10.2 mg/ml.

**Effect of carbon sources on the growth and L-glutamic acid production**

The results of the different carbon sources on the growth and L-glutamate acid production by the test isolates and *C. glutamicum* ATCC 13032 are presented in Table 3. Glucose was the best carbon source utilized by the test isolates and the reference strain with (growth range of 1.23 to 1.82 optical density and L-glutamic acid yield of 5.1 to 8.4 mg/ml).

Galacticol was poorly utilized by the test isolates and the reference strain (growth range of 0.38 to 0.70 optical density and L-glutamic acid was not produced by all the organisms). The least glucose utilized was by the test isolate *B. licheniformis* OG4 with an optical density and glutamic acid of 1.24 and 5.1 mg/ml, respectively, while *C. glutamicum* was the best glucose utilizing strain with an optical density of 1.82 and glutamic acid yield of 8.4 mg/ml (Table 3).

**Effect of nitrogen sources on the growth and L-glutamic acid production**

Investigations on the use of various nitrogen sources by the test isolates and the reference strain showed that, ammonium nitrate was best utilized with glutamate production ranged from 6.00 to 6.30 mg/ml, while asparagine was least utilized and the produced glutamate ranged from 3.40 to 3.60 mg/ml (Table 4). Growth range by the test isolates and the reference strain in ammonium nitrate was 0.56 to 0.88 optical density, while for asparagines, it was 0.42 to 0.46 optical density.

**Table 1.** Biochemical characteristics of micro-organisms isolated from some Nigeria fermented condiments.

| Source | Isolate code | Colony morphology | Cell characteristic | Endospore | Catalase | Voges proskauer | Methyl red | Starch | Motility | Citrate | Nitrate | Urease | Anaerobiosis | Growth in NaCl | | | Casein | Gelatin | Sugar fermentation | | | | | | | | | Probable identity |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | | Indole | 5% | 7% | 10 % | | | LA | AR | MA | GL | XY | RI | FR | MN | SU | |
| Dawadawa | DD1 | Cream dry surface, flat entire, 1-2 mm diameter | + Rods | E | + | + | + | + | + | + | + | + | - | - | + | + | + | + | + | + W | + | + | + | + | + | + | - | + | *Bacillus subtilis* |
| | DD2 | Cream, flat entire, 1-2 mm | + Rods | E | + | - | + | - | + | + | + | + | - | - | + | + | + | + | + | + W | + | + | + | + | + | + | - | + | *B.pumilus* |
| | DD3 | Cream, flat 1-2 mm diameter | + Rods | E | + | + | + | + | + | + | + | + | - | - | + | + | + | + | + | + W | + | + | + | + | + | + | - | + | *B. subtilis* |
| | DD4 | Cream, entire, flat, 1-2 mm diameter | + Rods | E | + | - | + | + | + | + | + | + | + | - | + | + | + | + | + | + W | + | + | + | + | + | + | - | + | *B. pumilus* |
| | DD5 | Cream, undulating, 1-2 mm diameter | + Long Rods | E | + | + | + | + | + | + | + | + | + | - | + | + | + | + | + | + W | + | + | + | + | + | + | + | + | *B. licheniformis* |
| | DD6 | Cream, flat, entire , 1- 2 mm diameter | + Short Rods | E | + | + | + | + | + | + | + | + | - | - | + | + | + | + | + | + W | - | + | + | + | + | + | + | + | *B.polymyxa* |
| Ugba | UG1 | Cream dirty, flat entire, 1-2 mm diameter | + Rods | E | + | + | + | + | + | + | + | + | - | - | + | + | + | + | + | + W | + | + | + | + | + | + | - | + | *B. subtilis* |
| | UG2 | Cream dirty,flat entire, 1-2 mm diameter | + Rods | E | + | + | + | + | + | + | + | + | - | - | + | + | + | + | + | + W | + | + | + | + | + | + | - | + | *B. subtilis* |
| | UG3 | Cream, dry surface, entire flat, 1-2 mm diameter | + Rods | E | + | - | + | - | + | + | + | + | - | - | + | + | + | + | + | + W | + | + | + | + | + | + | - | + | *B. pumilus* |

**Table 1.** Continued.

| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ogiri | UG4 | Cream, entire, flat, 2-3 mm diameter | + | Rods | E | + | - | + | - | + | + | + | + | - | - | + | + | + | + | + | + W | + | + | + | + | + | + | - | + | *B. pumilus* |
| | UG5 | Cream, entire flat, 1-2 mm diameter | + | Rods | E | + | + | + | + | + | + | + | + | - | - | + | + | + | + | + | + W | + | + | + | + | + | + | + | + | *B. pumilus* |
| | UG6 | Cream, entire, flat, 2-3 mm diameter | + | Rods | E | + | + | + | + | + | + | + | + | + | - | + | + | + | + | + | + W | + | + | + | + | + | + | + | + | *B. lichenifor mis* |
| | UG7 | Cream, entire flat, 1-2 mm diameter | + | Rods | E | + | - | + | + | + | + | + | + | + | - | + | + | + | + | + | + W | - | + | + | + | + | + | + | + | *B. lichenifor mis* |
| | UG8 | Cream, entire flat, 1-2 mm diameter | + | Rods | E | + | + | + | + | + | + | + | + | + | - | + | + | + | + | + | + W | - | + | + | + | + | + | + | + | *B.polymy xa* |
| | UG9 | Cream, entire, large, 2-3 mm diameter | + | Rods | E | + | + | + | + | + | + | + | + | + | - | + | + | + | + | + | + W | - | + | + | + | + | + | + | + | *B.polymy xa* |
| | OG1 | Cream, entire flat, 1-2 mm diameter | + | Rods | E | + | + | + | + | + | + | + | + | - | - | + | + | + | + | + | + W | + | + | + | + | + | + | - | + | *B. subtilis* |
| | OG2 | Cream, entire flat, 1-2 mm diameter | + | Rods | E | - | + | + | + | + | + | + | + | - | - | + | + | + | + | + | + W | + | + | + | + | + | + | - | + | *B. subtilis* |
| | OG3 | Cream, entire flat, 1-2 mm diameter | + | Rods | E | + | - | + | - | + | + | + | + | - | - | + | + | + | + | + | + W | + | + | + | + | + | + | - | + | *B. pumilus* |
| | OG4 | Cream, entire, flat, 2-3 mm diameter | + | Rods | E | + | - | + | + | + | + | + | + | - | - | + | + | + | + | + | + W | + | + | + | + | + | + | - | + | *B. pumilus* |
| | OG5 | Cream, entire flat, 1-2 mm diameter | + | Rods | E | - | + | + | + | + | + | + | + | - | - | + | + | + | + | + | + W | + | + | + | + | + | + | + | + | *B. lichenifor mis* |

**Table 1.** Continued.

| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| OG6 | Cream, entire, flat, 2-3 mm diameter | + | Rods | | E | + | + | + | + | + | + | + | + | - | - | + | + | + | + | + | + W | + | + | + | + | + | + | + | + | *B. licheniformis* |
| OG7 | Cream, entire flat, 1-2 mm diameter | + | Rods | | E | + | + | + | + | + | + | + | + | - | - | + | + | + | + | + | + W | + | + | + | + | + | + | + | + | *B. licheniformis* |

+ = Positive; LA = lactose; FR = fructose; GL = glucose; AR = arabinose; MN = mannose; XY = xylose; - = negative; MA = maltose; SU= sucrose; RI = ribose; W = weak reaction.

**Table 2.** Production of L-glutamic acid by *Bacillus* spp. Isolated from fermented condiments.

| Isolate | Source | Identity | L-glutamic acid yield (mg/ml) |
|---|---|---|---|
| DD1 | Dawadawa | *B. subtilis* | 8.3 ± 0.05 |
| DD2 | Dawadawa | *B. pumilus* | 8.2 ± 0.04 |
| DD3 | Dawadawa | *B. subtilis* | 8.2 ± 0.05 |
| DD4 | Dawadawa | *B. pumilus* | 8.4 ± 0.04 |
| DD5 | Dawadawa | *B. licheniformis* | 6.4 ± 0.04 |
| DD6 | Dawadawa | *B. polymyxa* | 6.2 ± 0.04 |
| UG1 | Ugba | *B. subtilis* | 8.5 ± 0.04 |
| UG2 | Ugba | *B. subtilis* | 6.4 ± 0.03 |
| UG3 | Ugba | *B. pumilus* | 7.2 ± 0.04 |
| UG4 | Ugba | *B. pumilus* | 7.0 ± 0.05 |
| UG5 | Ugba | *B. pumilus* | 5.8 ± 0.04 |
| UG6 | Ugba | *B. licheniformis* | 5.9 ± 0.03 |
| UG7 | Ugba | *B. licheniformis* | 5.6 ± 0.05 |
| UG8 | Ugba | *B. polymyxa* | 5.4 ± 0.04 |
| UG9 | Ugba | *B. polymyxa* | 6.0 ± 0.03 |
| OG1 | Ogiri | *B. subtilis* | 6.7 ± 0.02 |
| OG2 | Ogiri | *B. subtilis* | 7.1 ± 0.02 |
| OG3 | Ogiri | *B. pumilus* | 8.0 ± 0.02 |
| OG4 | Ogiri | *B. licheniformis* | 8.4 ± 0.01 |
| OG5 | Ogiri | *B. licheniformis* | 5.0 ± 0.05 |
| OG6 | Ogiri | *B. licheniformis* | 5.4 ± 0.02 |
| OG7 | Ogiri | *B. polymyxa* | 8.2 ± 0.04 |
| ATCC 13032 | Reference strain | *C. glutamicum* | 10.2 ± 0.03 |

Value represents the mean score (n=3) ± S.D.

**Table 3.** Effect of carbon sources on growth (OD at 540 nm) and glutamic acid production by the test isolates at 32°C.

| Isolate | Arabinose | Xylose | Glucose | Fructose | Maltose | Galactose | Lactose | Sucrose | Starch | Mannitol | Galactitol | Control |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *B. subtilis* | [a]0.71± 0.01 | 0.07± 0.11 | 1.23± 0.01 | 0.32± 0.02 | 0.03± 0.01 | 0.57± 0.11 | 0.09± 0.01 | 0.25± 0.01 | 0.17± 0.01 | 0.12± 0.01 | 0.70± 0.01 | 0.24± 0.01 |
| UG1 | [b](3.8± 0.4) | (1.40± 0.12) | (5.6± 0.21) | (1.50± 0.03) | (1.40± 0.02) | (1.20± 0.12) | (1.50± 0.02) | (1.64± 0.02) | (0.04± 0.02) | (1.30± 0.02) | (0.00± 0.00) | (0.00± 0.00) |
| *B. pumilus* | [a]0.19± 0.01 | 0.81± 0.01 | 0.18± 0.01 | 0.10± 0.01 | 0.80± 0.01 | 0.58± 0.02 | 0.19±0.01 | 1.82± 0.01 | 0.31± 0.01 | 0.19± 0.01 | 0.38± 0.02 | 0.22± 0.01 |
| DD4 | [b](3.60± 0.03) | (2.4± 0.03) | (6.2± 0.13) | (1.30± 0.02) | (1.11± 0.01) | (1.60± 0.04) | (1.42± 0.02) | (4.2± 0.02) | (4.2± 0.02) | (1.45± 0.01) | (0.00± 0.00) | (0.00± 0.00) |
| *B. licheniformis* | [a]0.54± 0.01 | 0.76± 0.4 | 1.24± 0.01 | 0.72± 0.02 | 0.60± 0.02 | 0.60± 0.02 | 0.57± 0.01 | 0.50± 0.01 | 1.54± 0.02 | 0.42± 0.01 | 0.55± 0.01 | 0.13± 0.01 |
| OG4 | [b](3.60± 0.03) | (2.42± 0.06) | (5.1± 0.21) | (1.80± 0.03) | (1.80± 0.03) | (1.23± 0.03) | (2.40± 0.02) | (1.46±0.02) | (4.6± 0.03) | (1.52± 0.01) | (0.00± 0.01) | (0.00± 0.00) |
| *B. polymyxa* | [a]0.47± 0.01 | 0.25± 0.11 | 1.63± 0.01 | 0.82± 0.01 | 0.50± 0.04 | 0.54± 0.02 | 0.77± 0.01 | 1.94± 0.03 | 0.67± 0.01 | 0.30± 0.00 | 0.61± 0.01 | 0.23± 0.01 |
| OG7 | [b](3.40± 0.13) | (2.50± 0.04) | (6.50.02) | (2.20± 0.02) | (1.41± 0.02) | (2.20± 0.03) | (1.56± 0.02) | (6.2± 0.04) | (1.00± 0.02) | (2.8± 0.02) | (0.00±0.00) | (0.00± 0.00) |
| *C. glutamicum* | [a]0.59± 0.01 | 0.76± 0.04 | 1.82± 0.01 | 1.52± 0.03 | 0.70± 0.02 | 0.64± 0.03 | 0.47± 0.01 | 1.98± 0.02 | 0.56± 0.01 | 0.96± 0.01 | 0.64± 0.01 | 0.41± 0.01 |
| ATCC 13032 | [b](3.70± 0.05) | (2.80± 0.04) | (8.4± 0.32) | (3.60± 0.02) | (1.48± 0.02) | (2.7± 0.02) | (2.40± 0.02) | (6.4± 0.03) | (1.20± 0.02) | (3.00± 0.02) | (0.01±0.00) | (0.00± 0.00) |

Values represent the mean score (n = 3) ± S.D. a = Growth of the isolates (optical density); b = glutamic acid yield (mg/ml).

**Table 4.** Effect of different nitrogen sources on growth (O.D at 540 nm) and glutamic acid production (mg/ml) by the test isolates at 32°C.

| Isolate | $KNO_3$ | $NH_4CL$ | $NH_4No_3$ | Urea | Asparagine | Alanine | Control |
|---|---|---|---|---|---|---|---|
| *B. subtilis* UG1 | [a]0.25± 0.01 | 0.57± 0.02 | 0.88± 10.2 | 0.76± 0.01 | 0.42± 0.02 | 0.43± 0.02 | 0.42± 0.01 |
| | [b]6.4± 0.05 | 5.41± 0.01 | 5.41± 0.01 | 5.61± 0.01 | 6.1± 0.01 | 6.1± 0.01 | 7.0± 0.01 |
| | [c](4.3± 0.01) | (3.60± 0.02) | (6.00± 0.01) | (6.40± 0.02) | (3.6± 0.02) | (3.1± 0.02) | (0.001± 0.00) |
| *B. pumilus* DD4 | [a]0.35± 0.02 | 0.54± 0.03 | 0.56± 0.03 | 0.76± 0.03 | 0.43± 0.01 | 0.32± 0.01 | 0.30± 0.01 |
| | [b]6.2± 0.02 | 5.6± 0.03 | 5.5± 0.02 | 5.2± 0.03 | 6.2± 0.02 | 6.3± 0.02 | 7.0± 0.02 |
| | [c](3.8± 0.01) | (3.8± 0.02) | (6.20± 0.02) | (6.4±0.02) | (3.60±0.01) | (3.20± 0.01) | (0.00± 0.00) |
| *B. licheniformis* OG4 | [a]0.38± 0.05 | 0.50± 0.01 | 0.57± 0.05 | 0.57± 0.01 | 0.46± 0.02 | 0.62± 0.02 | 0.31± 0.01 |
| | [b]6.2± 0.02 | 5.8± 0.02 | 5.8± 0.01 | 4.8± 0.01 | 5.4± 0.05 | 5.8± 0.01 | 7.0± 0.01 |
| | [c](3.8± 0.01) | (3.60± 0.01) | (6.30± 0.02) | (6.20± 0.02) | (3.40± 0.02) | (3.30± 0.02) | (0.00± 0.00) |
| *B. polymyxa* OG7 | [a]0.36± 0.02 | 0.60± 0.05 | 0.56± 0.01 | 0.63± 0.02 | 0.42± 0.01 | 0.42± 0.01 | 0.42± 0.01 |
| | [b]6.4± 0.01 | 5.8±0.05 | 6.1± 0.02 | 5.2± 0.02 | 5.7± 0.05 | 5.6± 0.02 | 7.0± 0.01 |
| | [c](3.60± 0.01) | (4.20± 0.02) | (6.20±0.03) | (6.42± 0.03) | (3.6± 0.01) | (3.50± 0.02) | (0.00± 0.00) |
| *C. glutamicum* ATCC 13032 | [a]0.42± 0.02 | 0.82± 0.821 | 0.88±0.01 | 0.76± 0.02 | 0.46± 0.02 | 0.88± 0.01 | 0.38± 0.01 |
| | [b]6.6± 0.02 | 6.2± 0.03 | 6.9± 0.01 | 5.8± 0.02 | 6.2± 0.01 | 5.8±0.02 | 7.0± 0.01 |
| | [c](4.2± 0.01) | (4.80± 0.02) | (6.50± 0.02) | (6.10± 0.01) | (3.40± 0.01) | (3.50± 0.01) | (0.00± 0.00) |

Values represent the mean scores (n = 3) S.D. a= Growth of the isolates (optical density); b = pH of the medium; c = glutamic acid yield (mg/ml).

## DISCUSSION

The involvement of the various types of *Bacillus* species in the fermentation of vegetable proteins had earlier been reported by Odunfa (1985). *Bacillus* is mostly found in vegetable proteins, since they are probably the survivors of production and fermentation process of these condiments due to their spore forming ability.

Reports by Dike and Odunfa (2001) on the occurrence of *Bacillus* species in vegetable fermented foods showed that *Bacillus* was isolated from the fermented plant materials. In this study, a total of 22 strains of *Bacillus* were isolated and characterized from some traditional fermented vegetable protein seeds in Nigeria. *B. subtilis, B. pumilus, B. polymyxa* and *B. licheniformis* were the dominant species isolated. The isolated *Bacillus* species were used as starter cultures in the production of L-glutamic acid. The high number of *B. subtilis* recorded in this study could be due to the fact that, *B. subtilis* from vegetable proteins are proteolytic and they grow rapidly and reach mid-log phase in 3 to 4 h (Preston, 1981). The identification of the different types of *Bacillus* species in this study could be due to the fact that, majority of the substrates used in the preparation of vegetable protein are of different plant origin and each particular plant species provide a unique environment in terms of competing microorganisms, natural plant, antagonists type, availability and concentration of substrates and various physical factor. Two conditions allow for the development of characteristic epiphytic fermentation micro-organisms when plant materials are harvested and prepared for fermentation.

The *Bacillus* species constitute an important group of organisms particularly in the food industry. The reason for the widespread use of *Bacillus* in the preparation of foods and other fermentation processes are; production of desired flavor through enhancement of L-glutamic acid synthesis and production of extracellular enzymes particularly alpha and beta amylases which finds considerable commercial applications in the brewery, textile and paper industries (Ramesh and Losane, 1990; Forgarty et al., 1991; Oguntimerin, 1993). From literature, till date, there is no detailed information on the use of different *Bacillus* species from vegetable proteins as starters in the production of L-glutamic acid. Previous studies have been generated towards gathering information in general on *Bacillus* species in enzymes production. Hence, these findings presented the detailed information about the different types of *Bacillus* species from vegetable proteins in their use as starter cultures.

The various isolated *Bacillus* strain were confirmed to be producers of L-glutamic acid through thin layer chromatographic assay on thin layer plates. The Rf value of this sample products was compared with the standard L-glutamic acid. The quantity produced differed from one organism to the other as well as their source of isolation. Ogbadu et al. (1990) also confirmed L-glutamic acid from some strains of *Bacillus* and quantities' produced varied between strains of the same species of *Bacillus* as well as their source of isolation. This result confirms the flavor enhancement of condiments.

Based on the yield of L-glutamic acid produced by the 22 strains of *Bacillus* isolated from vegetable proteins, selection was made. *B. subtilis,* UG1 from "Ugba" and *B. pumilus* DD4 from "Ogiri" produced L-glutamic acid that compared favourably with of the L-glutamic acid produced by the reference strain *C. glutamicum* ATCC 13032 (10.2 mg/ml). However, glutamic acid yields by the test isolates compared well with those of *Bacillus* species as quoted by previous workers (Chatopadhyay and Banerjee, 1978; Ogbadu et al., 1990, Madhavan and Ashok, 1996).

Among the carbon sources tested, glucose was best utilized for growth and L-glutamic acid production and no glutamate was produced in the presence of galacticol. Thus, the observation agrees with the works of Ko and Gross (1998), Yamada et al. (1978) and Richard and Ramanchandra (1973).

The ample supply of a suitable nitrogen source is essential for L-glutamic acid fermentation. Among the various nitrogen sources, ammonium nitrate and urea were found to be the most suitable and as paragine was least. However, absence of a suitable nitrogen source led to the inability of any of the strains to produce L-glutamic acid. The rapid assimilation of the nitrogen compound in the medium accumulates L-glutamic acid with a sudden fall in the pH value of the fermenting medium (Okada et al., 1961; Nakamishi et al., 1975; Hirose et al., 1985).

## Conclusions

This study has established that organisms which include *B. subtilis, B. licheniformis, B. polymyxa* and *B. pumilus* isolated from vegetable proteins are capable of L-glutamic acid.

The amount of L-glutamic acid produced by these isolates compared favourably with that produced by the reference strain *C. glutamicum* ATCC 13032. The use and establishment of these isolates as starter cultures for L-glutamic acid will thus, be of immense benefits to our food industries especially those that depend on the flavor enhancement of L-glutamic acid in their products. This will save these industries the huge foreign exchange which they currently spend on the importation of this item.

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