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Research Article

Antibacterial and antioxidant activity of Bacillus species isolated from fermented Parkia biglobosa (iru) and Ricinus communis (ogiri) -African traditionally fermented food condiments

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INTRODUCTION

Iru and Ogiri are among the two most popular indigenous traditionally fermented condiments produced from leguminous proteins (Omafuvbe et al., 2004). Fermented African locust bean (Parkia biglobosa) is called iru in Yoruba and dawadawa in Hausa respectively (Odunfa et al., 1998). Ogiri is obtained by fermenting melon seeds (Citrullus vulgaris), fluted pumpkin (Telferia occidentallis) and castor oil seeds (Ricinus communis) (Olumuyiwa et al., 2004) which is mostly consumed among the Igbos. These seeds are used in the production of ogiri-egusi, ogiri-ugu and ogiri-igbo/isi (Olumuyiwa et al., 2004; Achi et al., 2005) which can be used as protein supplements and as a functional ingredients in food preparation. Soetan et al. (2014) reported that traditional fermented foods contain high nutritive value, better digestibility, flavors, aroma and texture. The substrates used for the fermentation of these condiments harbour diverse microorganisms from the environment which helps to transform the chemical constituents of the raw materials into useful products. The advantages of fermenting the substrates include: enhance nutritive value of the products; enrich bland diets with improved flavor and texture; fortify food products with essential amino acids, health promoting bioactive compounds, vitamins and minerals; degrade undesirable compounds and anti-nutritional factors; impart antioxidant and antimicrobial properties; improve digestibility and stimulate probiotic functions (Ling et al., 2013). Fermentation also results in lower proportion of dry matter in the food condiments and increases the concentration of vitamins, minerals and protein when measured on dry weight basis (Savadogo et al., 2011).

The genus Bacillus are Gram-positive, rod-shaped bacteria, belonging to the phylum Firmicutes. Antimicrobial metabolites have been widely documented to be produced among the genera Streptomyces and Bacillus (Arasu et al., 2013). Bacillus species are good sources of bioactive compounds, notably antibiotics, therapeutic proteins, enzyme inhibitors and pharmacologically active agents (Huang et al., 2009). Lipopeptides such as surfactin, iturin and fengycin have been reported to be recovered from Bacillus species and characterized (Huang et al., 2009). However, most of these bioactive compounds are active against Gram-positive bacteria, some of them have a wide range of bio-activity against Gram-negative and filamentous fungi (Sirtori et al., 2008). In addition, they have also been used as bio-control agents due to their antagonistic and repressive activities against disease causing organisms such as fungi and bacteria (Youcef-Ali et al., 2014).

The search for natural biological agents that have antibacterial and antioxidant activities is a new research trend in the fields of biology, medicine and food sciences (Afify et al., 2012). Antioxidants play important roles in food as a health protecting and promoting constituent. Antioxidant reduce the risk of chronic diseases such as cancer and heart problems. Proteinous food condiments are natural sources of antioxidants such as vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens which have the ability to prevent the risk of infections and diseases (Tatiana et al., 2018). This research was therefore designed to investigate the antibacterial and antioxidant activity of Bacillus species isolated from spontaneously fermented food condiments.

MATERIALS AND METHODS

Sample collection

Thirty (30) samples of fermented food condiments comprising 10 samples of each of Iru woro, Iru pete and Ogiri were obtained from different local markets including Owode, Igbona, Oja-oba and Sasa in Osogbo, Osun State, Nigeria.

Preparation of samples

One (1g) of each sample was weighed and added into a test tube containing 9ml of Ringer solution. The mixture was shaken thoroughly on rotary shaker to obtain stock solution and 6 folds serial dilutions were made. Exactly 0.1ml of 10-3 and 10-5 of each diluted samples were introduced into sterile plates and

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molten nutrient agar was poured and allowed to solidify and the plates were incubated for 24 hours at 37°C.

Distinct colonies obtained after incubation were re-streaked on nutrient agar plates and incubated for 24 hours at 37°C to obtain pure cultures. Pure cultures of the different organisms were sub-cultured and preserved on agar slants and refrigerated at 4°C.

CHARACTERIZATION AND IDENTIFICATION OF ISOLATES

Morphological characterization

The cultural characteristics of the pure isolates on the agar plates were observed by checking the appearance, Gram staining and spore staining reactions.

Biochemical characterization

The biochemical characteristics of the pure isolates were observed using citrate, indole, catalase, coagulase, oxidase and sugar fermentation tests (lactose, mannitol, sucrose, maltose and glucose).

Antibacterial activity

The antibacterial activity of the pure isolates against pathogenic bacteria including Staphylococcus aureus (NCTC 6571) and E. coli (ATCC 25922) obtained from Obafemi Awolowo University, Ile-Ife and Corynebacterium diphtheria (ATCC 13813), Proteus mirabilis (ATCC 7002) and Klebsiella pneumonia (ATCC 43816) obtained from Nigeria Institute of Medical Research (NIMR), Lagos were investigated by using agar-well diffusion method. Standardization of the broth cultures was done according to the method of Cheesbrough, (2000) by diluting l ml of broth culture to 5ml of nutrient broth and visually comparing the turbidity to that of 0.5 Macfarland turbidity standards after incubating at 37 for 3-5 hours. Nutrient agar was poured into sterilized Petri-dishes and allowed to solidify for 30 minutes. The test organism was inoculated onto the sterile plates by seeding with sterile swab sticks. Wells of 9mm diameter were aseptically bored using sterile cork borer . On each agar plate, 0.3ml of the concentration was added to the wells and gentamycin was used as a positive control. The plates were then incubated at 37°C for 24hours. Effect of the isolates metabolites was assessed by measuring of zones of inhibition (mm) and then compared with the standard gentamycin (CLSI, 2018).

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

MIC

MIC of the pure isolates was determined using the broth dilution method. Test tubes labeled 1-5 were used for each isolate. Each of the test tubes contained 5ml of nutrient broth and 5ml of the appropriate concentration of each isolate was introduced into tube one and mixed thoroughly. 5ml of the content of tube 1 was introduced into tube 2 and the procedure was repeated for the remaining test tubes except tube 5 (control). To each of the test tubes, 0.l ml of broth cultures of the test organism was added. All the tubes were incubated at 37°C for 18-24 hours, after which they were examined for bacterial growth (Doughari, 2006).

MBC

MBC was determined by selecting the tubes that showed no growth during the MIC determination. One loopful from each of these tubes was sub-cultured onto the surface of culture free nutrient agar plates and incubated for 24hrs at 37°C. The lowest concentration at which no growth was observed on the agar plate was recorded as the MBC.

ANTIOXIDANT ACTIVITY

2,2- diphenyl- 1- picrylhydrazyl (DPPH) Radical Scavenging Activity Assay

The scavenging effect of selected pure isolates on the free radical 2,2- diphenyl- 1- picrylhydrazyl (DPPH) was measured in accordance with the slightly modified method of Lin and Chang, (2000). A sample (Nutrient broth, 1 mL) and a freshly prepared 2,2- diphenyl- 1- picrylhydrazyl (DPPH) solution (0.2mM, 1m) were mixed. The mixture was vigorously shaken and left to react for 30 minutes in the dark at room temperature, The control sample contained deionized water instead of the sample solution. The scavenged DPPH was then monitored by determining the absorbance at 517 nm using Visible Spectrophotometer (BOSCH 712G). The radical scavenging activity was quantified as units/ml (U/ml) by using the expression

$$\text{DPPH Scavenged (\%)} = [(\text{A control} - \text{A test}) / \text{A control}] \times 100$$

Where A control is the absorbance of the control reaction and A test is the absorbance in the presence of the sample of the extracts.

Reducing power assay

The reducing activity was determined as described by Lin and Yen (1999) with slight modification. A sample (Nutrient broth, 0.5 ml) was mixed with potassium ferricyanide (1%, 0.5 ml) and Phosphate-buffered saline (pH 6.6, 0.5 ml). Subsequently, the mixture was heated at 50ºC for 20 min and allowed to cool. Upon cooling, 0.5 ml of 10% trichloroacetic acid (TCA) was added to the mixture and then centrifuged at 3000g for 5 min. The upper layer (1 ml) was mixed with ferric chloride (0.1%, 1 mL) and allowed to react for 10 min. The absorbance of the mixture was obtained at 700 nm by Visible Spectrophotometer (BOSCH 712G).

Thin layer chromatography (TLC) analysis of fermented Parkia biglobosa (iru) and Ricinus communis (ogiri)

Each of the samples were dissolved in 2ml of distilled methanol to allow the extraction of the Bacillus species metabolites in the sample. 2mg of the samples were weighed in a dish and 2ml of distilled methanol were added. The sample solutions were mixed thoroughly until a homogenous mixture was obtained. TLC is a procedure that is used to separate compounds by their rate of movement therough a thin layer of silica gel coated on a glass plate. Developing tank: 3ml of distilled N: Hexane, 6ml of distilled ethyl acetate, 9ml of distilled methanol were used based on capillary action of the samples on plate (ratio: 1:2:3) observed under the UV- visible light (Meena et al., 2014).

RESULTS

Identification of bacterial isolates

Table 1 shows the Gram's reaction and biochemical characteristics of bacteria isolated from ogiri. Two Bacillus species were isolated and identified as : B. subtilis, B. mycoides. Other bacteria that were isolated and identified include: Enterococcus sp, Listeria sp and Micrococcus sp.. Only the Bacillus species were used for further studies.

Table 1: Gram's reaction and biochemical characteristics of bacterial isolates from ogiri

Isolates code	Catalase	Oxidase	Spore	Indole	Coagulase	Citrate	Lactose	Mannitol	Sucrose	Maltose	Glucose	Motility	Gram's reaction	Propable
OG A3	+	-	+	-	-	+	+	+	+	-	+	+	+	B.mycoides
OG A5	+	-	-	-	-	+	+	+	-	+	+	+	+	Enterococcus sp
OG B3	+	-	+	-	-	+	+	+	+	+	+	+	+	B.subtilis
OG B5	+	-	-	-	-	-	+	+	-	+	+	+	+	Listeria sp
OG C3	+	-	+	-	-	+	+	+	+	+	+	+	+	B. subtilis
OG C5	+	-	+	-	-	+	+	+	+	+	+	+	+	B. subtilis
OG D3	+	-	+	-	-	+	+	+	+	+	+	+	+	B. subtilis
OG D5	+	-	-	-	-	+	+	+	-	+	+	-	+	Micrococcus sp
OG E3	+	+	-	-	-	+	+	-	-	-	+	+	+	Listeria sp
OG E5	+	-	+	-	-	+	+	+	+	+	+	+	+	B. subtilis
OG F3	+	-	-	-	-	-	-	-	-	-	+	-	+	Micrococcus sp
OG F5	+	-	-	-	-	-	-	+	+	-	+	-	+	Enterococcus sp

KEY: += Growth; -= No growth

The Gram's reaction and biochemical characteristics of bacteria isolated from iru pete is presented in Table 2. Three Bacillus species were isolated and identified as B. laterosporus, B. licheniformis and B. subtilis. Other bacteria that were isolated and identified include: Enterococcus sp, Listeria sp and Micrococcus sp. Only the Bacillus species were used for further studies.

Table 3 shows the Gram's reaction and biochemical characteristics of bacteria isolated from iru pete. Three Bacillus species were isolated and identified as: B. subtilis, B.

mycoides and B. licheniformis. Other bacteria that were isolated and identified are: Corynebacterium sp and Listeria sp.

Table 2: Gram's reaction and biochemical characteristics of bacterial isolates from iru pete

Isolates code	Catalase	Oxidase	Spore	Indole	Coagulase	Citrate	Lactose	Mannitol	Sucrose	Maltose	Glucose	Motility	Gram's reaction	Probable Organism
IP A3	+	-	+	-	-	+	-	+	+	+	+	+	+	*B. subtilis*
IP A5	+	-	-	-	-	+	+	+	-	+	+	-	+	Micrococcus sp
IP B3	+	+	-	-	-	+	+	-	-	-	+	+	+	Listeria sp
IP B5	+	-	+	-	-	+	-	+	+	+	+	+	+	B. licheniformis
IP C3	+	-	-	+	-	+	-	+	+	+	+	+	+	B. licheniformis
IP C5	+	+	-	+	-	+	-	+	+	-	+	+	+	Enterococcus sp
IP D3	+	+	-	-	-	+	+	-	-	-	+	+	+	Listeria sp
IP D5	+	+	-	-	-	+	-	+	+	+	+	+	+	B. licheniformis
IP I3	+	-	+	-	-	+	+	+	+	-	+	+	+	B. laterosporus
IP E5	+	+	-	-	-	+	+	-	-	-	+	+	+	Listeria sp
IP H3	+	-	+	-	-	+	+	+	+	+	+	+	+	B. subtilis
IP F5	+	-	-	+	-	+	-	+	+	+	+	+	+	B. licheniformis
IP G3	+	+	-	-	-	+	-	-	-	-	+	-	+	Corynebacterium sp

Table 3: Gram's reaction and biochemical characteristics of bacterial isolates from iru woro

Isolates code	Catalase	Oxidase	Spore	Indole	Coagulase	Citrate	Lactose	Mannitol	Sucrose	Maltose	Glucose	Motility	Gram's reaction	Probable Organism
IW A3	+	-	+	-	-	+	+	+	+	+	+	+	+	*B. subtilis*
IW A5	+	+	+	-	-	+	-	+	+	+	+	+	+	*B. licheniformis*
IW B3	+	+	-	-	-	+	+	-	-	-	+	+	+	Listeria sp
IW B5	+	-	+	-	-	+	+	+	+	-	+	+	+	*B. mycoides*

IW C3	+	+	-	-	-	+	-	-	-	-	+	-	+	*Coryneb acterium sp*
IW C5	+	+	+	-	-	+	+	+	+	-	+	+	+	*B. mycoides*
IW D3	+	+	-	-	-	+	+	-	-	-	+	+	+	Listeria sp
IW D5	+	+	-	-	-	+	-	-	-	-	+	-	+	*Coryneb acterium sp*
IW E3	+	+	-	-	-	+	-	-	-	-	+	-	+	*Coryneb acterium sp*
IW E5	+	+	-	-	-	+	+	-	-	-	+	+	+	Listeria sp
IW F3	+	-	+	-	-	+	-	+	+	+	+	+	+	B. lichenif ormis
IW F5	+	-	+	-	-	+	-	+	+	+	+	+	+	B. lichenif ormis
IW G3	+	+	-	-	-	+	-	-	-	-	+	-	+	Coryne bacteriu m sp

Figure 1 shows the percentage susceptibility pattern of the isolated Bacillus species metabolites against test organisms. P.mirabilis was 43% resistant and 57% susceptible, .E. coli and C. diphtheria were 86% resistant and 14% susceptible, while S. aureus and K. pneumoniae were 100% resistant and not susceptible to the isolated Bacillus species.

The zones of inhibition produced by Bacillus species metabolites against test organisms during agar well diffusion method is presented in Table 4. It was observed that OG B3 (B. subtilis) had the highest zone of inhibition of 23 mm against P. mirabilis and IP I3 (B. laterosporus) had the least zone of inhibition (13 mm) against E. coli.

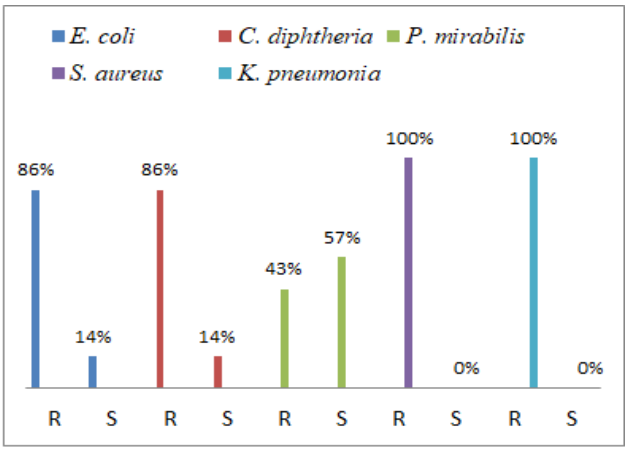

Figure 1: Percentage susceptibility pattern of Bacillus species metabolites against test organisms

Test Organism	OG A3	IW A3	IW B5	IP I3	OG B3	IP B5	IP H3	GT(30µg)
E. coli	0	16 mm	0	13 mm	0	14 mm	0	19 mm
C. diphtheriae	0	0	0	20 mm	17 mm	0	0	22 mm
P. mirabilis	0	17 mm	16 mm	20 mm	23 mm	18 mm	19 mm	18 mm
S. aureus	0	0	0	0	0	0	0	20 mm
K. pneumoniae	0	0	0	0	0	0	0	17 mm

Table 4: Zones of inhibition produced by Bacillus species metabolites against test organisms during the agar well diffusion method

KEY: GT: Gentamycin, OG: Ogiri, IW: Iru woro, IP: Iru pete

Table 5 shows the minimum inhibitory concentration at which the Bacillus species isolated from iru woro, iru pete and ogiri inhibited the growth of test organisms.

IW A3, IW B5 and IP I3 showed the MIC at which they inhibited the test organisms. OG A3 did not show any zone of inhibition.

The minimum bactericidal concentration at which the Bacillus species metabolites isolated from iru woro, iru pete and ogiri produced substances that killed test organisms is presented in Table 6.

It was observed that IW A3 showed the MBC at which it killed the test organisms but OG A3, IP I3 and IW B5 did not produce any metabolite that could kill the test organisms.

Test organisms	**OG A3**				**IW A3**	**IW B5**	**IP I3 (10-1)**									
(mg/ml)	35	25	15	10	37	30	25	15	36	24	16	10	38	25	18	11
E. coli	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+
C. diphtheriae	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+
P. mirabilis	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+
S. aureus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
K. Pneumoniae	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table 5: Minimum inhibitory concentration of Bacillus species against test organisms at 530 nm

Test organisms	**OG A3**				**IW A3**	**IW B5**	**IP I3 (10-1)**									
(mg/ml)	37	30	25	15	35	25	20	15	36	24	16	10	38	25	18	11
E. coli	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+
C. diphtheriae	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
P. mirabilis	-	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+
S. aureus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
K. Pneumoniae	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table 6: Minimum bactericidal concentration of Bacillus species metabolites against test organisms at 530nm

Table 7 shows the radical scavenging activity using 2,2- diphenyl-1- picrylhydrazyl (DPPH) at which OG B3 present 65.80% and IP B5 present 27.52% antioxidant properties that acted against

the activity of free radicals. OG B3 produced a higher antioxidant than IP B5.

Sample	Absorbance 1	Absorbance 2	Absorbance 3	Mean	DPPH Scavenged %
OG B3	0.25	0.252	0.251	0.251	65.8
IP B5	0.53	0.533	0.535	0.532	27.52
Control	0.73	0.74	0.731	0.734	0

The reducing power (i.e., the rate at which the Bacillus spp metabolites acted against the activity of free radicals) with OG B3 having a higher reducing power than other Bacillus species is presented in Table 8

Sample	Absorbance 1	Absorbance 2	Absorbance 3	Mean
OG B3	0.68	0.689	0.679	0.683
IP B5	0.313	0.299	0.314	0.309
Control	0.113	0.114	0.112	0.113

Table 8: Reading for reducing power at 700 nm from the visible spectrophotometer

Plate 1 shows the TLC of extracted lipopeptide by Bacillus subtilis OG B3 which produced a blue violet colour, indicating the presence of iturin as compared to the standard obtained from literature (Meena et al., 2014). Iturin is a cyclic antibiotic that can be used as a biocontrol and antifungal agent.

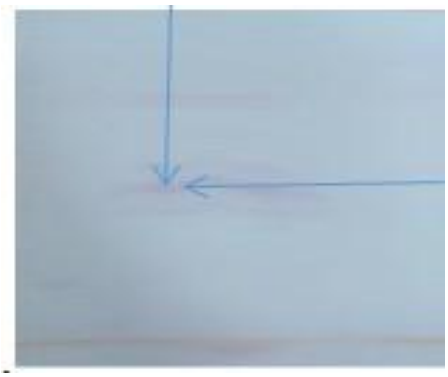

Plate 1: Thin layer chromatography sheet which indicates the presence of iturin

DISCUSSION

The result of this study provides information on the different types of bacteria, with emphasis on Bacillus species isolated from locally fermented condiments, antibacterial activity of the Bacillus species against test pathogens implicated in nosocomial infections and susceptibility pattern of the Bacillus species metabolites against the test organisms. Minimum inhibitory concentration and minimum bactericidal concentration was also determined. Antioxidant activity of selected Bacillus strains and the identification of B. subtilis metabolite using the thin layer chromatography method was also carried out. The different types of microorganisms isolated from ogiri includes: B. mycoides, Enterococcus sp, B. subtilis, Micrococcus sp and Listeria sp; iru pete: Enterococcus sp, B.licheniformis, Listeria sp and B. laterosporus; iru pete: B. licheniformis, B. mycoides, Listeria sp and Corynebacterium sp. Several researcher have reported that Bacillus species are the most predominant organisms involved in the fermentation of condiments (Achi, 2005; Sanni et al., 2002; Afify et al., 2012; Soetan et al., 2014; Tatiana et al., 2018). However, other species of bacteria isolated from the condiment samples might due to the contamination of the utensils and water used for the fermentation process. Several authors have confirmed that B. subtilis is the most predominant Bacillus strain isolated from traditionally fermented condiments (Oguntoyinbo et al., 2010; Dajanta et al., 2011; Lawal et al., 2011 and Adebayo et al., 2018). However, the results obtained from this work is in agrees with previous reports as B. mycoides and B. subtilis was more prevalent in ogiri, B. subtilis and B. licheniformis in iru pete while B. subtilis, B. mycoides and B. licheniformis was more predominant in iru woro. However, B. subtilis was the most predominant of all the Bacillus species isolated

The antibacterial susceptibility pattern of the isolated Bacillus species against test pathogens; Escherichia coli, Corynebacterium diphtheria, Proteus mirabilis, Staphylococcus aureus, Klebsiella pneumoniae was determined using the agar-well diffusion method. E.coli was susceptible to Bacillus isolates: IWA3, IPI3 and IPB5 showing following zones of inhibition 16mm, 13mm and 14mm respectively. C. diphtheriae was susceptible to IPI3 and OGB3 with the corresponding zones of inhibition: 20mm and 17mm respectively. S. aureus and K.pneumoniae were resistant to all the Bacillus isolates. However, P. mirabilis was susceptible to all the selected Bacillus isolates: IWA3, IWB5, IPI3, OGB3, IPB5 and IPH3 having the corresponding zones of inhibition: 17mm, 16mm, 20mm, 23mm, 18mm and 19mm respectively. The highest antibacterial activity was demonstrated against P. mirabilis whose control (gentamycin) showed 18mm zone of inhibition. This suggests that OGB3 is a potential antimicrobial agent which

comparatively has better potency than gentamycin. Kumar et al. (2015) had earlier reported the ability of many Bacillus strains to produce highly potent antibiotics. This is conformity with the results obtained from this work as the Bacillus species demonstrated high antibacterial activity against nosocomial implicated pathogens. Kuta, (2008) reported 5mm and 6mm Bacillus species zones of inhibition against S. aureus and K. pneumoniae respectively. Kumar et al. (2015) also documented 20mm zones of inhibition against S. aureus and K. pneumoniae. However, no antibacterial activity was recorded for S. aureus and K.pneumoniae as the two organisms were resistant to all the Bacillus species. The results of the minimum inhibitory concentration showed that IWA3 and IPI3 exhibited MIC of 3.7mg/ml and 3.8mg/ml against E.coli respectively; IPI3 showed MIC of 2.5mg/ml and 3.8mg/ml against C. diphtheriae; IWA3 and IWB5 showed 2.5mg/ml and 3.6mg/ml against P.mirabilis respectively. However, no MIC record was recorded for S.aureus and K. pneumoniae. In addition, only IWA3 showed MBC of 3.5mg/ml and 3.7mg/ml against E. coli and P.mirabilis respectively. Shine et al. (2015) had earlier documented similar results.

Antioxidants are substances such as thiols and ascorbic acid (Vitamin C) produced by microorganisms such as B. subtilis, B. pumilus and B. licheniformis that can prevent or slow down chemical reactions that may damage cells caused by free radicals such as: nitric oxide (O2), nitrous oxide and peroxnitrite. These antioxidants can also be used in food preservation to increase shelf life. DPPH free radical scavenging is one of the mostly used methods in antioxidative assay. This method was employed to determine the antioxidant activity of OGB3 and IPB5 crude extracts by measuring the change in absorbance at 517 nm. OGB3 (B. subtilis isolated from ogiri) had higher antioxidative activity (65.80%) than IPB5 (B. licheniformis isolated from iru pete) (27.52%). This results is in line with the the report of several authors that have documented similar results (Lin and Yen, 1999; Katekan et al., 2011; Afify et al., 2012; Kumar et al., 2015). Katekan et al. (2011) recorded antioxidant activities of 65.65% and 62.12% from naturally fermented Thua Nao (a Thailand fermented soybean condiment). However, other bacteria such as Lactobacillus rhamnosus, Lactobacillus retueria (ATCC 20016) and Bifidobacterium breve (ATCC 15700) have been documented by Afify et al. (2012) to demonstrate very high antioxidant activity with the corresponding values: 91.71%, 96.74% and 86.99% respectively. Shine et al. (2015) also reported an antioxidant activity of 67.33% from B. amyloliquifaciens isolated from a silage. Members of the genus Bacillus have been reported to produce lipopeptides that demonstrates antagonistic effect against food borne pathogens, pathogens implicated in nosocomial infections as well as plant pathogens. Different lipopeptides such as iturin, fengycin and surfactin have been produced by B. subtilis, B. amyloliquifaciens, B. pumilus and other members of the Bacillus species. The thin layer chromatography method was employed in this work to extract a lipopeptide from OGB3 (B. subtilis isolated from ogiri). After extraction, the lipopeptide was identified as iturin. Baruzzi et al. (2011) had earlier reported that iturin produced by B. subtilis possesses strong anti-fungal activity against a wide range of plant fungal pathogens such as Fusarium graminearum, Rhizoctonia solani, Aspergillus flavus and post harvest pathogens such as Botritis cinerea and Penicillium expansum. Pyoung et al. (2010) and Youcef-Ali et al. (2014) also documented the production of iturin and other lipopeptides such as pumilin, lichenysins and subtilosin from B. subtilis, B. mojavensis and B. pumilus which exhibited strong anti-fungal, anti-parasitic and antibacterial activity against pathogenic microorganisms.

CONCLUSION

From the results obtained from this work, it can be concluded that B. subtilis (OGB3) can produce a potential antimicrobial agent that can be used against microorganisms implicated in nosocomial infections. However, it can also be used as a stater culture for the production of condiments. In addition, the lipopeptides produced can be employed as an anti-fungal agent in plants and also as a biopreservative in foods.

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