



Research Article

# Antimicrobial and Antioxidant Activities of Novel Marine Bacteria (*Bacillus* 2011SOCCUF3) Isolated from Marine Sponge (*Spongia officinalis*)

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## Abstract

**Background:** *Bacillus* species represent a rich source of new bioactive metabolites that can combat diseases.

**Methods:** *Bacillus* strain was isolated from the marine sponge *Spongia officinalis* and routinely maintained on marine broth. The bacteria strain was identified as *Bacillus* 2011SOCCUF3 using 16S rDNA sequencing. The strain was cultured on Tryptone Casein Oat Soluble Starch (TCOATSS) media with continuous agitation for 4 days. The fermented broth was centrifuged, and the supernatant was mixed with 10% (w/v) of adsorbent resin (XAD-7HP and XAD-16N, 1:1) and shaken continuously at a reduced speed for 7 h; and the resin was collected by filtration through sintered glass funnel and washed with MilliQ water, and then eluted with methanol to obtain the extract. The extract was evaporated *in vacuo* at reduced temperature and pressure to obtain the dry extract. The dry extract was purified by vacuum liquid chromatography, eluting with methanol in acetone gradient. The *in vitro* antimicrobial and antioxidant activities were investigated using the agar-well diffusion, DPPH scavenging and the phosphomolybdate methods respectively.

**Results:** The extract and fractions showed good antimicrobial activities with minimum inhibitory concentration range of <1.0 mg/mL. The extract and fractions also exhibited good antioxidant activities with their IC<sub>50</sub> values been comparable to the standard.

**Conclusion:** Thus, a novel *Bacillus* strain isolated from the marine sponge (*Spongia officinalis*) obtained from Cortiou and Riou, France, exhibited promising antimicrobial and antioxidant activities.

## Introduction

Natural products play very crucial roles in drug discovery and development process and have been, for a long time, of immense importance because they represent a remarkable source of 'lead' compounds for new drug discovery.<sup>1</sup> Many synthetic drugs currently in use were derived from plants and animals.<sup>2</sup> A large percentage of therapeutic agents in use now, which are presented as antibiotics, anticancer, agents for immunosuppressive disorders, were developed from natural products obtained from plants, animals and microorganisms.<sup>3</sup> Several research reports have shown the extent to which natural products play a leading curative role in human medicine.<sup>4</sup> Humans, however, are still faced with life-threatening diseases such as the infectious diseases that are associated with antimicrobial resistance.<sup>5</sup> This and other problems, which culminate in disease treatment failure as well as unwanted reactions associated with existing medications, have clearly shown the

imperativeness of continuing research in order to develop new and better drugs.

Many natural product-derived therapeutic agents used presently are of terrestrial origin.<sup>6</sup> But the marine world remains largely unexplored even though it is considered as largely hosting biodiversity.<sup>7</sup> Marine bacteria have produced interesting biologically active compounds that can pave way for novel drug discovery as experiments carried out on both familiar and novel bacteria of marine origin provide evidences.<sup>8</sup> Some of the challenges that appear to have clogged the process of marine natural product drug discovery include supply problems and target identification. However, with improvement and innovation in the field of marine natural products, there is high expectation that they will lead to a new breakthrough.<sup>9</sup> Over the past two decades, there has been an increased growth rate in the chemistry of marine organisms, which

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has led to the discovery of new structures, many of which had not been observed among the compounds of terrestrial origin. More so, these new substances possess pharmacological properties that were hitherto unknown.<sup>10</sup> Marine organisms adapt to harsh marine environment conditions such as high or low temperatures, alkaline or acidic water, high pressure and limited substrate in the deep sea, and, thus, possess unique properties. These qualities have drawn the attention of researchers who are exploring marine microorganisms since there is the potential of their use in biotechnology.<sup>11</sup> *Bacillus* species known to produce antibiotics include *Bacillus marinus*, *Bacillus silvestris*, *Bacillus licheniformis*, *Bacillus amyloliquefaciens*, *Bacillus pumilus*, *Bacillus cereus* and *Bacillus subtilis*.<sup>12</sup> In recent years, marine *Bacilli* have become important in producing natural products that have shown a wide range of biological activities, including antibacterial, antioxidant and anticancer activities.<sup>13</sup> The present study was done to evaluate the *in vitro* antimicrobial and antioxidant activities of novel marine *Bacillus* 2011SOCCUF3 strain isolate and its purified fractions.

## Materials and Methods

### Chemicals

Methanol and acetone used were purchased from Sigma Aldrich, Germany. Tryptone, yeast extract and agar were purchased from Formedium LTD, England. Others include Casein Digest (Difco™) (Becton, Dickinson and Company, USA), starch soluble (Acros Organics, New Jersey, USA), Instant Ocean (Aquarium Systems, Sarrebourg, France), Amberlite® XAD 7HP (20 – 60 mesh) (Sigma Aldrich, USA), Amberlite® XAD 16N (20 – 60 mesh) (Sigma Aldrich, USA), Diaion HP20 ( $\geq 250 \mu\text{m}$  beads) (Alfa Aesar, England) and MilliQ water (Millipore Corporation, Bedford, MA, USA). DPPH solution and phosphomolybdate solution were prepared according standard procedures.

### Test microorganisms

The microorganisms used were clinical isolates maintained in the Department of Microbiology, University of Nigeria, Nsukka laboratory. They include gram-negative bacteria: *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*; gram-positive bacterium *Staphylococcus aureus* and fungi *Aspergillus niger* and *Candida albicans*.

### Experimental procedures

#### Isolation and identification of bacteria

The 2011SOCCUF3 strain was isolated from the marine sponge *Spongia officinalis* obtained in a scuba diving expedition at Cortiou and Riou, France by the Goss Research Group, School of Chemistry, University of Saint Andrews, UK. The 2011SOCCUF3 strain was determined to be most closely related to *Bacillus horneckiae* using 16S sequencing.

#### Cultivation of bacteria strain

The *Bacillus* 2011SOCCUF3 strain was cultured in 14

x 0.5 L volumes of Tryptone Casein Oat Soluble Starch (TCOATSS) media (4 g of tryptone, 4 g of casein, 10 g soluble starch, 10 g oats, 1000  $\mu\text{L}$  trace element in 1 L of MilliQ water) while shaking at 180 rpm for 4 days.

### Extraction procedure

At the end of the fermentation period, the fermented broth was centrifuged (8000 rpm, 1 h, 4°C) and the supernatant was mixed with 10% (*w/v*) of adsorbent resin (XAD-7HP and XAD-16N, 1:1) and shaken continuously at a reduced speed for 7 h. The resin was then collected by filtration through sintered glass funnel and washed with MilliQ water (5 L), and then eluted with methanol (3.0 L).<sup>14</sup> The extract was evaporated *in vacuo* at reduced temperature and pressure to obtain the dry extract (9.320 g).

### Purification of extract

The extract was purified using Vacuum Liquid Chromatography (VLC). Also, 9.0 g of the extract was triturated with 10.0 g of silica gel and loaded onto a glass column (150 x 1.5 cm, ID), and then eluted with methanol in acetone gradient (25 – 100%, 1 L each) to obtain the VLC fractions (BF1 – BF4).

### Antimicrobial assay

The Agar-well diffusion assay method described by Adeshina *et al.*<sup>15</sup> was used for the antimicrobial assay. The bacteria and fungi were first streaked onto nutrient agar and incubated overnight at 37°C and 27°C respectively. The single colony formed was then used to inoculate 10 mL of the nutrient agar and the culture was incubated in a Faster BH-EN 2004 Laminar flow cabinet overnight. After the incubation, 10% of the inoculum volume was used to inoculate 0.5% Muller-Hinton agar which had been cooled down to 40°C and then poured into an agar plate with cork-borer (6 mm diameter) placed on the agar. Different concentrations (2.5, 5, 10, 20 mg/mL in DMSO) of the samples were then loaded onto each disc in 10  $\mu\text{L}$  volumes; ciprofloxacin and fluconazole were used as a positive control and the negative control was DMSO solution. These plates were then incubated overnight at 37°C and 27°C respectively and the inhibition zone diameter (IZD) measured after 24 h and 48 h for bacteria and fungi respectively. The Minimum Inhibitory Concentrations (MICs) of the samples were determined from the plot of logarithmic of the sample concentrations to base 10 against the square of the corresponding IZDs.

### Antioxidant assay

The *in vitro* antioxidant assay of the samples was done using the DPPH radical scavenging activity and the Phosphomolybdate methods.

### DPPH radical scavenging activity

The *in vitro* antioxidant assay was done using the method as described by Agbo *et al.*<sup>16</sup> with slight modification. The samples were dissolved in methanol to give a concentration

of 1 mg/1000  $\mu$ L stock solution. Serial dilution (10 – 50 mg/mL) of the stock solution was obtained. 10  $\mu$ L of these solutions were added to 490  $\mu$ L DPPH solution (4.5 mg/100 mL) in an Eppendorf vial. The mixture was incubated for 10 min in the dark and observed for any colour change. The reduction of DPPH radical was quantified by measuring the absorbance at 517 nm using UV-Vis spectrophotometer (Perkin Elmer, Lambda 25). The absorbance of the DPPH blank solution and the positive control (ascorbic acid) were also determined. The percentage of DPPH radical scavenging activity was calculated using the formula below:

$$\% \text{DPPH Radical Scavenging activity} = \frac{A_c - A_s}{A_c} \times 100 \quad \text{Eq. (1)}$$

where  $A_c$  = absorbance of control;  $A_s$  = absorbance of sample. The  $IC_{50}$  of the test samples and positive control were thereafter determined for comparison.

#### Phosphomolybdate assay

The total antioxidant capacities (TAC) of the samples were determined by the phosphomolybdate method as described by Agbo *et al.*<sup>17</sup> with slight modification. Then 0.1 mL of various concentrations of the samples (10 - 100 mg/mL) were mixed with 1 mL of reagent solution (600 mm tetraoxosulphate (VI) acid, 28 mm sodium phosphate and 4 mm ammonium molybdate 1:1:1) in an Eppendorf vial. The vials were incubated in a water bath at 95°C for 1.5 h and cooled down to room temperature. The absorbencies of the mixture were determined at 765 nm against a blank containing 1 mL of the reagent solution and ascorbic acid as positive control. The total antioxidant capacity was estimated using the formula for the DPPH assay as previously described and the  $IC_{50}$  were values determined from the regression curve.

#### Statistical analysis

Descriptive statistics was used for the analysis of the results. All the experiments were done in triplicate and the results expressed as means  $\pm$  standard error of the mean. The 50% inhibitory concentration ( $IC_{50}$ ) values of the extract and VLC fractions were calculated using MS Excel.

## Results

### Isolation and identification of the *Bacillus* strain

2011SOCCUF3 strain was isolated from the marine sponge (*Spongia officinalis*) obtained from Cortiou and Riou, France. The isolate was purified by streak method on marine broth and preserved in a refrigerator maintained at - 80°C. The 16S genome sequencing of the isolate showed that it is closely related to *Bacillus horneckiae*.

### Cultivation and fermentation of the strain

2011SOCCUF3 strain was cultured on TCOATSS media with continuous agitating in a Faster BH-EN 2004 Laminar flow cabinet at 180 rpm for 4 days to allow for the fermentation of the *Bacillus* strain.

### Extraction and purification procedure

The fermented broths was adsorbed unto XAD resins (XAD-7HP and XAD-16N, 1:1) and shaken for 7 h and the resin collected by filtration through sintered glass funnel and washed thoroughly with enough MilliQ water and eluted with methanol to obtain the extract. The methanol was evaporated to obtain the dry extract which was purified using vacuum liquid chromatography eluting with a gradient of methanol in acetone.

### Antimicrobial activity

Agar-well diffusion method was used to evaluate the *in vitro* antimicrobial activity of the samples (Table 1). The extract and VLC fractions had strong antimicrobial activity against the tested organisms with MICs values lower than that of the standards used. The extract (0.108 mg/mL) at the concentration range of 2.5 – 20 mg/mL had highest MIC value against *C. albicans* which was approximately 15 times more than that of fluconazole (1.60 mg/mL) at same concentration. Also, BF2 showed strong antibacterial activity against *S. typhi* with MIC value of 0.083 mg/mL compared to the standard (ciprofloxacin = 2.38 mg/mL).

### Antioxidant activity

The *in vitro* antioxidant activity of the *Bacillus* extract and VLC fractions were assessed using the DPPH radical scavenging activity and the phosphomolybdate methods. The results of the DPPH assay showed a dose dependent scavenging activity with the extract having percentage inhibition of 38.89 – 49.14% (10 – 50 mg/mL) as compared to the standard (ascorbic acid) with inhibition percentage

**Table 1.** Minimum Inhibitory Concentrations of the Extract and Fractions.

Samples	Minimum Inhibitory Concentrations (MICs) (mg/mL)					
	<i>P. aeruginosa</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>S. typhi</i>	<i>C. albicans</i>	<i>A. niger</i>
BM	0.162	0.750	0.247	0.247	0.108	0.610
BF 1	0.163	0.881	0.785	0.908	0.628	2.505
BF 2	1.378	1.114	2.182	0.083	0.907	0.801
BF 3	0.674	1.009	0.610	0.907	0.907	0.750
BF 4	1.591	1.124	1.389	1.167	1.225	0.709
CPF	2.50	1.42	1.62	2.83	ND	ND
FCZ	ND	ND	ND	ND	1.60	1.25

BM = Extract, BF 1 – BF 4 = VLC fractions, CPF = ciprofloxacin, FCZ = fluconazole, ND = Not tested.

**Table 2.** Percentage Radical Scavenging Activity.

Samples	% Inhibition of Sample at different Concentrations (mg/mL)				
	10	20	30	40	50
BM	38.89±0.14	40.74±0.18	41.49±0.23	43.04±0.26	49.14±0.09
BF 1	20.56±0.07	21.45±0.03	22.45±0.07	23.71±0.12	25.64±0.23
BF 2	49.59±0.29	52.07±0.40	53.86±0.24	54.70±0.33	58.85±0.23
BF 3	21.63±0.08	22.24±0.10	23.01±0.20	23.61±0.04	25.37±0.08
BF 4	19.31±0.28	21.57±0.01	26.68±0.13	29.37±0.03	35.06±0.04
Ascorbic Acid	50.99±0.11	63.58±0.09	68.62±0.06	70.66±0.03	71.00±0.10

Results expressed as Mean ± SEM; n = 3, BM = Extract, BF 1 – BF 4 = VLC fractions.

**Table 3.** Percent Inhibition of the samples using Phosphomolybdate Assay.

% Inhibition of Sample	Concentration of Sample (mg/mL)				
	10	20	30	40	50
BM	5.53±0.01	12.14±0.01	16.83±0.00	22.42±0.02	28.73±0.01
BF 1	2.27±0.01	5.66±0.02	10.89±0.01	15.42±0.01	20.64±0.01
BF 2	17.45±0.02	21.96±0.01	28.04±0.03	33.44±0.05	37.77±0.01
BF 3	2.22±0.01	6.65±0.00	11.95±0.02	15.20±0.01	20.34±0.01
BF 4	4.56±0.04	9.46±0.01	14.19±0.02	18.94±0.01	24.45±0.03
Ascorbic Acid	15.43±0.02	24.68±0.02	31.64±0.01	37.59±0.02	45.49±0.03

Results expressed as Mean ± SEM; n = 3, BM = Extract, BF 1 – BF 4 = VLC fractions.

of 50.98 – 71.07% (Table 2). The VLC fractions had low radical scavenging activity when compared to the standard except BF2 with percentage inhibition of 49.60 – 58.85% (10 – 50 mg/mL). The total antioxidant capacity (TAC) of the extract and VLC fractions ranged from 5.53 – 37.78% (10 – 50 mg/mL) compared to the standard which showed 15.43 – 45.49% under same concentration (Table 3). It was observed that the TAC increased slowly with concentration even at the highest concentration of 50 mg/mL.

## Discussion

In this present study, we report the antimicrobial and antioxidant properties of a novel *Bacillus* strain. *Bacillus subtilis* is known to produce bioactive peptidolipid antibiotics like the Iturin group.<sup>18</sup> Iturin antibiotics like Bacillomycin F isolated from *Bacillus subtilis* has been reported to have antibacterial activity against *Staphylococcus aureus* (MIC = > 400 µg/mL).<sup>19</sup> Also, derivatives of Bacillomycin F isolated from *Bacillus subtilis* H215 showed promising antifungal activity against spoilage mould (*Byssoschlamys fulva* H25) which causes spoilage of juices and beverages.<sup>20</sup> But, to the best of our knowledge, no antimicrobial or antioxidant activity of *Bacillus* 2011SOCCUF3 strain has been documented.

The extract of *Bacillus* strain showed good antimicrobial activity against all the tested organisms with *C. albicans* having the least MIC of 0.108 mg/mL. This is followed by *S. aureus* and *S. typhi* with MIC of 0.247 mg/mL (Table 1). The VLC fractions also exhibited good antimicrobial activity against the test bacteria and fungi compared with the standards used. BF2 had the highest inhibition of *S. typhi* with MIC value of 0.083 mg/mL. BF1 possesses the highest activity at least on three test organisms viz; *P. aeruginosa*, *E. coli* and *C. albicans* with MIC values of 0.163, 0.881 and 0.628 mg/mL respectively. This indicated that the novel *Bacillus* 2011SOCCUF3 strain contains both antibacterial and antifungal bioactive metabolites. Our

findings agree with the work of Abad and co-worker that isolated metabolites of marine microbes and declared that they have the ability to produce activity against infectious diseases caused by bacteria, fungi and virus.<sup>21</sup> Methanol extract of *Spongia officinalis* has been reported to possess antimicrobial activity against some bacteria and fungi.<sup>22</sup> This was in agreement with our current findings that extract and VLC fractions of *Bacillus* 2011SOCCUF3 strain isolated from a marine sponge *Spongia officinalis* exhibited antimicrobial activities.

Free radicals, which are responsible for oxidative stress diseases, are beneficial as antibacterial, but cause damage to cellular proteins, membrane lipids, nucleic acid and death of cells in excess amount.<sup>23-25</sup> Studies have shown that extracts of *actinobacteria* isolate from soil or marine sediments possess strong antibacterial and antioxidant activities.<sup>26-28</sup> Based on this, we investigated the antioxidant properties of the extract and the VLC fractions. The extract and VLC fractions showed high antioxidant activity in the DPPH assay, evident in the low IC<sub>50</sub> values obtained (1.008 – 7.140 mg/mL) (Table 4).

**Table 4.** IC<sub>50</sub> (mg/mL) of the Extract and Fractions of *Bacillus* 2011SOCCUF3 strain.

Samples	IC <sub>50</sub> for DPPH Assay (mg/mL)	IC <sub>50</sub> for Phosphomolybdate Assay (mg/mL)
BM	2.035	44.55
BF 1	5.420	103.03
BF 2	1.008	30.80
BF 3	7.140	105.43
BF 4	2.517	90.01
Ascorbic Acid	0.521	20.15

BM = Extract, BF 1 – BF 4 = VLC fractions.

BF2 had the highest antioxidant activity with IC<sub>50</sub> value of 1.008 mg/mL, while BF3 had the least antioxidant activity

(IC<sub>50</sub> = 7.140 mg/mL). The total antioxidant activity was more for the extract and BF2 with IC<sub>50</sub> values of 44.55 and 30.80 mg/mL respectively (Table 4). Krishnan and Keerthi in their previous study reported that methanol extract of *Spongia officinalis* var. *ceylonensis* contained polyphenolic compounds like phenols.<sup>29</sup> Thus, the antioxidant properties of the extract and VLC fractions could be attributed to this metabolite since the *Bacillus* strain was isolated from this marine sponge.

### Conclusion

In this study, we reported the antimicrobial and antioxidant properties of a novel *Bacillus* strain. Work is on-going to purify the most active fractions (BF1 and BF2) with a view to isolate and characterize the bioactive compound responsible for the activities.

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### Conflict of interests

We declare that there is no conflict of interest.

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