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Original Research Article

Effect of *Irvingia* Fruit Kernel Supplement on the Growth of *Bacillus species*

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Abstract: The kernels of *Irvingia specie* have been reported to used as soup thickener in some parts of Nigeria and also rich in protein, glucose, fat and oil and fatty acids. Hence, this research aims to assess the effect of *Irvingia* fruit kernel on the mean population density of *Bacillus spp.* Eleven isolates were identified as suspected *Bacillus species* using standard microbiological and biochemical testing techniques. *Irvingia* broth, Irvingia broth/tryptone (50/50) and tryptone soya broth were prepared, and *Bacillus* isolates were inoculated into the different broths and incubated at 30^0C for 14 days. All isolates were Gram positive, indole and oxidase negative, citrate negative (except isolate M1) and catalase negative (except Isolates F2,F3,FW2, FW3 and FW4). The maximum mean population density of *Bacillus spp* in *Irvingia* and Irvingia/Tryptone broths in Day1,3,7,10 and 14 were 3.1 x 10^8 cfu/ml (Irvingia/Tryptone), 3.1 x 10^8 cfu/ml (*Irvingia*), 3.1 x 10^8 cfu/ml (*Irvingia* and Irvingia/Tryptone), 4.0x10^8 cfu/ml (*Irvingia* and Irvingia/Tryptone) and 4.0x10^8 cfu/ml (*Irvingia*) respectively. The findings in this research signifies that *Irvingia* and *Irvingia*/Tryptone broths can support the growth of *Bacillus spp* just like tryptone soya broth.

Keywords: *Irvingia* fruit waste, *Irvingia* broth, *Bacillus species*, Mean population density.

INTRODUCTION

The African bush mango has a fleshy part and a nut consisting of a hard shell and kernel or seed [1] and people in the southern and eastern part of Nigeria use the kernels for preparing the leafy vegetables, chili powder, smoked fish, crayfish, meat, spices and other additives into a thick, gelatinized, slimy Ogbono or Draw soup eaten with eba or foo-foo [2, 3]. The kernels are rich source of fat, oil and protein [4]. Secondary metabolites are not essential for cell growth, but serve as a survival strategy during adverse conditions, and are synthesized at the late or stationary phase of their growth, and this can be activated by environmental stress, and limited growth conditions [5]. *Bacillus* are a major group of soil bacteria, more widely distributed and more numerous than the other bacteria isolated from soil samples. The microbe is Gram negative with endospore-forming ability [6, 7] and synthesize secondary metabolites with different structures and functions, including remarkable antimicrobial activities. Secondary metabolite production is affected by some physical (temperature, pH and incubation period) and nutritional factors (carbon, nitrogen and mineral) [8]; which ensure the fermentation conditions suitable for bacterial growth and metabolites production [9]. Antibiotics formation is naturally genetic but expression can be influenced greatly by environmental manipulations, like exhaustion of nutrient, addition of an inducer and/or by a decrease in growth rate [10]. Production of antibiotics is also regulated by nutrients like nitrogen, phosphorous and carbon source, including metals, growth rate, feedback control and enzyme inactivation [11]. Glucose is an excellent carbon source for growth but it interferes with the formation of many antibiotics. Macrocyclic polyketides produced by type I and II PKSs and the production of polyketydes is also suppressed by different carbon sources.

MATERIALS AND METHODS

Collection and Processing of Soil Samples

Eleven soil samples were collected from receded marine and fresh water swamps and a farm in Ogbolomabiri, Abobiri and Ogbia town respectively, all in Bayelsa State East Senatorial district, Nigeria. Samples were taken from nine points which are 10m apart from each other and three points each were then pooled together to get representative sample one, two

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and three for each of the environments. With a clean trowel surface debris was scrapped off followed by mixing the top 6- 12inches of soil (where most microbes are concentrated and microbial activity take place) and then placing samples into sterile black polythene bag. Air drying samples in hot air oven at 45^{0}C for 1 hr was done to reduce the population of bacteria other than *Bacillus* [12], because spores of *Bacillus* are more resistance to desiccation as compared to gram negative bacteria. A gram of soil sample was suspended in sterile water, and made up to 10ml and 0.1ml was inoculated on starch casein agar (SCA) supplemented with tetracycline (5µg/ml) and ketoconazole (100µg/ml) [13] and incubated at 35^{o}C for 7 days. Absorbance was measured with a spectrophotometer (600 nm) and mean population density estimated for day1, 3, 7,10 and 14.

Gram staining and Biochemical Characterization

Eleven (11) selected isolates were subjected to Gram staining and biochemical tests such as catalase, oxidase, citrate, starch hydrolysis and indole [14].

Optical density of isolates (Absorbance)

Fifty grams of *Irvingia* seeds were aseptically suspended in 200ml distilled water to prepare the *Irvingia* broth [15], while tryptone soya broth was prepared according to manufacturers instruction. All broths were further supplemented with tetracycline (5µg/ml) and ketoconazole (100µg/ml) to inhibit the growth of bacteria and fungi. One to three colonies of the different *Bacillus* isolates were inoculated into 10mls of the Tryptone soya broth (TSB), *Irvingia* seeds broth and *Irvingia*/Tryptone soya broth (50/50 volume), followed by incubation at 35^{0}C for 24 hours. Five milliliters from each broth was then transferred into 75mls of the sterile broths in a 250ml erlenmeyer flask and incubated for 14 days. Absorbance was measured with a spectrophotometer (600 nm) on day1, 3, 7,10 and 14 [16].

Optimization of culture conditions for antibiotic production

The *Bacillus* isolates were cultured in tryptone soy broth, Irvingia broth, tryptone/irvingia broth, tryptone broth exposed to ultra violet irradiation and tryptone soy broth [17, 18]. The *Irvingia* broth and tryptic soy broth were both incubated a for 14 days at 30°C and these were shaken at least twice daily and also, absorbance of each culture was taken on days 1,3,7,10 and 14 [19].

RESULTS

Table 1: Bacterial Isolate Code and Selected Biochemical Tests

Sample source	Bacteria Isolate Code	Gram reaction	Catalase	Oxidase	Citrate	Starch hydrolysis	Indole	Remark
Marine	M1	Gram +ve bacilli	Negative	Negative	Positive	Negative	Negative	StbB
Marine	M2	Gram +ve bacilli	Negative	Negative	Negative	Negative	Negative	StbB
Marine	M3	Gram +ve bacilli	Negative	Negative	Negative	Positive	Negative	StbB
Marine	M4	Gram +ve bacilli	Negative	Negative	Negative	Negative	Negative	StbB
Farm	F1	Gram +ve bacilli	Negative	Negative	Negative	Negative	Negative	StbB
Farm	F2	Gram +ve bacilli	Positive	Negative	Negative	Positive	Negative	StbB
Farm	F3	Gram +ve bacilli	Positive	Negative	Negative	Positive	Negative	StbB
Fresh	FW1	Gram +ve bacilli	Negative	Negative	Negative	Positive	Negative	StbB
Fresh	FW2	Gram +ve bacilli	Positive	Negative	Negative	Positive	Negative	StbB
Fresh	FW3	Gram +ve bacilli	Positive	Negative	Negative	Negative	Negative	StbB
Fresh	FW4	Gram +ve bacilli	Positive	Negative	Negative	Positive	Negative	StbB

StbB=Suspected to be *Bacillus*

Table 2: Mean Absorbance and population Density (Cfu/ml) of *Bacillus species* on Day 1

Treatment	F1	F2	F3	FW1	FW2	FW3	FW4	M1	M2	M3	M4
IRVINGIA	0.939	0.524	0.827	0.857	1.318	1.164	0.745	1.018	0.855	0.911	0.934
TSB	0.441	0.654	0.512	0.764	0.06	0.517	0.426	0.491	0.503	0.468	0.558
TSB/IRVINGIA	0.929	0.97	0.826	0.975	1.411	1.163	1.517	0.86	1.031	1.047	0.931
UV	0.401	0.495	0.589	0.587	0.486	0.516	0.695	0.4	0.396	0.79	0.61
IRVINGIA	$1.9x10^8$	$1.0x10^8$	$1.7x10^8$	$1.8x10^8$	$2.7x10^8$	$2.4x10^8$	$1.5x10^8$	$2.1x10^8$	$1.8x10^8$	$1.9x10^8$	$1.9x10^8$
TSB	$9.2x10^7$	$1.3x10^8$	$1.1x10^8$	$1.6x10^8$	$1.6x10^7$	$1.1x10^8$	$8.9x10^7$	$1.1x10^8$	$1.0x10^8$	$9.7x10^7$	$1.2x10^8$
TSB/IRVINGIA	$1.9x10^8$	$1.9x10^8$	$1.7x10^8$	$2.0x10^8$	$2.9x10^8$	$2.4x10^8$	$3.1x10^8$	$1.8x10^8$	$2.1x10^8$	$2.1x10^8$	$1.9x10^8$
UV	$8.4x10^7$	$1.0x10^8$	$1.2x10^8$	$1.2x10^8$	$1.0x10^8$	$1.1x10^8$	$1.4x10^8$	$8.4x10^7$	$8.3x10^7$	$1.6x10^8$	$1.3x10^8$

KEY: TSB=Tryptic soy broth; UV=Ultraviolet radiation treated broth; Values= Absorbance and Colony forming unit/ml

The maximum mean population density of *Bacillus* isolates on Day 1 was as follows: Isolate FW2 ($2.9x10^8$cfu/ml in TSB/IRVINGIA and $2.7x10^8$ cfu/ml in *Irvingia*); Isolate F2 ($1.3x10^8$ cfu/ml in TSB); Isolate

FW4 ($3.1x10^8$ cfu/ml in TSB/IRVINGIA), and isolate M3 ($1.3x10^8$ cfu/ml in ultraviolet radiation treated TSB broth). Almost all isolates incubated in *Irvingja* and TSB/IRVINGIA) broths had higher mean colony forming unit than TSB and Ultraviolet radiation treated broths.

Table 3: Mean Absorbance and Population density (cfu/ml) of *Bacillus species* on Day 3

Treatment	F1	F2	F3	FW1	FW2	FW3	FW4	M1	M2	M3	M4
IRVINGIA	1.036	1.408	1.059	1.318	1.476	1.539	1.241	1.113	1.399	1.315	1.244
TSB	0.975	1.052	0.96	0.059	1.552	1.119	0.776	0.498	0.827	0.545	0.447
TSB/IRVINGIA	0.16	1.36	1.387	1.387	1.457	1.324	1.119	0.986	1.447	1.374	1.176
UV	0.498	0.72	0.758	0.642	0.696	0.647	0.695	0.412	0.498	0.859	0.745
IRVINGIA	$2.1x10^8$	$2.8x10^8$	$2.2x10^8$	$2.7X10^8$	$3.0X10^8$	$3.1X10^8$	$2.5X10^8$	$2.3X10^8$	$2.9X10^8$	$2.7X10^8$	$2.5X10^8$
TSB	$2.0x10^8$	$2.1x10^8$	$2.0x10^8$	$1.2X10^7$	$3.2X10^8$	$2.3X10^8$	$1.6X10^8$	$1.0X10^8$	$1.7X10^8$	$1.1X10^8$	$9.1X10^7$
TSB/IRVINGIA	$3.2x10^7$	$2.8x10^8$	$2.8x10^8$	$2.8x10^8$	$3.0X10^8$	$2.7X10^8$	$2.3X10^8$	$2.0X10^8$	$3.0X10^8$	$2.8X10^8$	$2.4X10^8$
UV	$1.0x10^8$	$1.5x10^8$	$1.5x10^8$	$1.3X10^8$	$1.4X10^8$	$1.3X10^8$	$1.4X10^8$	$8.4X10^8$	$1.0X10^8$	$1.5X10^8$	$1.5X10^8$

KEY: TSB=Tryptic soy broth; UV=Ultraviolet radiation treated broth; Values= Absorbance and Colony forming unit/ml

The maximum mean population density of *Bacillus* isolates on Day 3 in four different broths was as follows:
Isolate FW3 ($3.1x10^8$ cfu/ml in *Irvingia*); Isolate FW2 ($3.2x10^8$ cfu/ml in TSB and $3.0x10^8$ cfu/ml in TSB/IRVINGIA), and isolate F2, F3.M3 and M4 ($1.5x10^8$ cfu/ml in ultraviolet radiation treated TSB broth).

All isolates except FW2 that were incubated in *Irvingja* and TSB/IRVINGIA) broths had higher mean colony forming units than TSB and Ultraviolet radiation treated broths.

Table 4: Mean Absorbance and Population density (cfu/ml) of *Bacillus species* on Day 7

Treatment	F1	F2	F3	FW1	FW2	FW3	FW4	M1	M2	M3	M4
IRVINGIA	1.411	1.452	1.356	1.447	1.531	1.393	0.658	1.305	1.443	1.539	1.491
TSB	0.975	1.619	1.552	1.447	1.564	1.343	1.356	1.147	1.144	1.058	1.017
TSB/IRVINGIA	1.328	1.404	1.488	1.488	1.514	1.45	1.347	1.462	1.454	1.507	1.299
UV	0.587	0.885	0.974	0.751	0.75	0.769	0.785	0.51	0.615	1.006	0.953
IRVINGIA	$2.9x10^8$	$3.0x10^8$	$2.8x10^8$	$3.0x10^8$	$3.1x10^8$	$2.8x10^8$	$1.3x10^8$	$2.7x10^8$	$3.0x10^8$	$3.1x10^8$	$3.02x10^8$
TSB	$2.0x10^8$	$3.3x10^8$	$3.2x10^8$	$3.0x10^8$	$3.2x10^8$	$2.7x10^8$	$2.8x10^8$	$2.3x10^8$	$2.3x10^8$	$2.2x10^8$	$2.07x10^8$
TSB/IRVINGIA	$2.7x10^8$	$2.9x10^8$	$3.0x10^8$	$3.0x10^8$	$3.1x10^8$	$3.0x10^8$	$2.7x10^8$	$3.0x10^8$	$3.0x10^8$	$3.1x10^8$	$2.64x10^8$
UV	$1.2x10^8$	$1.8x10^8$	$2.0x10^8$	$1.5x10^8$	$1.5x10^8$	$1.6x10^8$	$1.6x10^8$	$1.0x10^8$	$1.3x10^8$	$2.1x10^8$	$1.95x10^8$

KEY: TSB=Tryptic soy broth; UV=Ultraviolet radiation treated broth; Values= Absorbance and Colony forming unit/ml

The highest mean colony counts recorded in the broths on Day 7 were as follows: Isolate FW2 and M4 (3.1x108 cfu/ml in Irvingia); Isolate F2 (3.3x108 cfu/ml in TSB); Isolate FW2 and M4 (3.1x108 cfu/ml in TSB/IRVINGIA), and isolate M4 (2.1x108 cfu/ml in ultraviolet radiation treated TSB broth).

Isolates F1, FW3, M1, M2, M3 and M4 incubated in Irvingja and TSB/IRVINGIA) broths had higher mean colony forming units than TSB and Ultraviolet radiation treated broths

Table 5: Mean Absorbance and Population density (cfu/ml) of *Bacillus species* on Day 10

Treatment	F1	F2	F3	FW1	FW2	FW3	FW4	M1	M2	M3	M4
IRVINGIA	1.372	1.46	1.443	1.531	1.821	1.963	1.34	1.337	1.337	1.515	1.306
TSB	0.975	1.58	1.612	1.531	1.505	1.963	1.357	1.807	1.506	1.716	1.292
TSB/IRVINGIA	1.356	1.45	1.588	1.588	0.837	1.523	1.347	1.324	1.479	1.456	1.342
UV	0.761	0.932	1.035	0.842	0.837	0.831	0.821	0.56	0.722	1.08	1.201
IRVINGIA	$2.8x10^8$	$3.0x10^8$	$3.0x10^8$	$3.1x10^8$	$3.7x10^8$	$4.0x10^8$	$2.7x10^8$	$2.7x10^8$	$2.7x10^8$	$3.1x10^8$	$2.7x10^8$
TSB	$2.0x10^8$	$3.2x10^8$	$3.3x10^8$	$3.1x10^8$	$3.1x10^8$	$4.0x10^8$	$2.8x10^8$	$3.7x10^8$	$3.1x10^8$	$3.5x10^8$	$2.6x10^8$
TSB/IRVINGIA	$2.8x10^8$	$3.0x10^8$	$3.2x10^8$	$3.2x10^8$	$1.7x10^8$	$3.1x10^8$	$2.7x10^8$	$2.7x10^8$	$3.0x10^8$	$3.0x10^8$	$2.7x10^8$
UV	$1.6x10^8$	$1.9x10^8$	$2.1x10^8$	$1.7x10^8$	$1.7x10^8$	$1.7x10^8$	$1.7x10^8$	$1.1x10^8$	$1.5x10^8$	$2.2x10^8$	$2.5x10^8$

KEY: TSB=Tryptic soy broth; UV=Ultraviolet radiation treated broth; Values= Absorbance and Colony forming unit/ml

The highest mean colony counts recorded in the broths on Day 10 were as follows: Isolate FW3 (4.0x108 cfu/ml in Irvingia); Isolate FW3 (3.5x108 cfu/ml in TSB); Isolate FW1 (3.2x108 cfu/ml in TSB/IRVINGIA), and isolate M4 (2.5x108 cfu/ml in ultraviolet radiation treated TSB broth).

Table 6: Mean Absorbance and Population density (cfu/ml) of *Bacillus species* on Day 14

Treatment	F1	F2	F3	FW1	FW2	FW3	FW4	M1	M2	M3	M4
IRVINGIA	1.483	1.595	1.551	0.857	1.477	0.957	1.727	1.455	1.608	1.611	1.885
TSB	1.552	1.595	1.482	0.512	0.523	0.607	1.667	1.569	1.446	1.769	1.999
TSB/IRVINGIA	1.449	1.511	1.512	1.011	0.929	1.57	1.667	1.464	1.533	1.515	1.782
UV	0.892	1.341	1.214	0.943	0.963	0.965	0.498	0.602	0.812	1.232	1.345
IRVINGIA	$3.0x10^8$	$3.3x10^8$	$3.2x10^8$	$1.7x10^8$	$3.0x10^8$	$2.0x10^8$	$3.5x10^8$	$3.0x10^8$	$3.3x10^8$	$3.3x10^8$	$4.0x10^8$
TSB	$3.2x10^8$	$3.3x10^8$	$3.0x10^8$	$1.0x10^8$	$1.1x10^8$	$1.2x10^8$	$3.4x10^8$	$3.2x10^8$	$3.0x10^8$	$3.6x10^8$	$4.1x10^8$
TSB/IRVINGIA	$3.0x10^8$	$3.1x10^8$	$3.1x10^8$	$2.1x10^8$	$1.9x10^8$	$3.2x10^8$	$3.4x10^8$	$3.0x10^8$	$3.1x10^8$	$3.1x10^8$	$3.6x10^8$
UV	$1.8x10^8$	$2.7x10^8$	$2.5x10^8$	$1.9x10^8$	$2.0x10^8$	$2.0x10^8$	$1.0x10^8$	1.2x108	$1.6x10^8$	$2.5x10^8$	$2.7x10^8$

KEY: TSB=Tryptic soy broth; UV=Ultraviolet radiation treated broth; Values= Absorbance and Colony forming unit/ml

At Day 14 isolate M1 fermented in tryptone soy broth (exposed to Ultraviolet irradiation) recorded the lowest absorbance (0.602), while isolate M4 (TSB) had the highest absorbance of 1.999. The highest absorbance in Irvingia broth was recorded by isolate M4 (1.855), and that of TSB/IRVINGIA broths was also exhibited by isolate M4 (1.782).

DISCUSSION

Table 1 depicts that all isolates were Gram negative bacilli showing negative reaction to indole and oxidase tests. Catalase test results were negative except for isolates F2, F3, FW2, FW3 and FW4; while all were citrate negative apart from isolate M. Isolates M3, F2, F3, FW1, FW2 and FW4 were positive to starch hydrolysis test. A study reported that two *Bacillus* isolates (Bt VKK-AC1, Bs VKKSL1 and one reference strain Btk HD-1) subjected to biochemical test were all catalase positive [20] like isolates F2, F3, FW2, FW3 and FW4. Biochemical test was carried out on three isolates from different water sources (Sepclean, Sediment river and Sediment sea isolates) revealed that all isolates were indole negative and citrate positive, while only the Sepclean isolate was oxidase positive [21].

This agrees with the findings in this study as all isolates were also indole negative and only isolate M1was citrate positive. It was opined that enrichment techniques are now in place to enhance the growth of desirable bacteria from natural habitats [22]. Thus, the absorbance and population density of *Bacillus* isolates in Tryptone Soy, *Irvingia* and Tryptone/*Irvingia* broths were investigated in this research. On Day1 isolate FW4 exhibited a maximum mean absorbance and population density of 1.517 with $3.1x10^8$ cfu/ml respectively in TSB/IRVINGIA; followed by isolate FW2 (1.411 with $2.9x10^8$cfu/ml) in TSB/IRVINGIA, and 1.318 with $2.7x10^8$ cfu/ml in *Irvingia*.

On Day 3 isolate FW2 recorded 1.55 with $3.2x10^8$ cfu/ml in TSB; followed by isolate FW3 (1.539 with $3.1x10^8$ cfu/ml in *Irvingia*); and isolates M2 and FW2 recording 1.447 with $3.0x10^8$ and 1.457 with $3.0x10^8$ cfu/ml respectively in TSB/IRVINGIA broth. On Day7 isolate F2 demonstrated maximum absorbance and mean population density of 1.619 with $3.3x10^8$ cfu/ml in TSB; followed by Isolate FW2 (1.564 with $3.2x10^8$cfu/ml in TSB; 1.531 with $3.1x10^8$cfu/ml in *Irvingia*; 1.514 with $3.1x10^8$cfu/ml in TSB/IRVINGIA); and M4 (1.507 with $3.1x10^8$ cfu/ml in TSB/IRVINGIA).

On Day10 isolate FW3 recorded maximum absorbance and mean population density of 1.963 with $4.0x10^8$ cfu/ml in both *Irvingia* and TSB broths; followed by isolate FW2 (1.821 with $3.7x10^8$ cfu/ml in *Irvingia*); and isolate M3 (1.716 with $3.5x10^8$cfu/ml) in TSB broth. On Day14 isolate M4 recorded maximum absorbance and mean population density of 1.999 with $4.1x10^8$cfu/ml in TSB; 1.885 with $4.0x10^8$cfu/ml in *Irvingia* and 1.782 with $3.6x10^8$ in TSB/IRVINGIA broth. This result implies that *Irvingia* and TSB/IRVINGIA broths can also support the growth *Bacillus species* as TSB broth. The maximum mean population density for isolates F1 ($3.0x10^8$cfu/ml), F2 ($3.3x10^8$cfu/ml) and F3($3.2x10^8$cfu/ml) fermented in *Irvinga* broth was observed on Day 14. The maximum mean population density for isolates FW1($3.1x10^8$cfu/ml), FW2 ($3.7x10^8$cfu/ml) and FW3 ($4.0x10^8$cfu/ml) also fermented in *Irvingia* broth was observed on Day10; while that for FW4 ($3.4x10^8$cfu/ml) was observed on Day14.

The maximum mean population density for isolates M1 ($3.0x10^8$cfu/ml), M2 ($3.3x10^8$cfu/ml), M3 ($3.3x10^8$cfu/ml) and M4 ($4.0x10^8$cfu/ml) also fermented in *Irvingia* broth was observed on Day14. The maximum mean population density for isolates F1 ($3.2x10^8$cfu/ml) and F2 ($3.3x10^8$cfu/ml) fermented in TSB broth was observed on Day 14, while that of F3($3.3x10^8$cfu/ml) was observed on Day 10. The maximum mean population density for isolates FW1 ($3.1x10^8$cfu/ml) fermented in TSB broth was observed on Day 10, FW2 ($3.2x10^8$cfu/ml) was observed on Day7, FW3 ($4.0x10^8$cfu/ml) was observed on Day10, while that for FW4 ($3.4x10^8$cfu/ml) was observed on Day 14.

The maximum mean population density for isolates M1 ($3.0x10^8$cfu/ml), M2($3.0x10^8$cfu/ml), M3($3.0x10^8$cfu/ml) and M4($3.0x10^8$cfu/ml) fermented in TSB/IRVINGIA broth was observed on Day14. This goes further to confirm that almost all the isolates demonstrated a maximum mean population density from Day7 to 14; which may be due to the fermentation time and the carbohydrate component in *Irvingia* and Tryptone soy broth. The results from this study could be related to that of research report where marine broth after

30 days incubation showed the highest number of actinomycetes ($4.40 \pm 0.80 \times 10^2$ cfu/g), because marine broth repairs damaged cells and induces spore germination which promote the growth of actinomycetes [23] and isolation of novel actinomycetes can be aided by extending the incubation period to about 30 days [24, 25].

CONCLUSION

Apart from indole and oxidase tests that was shown to be negative for *Bacillus species* in this study and by another researcher, different reactions can be observed via citrate, catalase and starch hydrolysis tests, therefore biochemical tests should not completely be relied upon in the identification of bacillus isolates. *Irvingia* broth and *Irvingia* added to tryptone soy broth increased the mean absorbance and population density of Bacillus isolates in this study. This goes on to imply that *Irvingia* fruit Kernel can also support the growth of *Bacillus species* like tryptone soy broth.

RECOMMENDATION

The findings from this research recommend as follows:

- Molecular identification of *Bacillus species* should be the gold standard for identification.
- Irvingia fruit waste (kernel) can be developed as a culture media for isolation *Bacillus species*.

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