CHAPTER THREE

RESULTS

3.1 Bacteriological characteristics of unknown colonies isolated from IMO4 soil

3.1.1 Colony morphology

A total of one hundred and thirty nine (139) bacterial colonies were isolated and screened for hemolytic colonies from agricultural soil extract using BHI blood agar. The colonies were smooth with entire edge, increasing in size from 3-5 to 6 to 8 mm with continued incubation more than 24 hours at 37°C (Figure 5). Some of them produced watery mucoid colonies (Figure 6A) while some produced rough colonies (Figure 6B & F). Some colonies were concentric with internal diameter of 3-5 mm, irregular margin with flat elevation (Figure 6B). Some isolates were observed as rounded white-shiny colonies (Figure 6D). The edge of the colony was smooth with drop-like elevation (Figure 7).Various colonial morphological types (Figure 7-9) were observed among the soil isolates included rough, smooth, glistening, mucoid, grayish-white and transparent. Some colonies were appeared as circular with internal diameter ranging from 5-6 mm when grown on BHI blood agar medium.. A strong haemolysis was observed among the unknown soil bacterial isolates. Table 2 shows the colonial morphology on BHI blood agar of the 139 isolates used in this study. Figure 10 and Table 2 summarizes the results of colony morphology types from the 139 unknown soil bacteria isolates prior to Gram staining. All the hemolytic colonies were observed in this study were β-hemolytic type and none of them were α-hemolytic type.

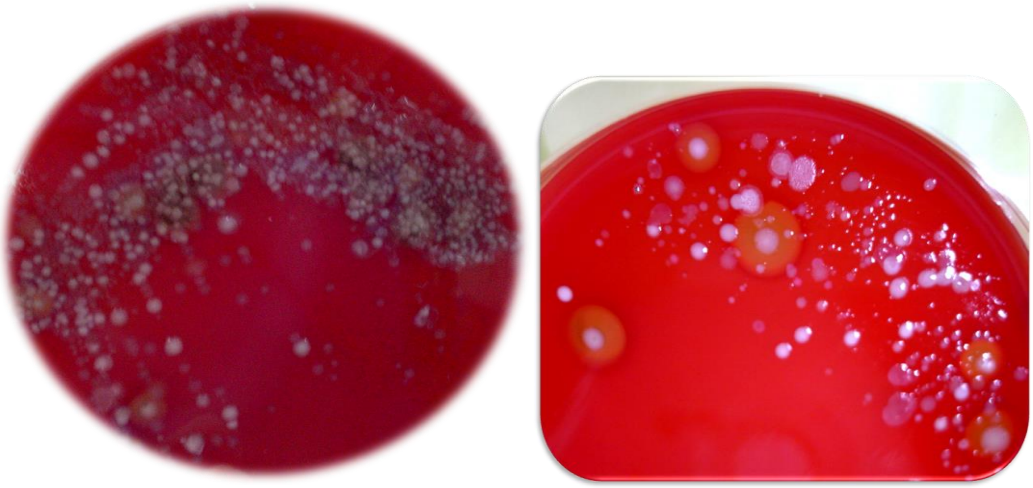

Figure 5: Petri-dish showing colonies present in agricultural soil extract distributed (left) on BHI blood medium after an overnight incubation at 37^{o}C showing various types of mixed colonies: small, large, haemolytic, non haemolytic, rough, mucoid and smooth (right)

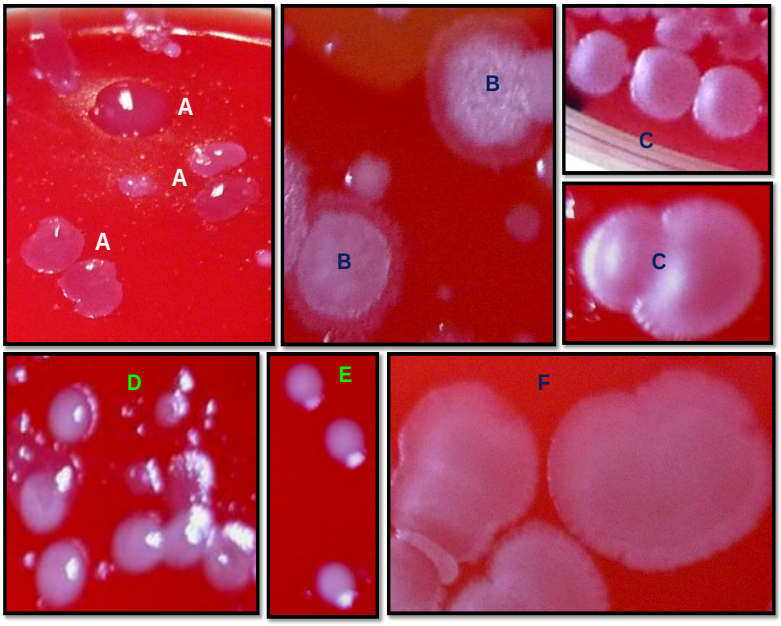

Figure 6: Various types of colonial morphology of unknown soil bacterial isolates- A; Mucoid colonies, B; Rough colonies with flat surface with irregular edges; C & F; Rough colonies with center-point surface and irregular edges, D & E; Smooth colonies with rounded shape

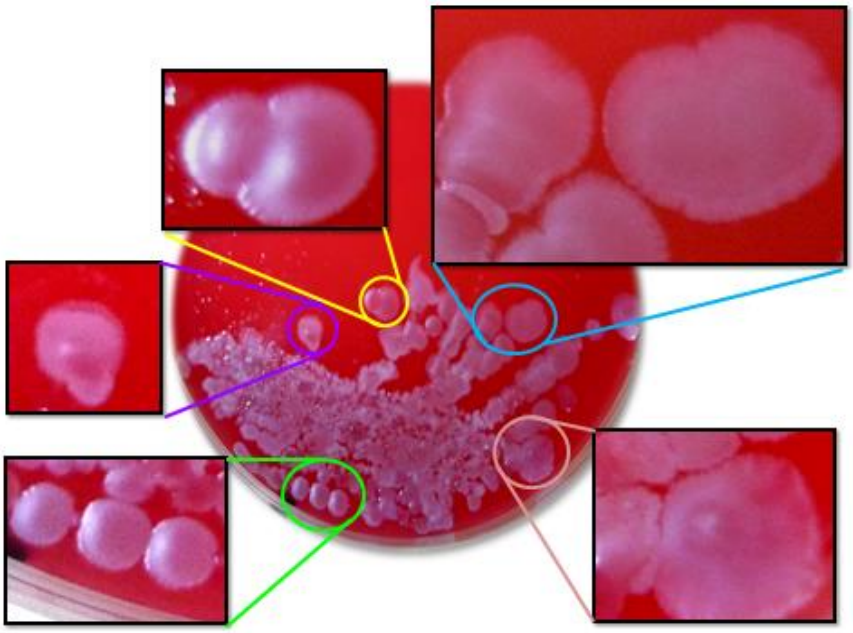

Figure 7: Petri-dish showing rough colonies of unknown representative soil bacterium growth on BHI blood agar medium. The colonies formed are raised white circle (drop-like colonies)

Figure 8: Representative of white-grayish rough colonies with strong haemolytic activity on BHI blood agar medium.

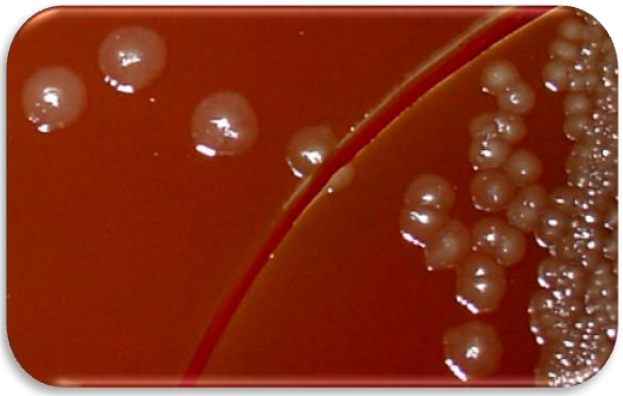

Figure 9: Representative mucoid, white-creamy, rounded colonies on BHI blood agar medium after 24 hours incubation.

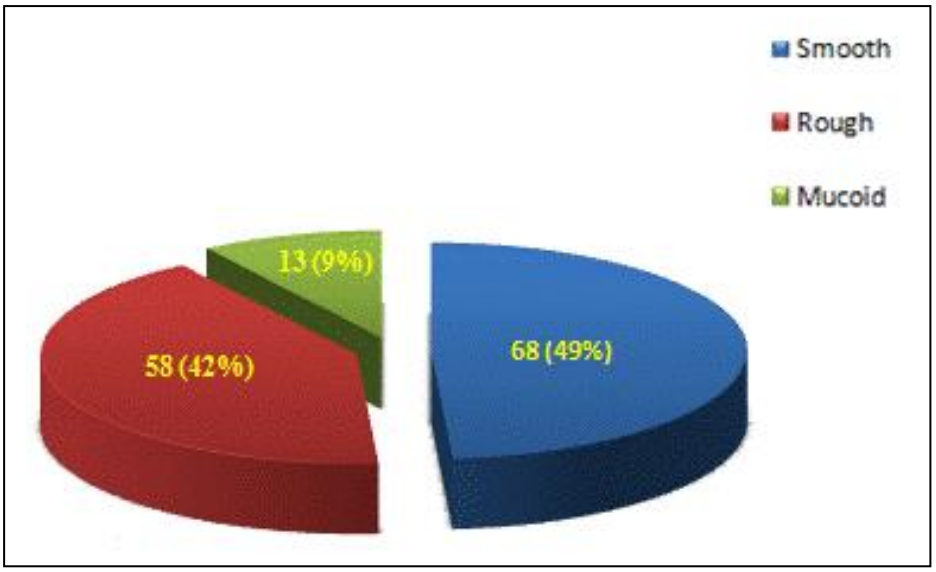

Figure 10: Pie chart illustrating the soil bacterial colony types among the 139 isolates with 49% of smooth, 42% of rough and 9% of mucoid colonies.

Table 2: Colony morphology surface appearance (smooth, rough and mucoid) of 139 unknown microorganism isolated from agricultural soil

Colony No.	Colony morphology	Colony No.	Colony morphology	Colony No.	Colony morphology
SO4/1	S	SO4/66	R	SO4/113	S
SO4/2	S	SO4/67	S	SO4/114	R
SO4/3	S	SO4/68	R	SO4/115	R
SO4/4	S	SO4/69	S	SO4/116	S
SO4/5	R	SO4/70	M	SO4/117	S
SO4/6	R	SO4/71	R	SO4/118	S
SO4/7	S	SO4/72	S	SO4/119	R
SO4/8	R	SO4/73	M	SO4/120	R
SO4/9	R	SO4/74	R	SO4/121	S
SO4/10	S	SO4/75	S	SO4/122	S
SO4/11	R	SO4/76	S	SO4/123	R
SO4/12	S	SO4/77	S	SO4/124	S
SO4/13	S	SO4/78	R	SO4/125	R
SO4/14	S	SO4/79	R	SO4/126	R
SO4/15	S	SO4/80	R	SO4/127	R
SO4/16	R	SO4/81	R	SO4/128	R
SO4/17	S	SO4/82	S	SO4/129	R
SO4/18	S	SO4/83	S	SO4/130	R
SO4/19	S	SO4/84	S	SO4/131	M
SO4/20	R	SO4/85	R	SO4/132	R
SO4/21	R	SO4/86	R	SO4/133	M
SO4/22	S	SO4/87	S	SO4/134	R
SO4/23	S	SO4/88	S	SO4/135	R
SO4/24	R	SO4/89	R	SO4/136	R
SO4/25	S	SO4/90	R	SO4/137	R
SO4/26	R	SO4/91	S	SO4/138	M
SO4/27	S	SO4/92	S	SO4/139	R
SO4/28	S	SO4/93	S	SO4/140	R
SO4/29	S	SO4/94	S	SO4/141	R
SO4/30	R	SO4/95	R	SO4/142	S
SO4/31	S	SO4/96	S	SO4/143	S
SO4/32	S	SO4/97	M	SO4/144	R
SO4/33	S	SO4/98	S	SO4/145	S
SO4/34	R	SO4/99	S	SO4/146	M
SO4/35	S	SO4/100	R	SO4/147	S
SO4/36	M	SO4/101	M	SO4/148	S
SO4/37	R	SO4/102	R	SO4/149	M
SO4/38	S	SO4/103	M	SO4/150	M
SO4/39	S	SO4/104	S	SO4/151	R
SO4/40	R	SO4/105	S	SO4/152	S
SO4/42	S	SO4/106	R	SO4/153	S
SO4/43	S	SO4/107	R	SO4/154	S
SO4/44	R	SO4/108	R	SO4/155	S
SO4/45	R	SO4/109	R	SO4/156	S
SO4/59	R	SO4/110	R	SO4/157	S
SO4/61	S	SO4/111	S		
SO4/63	M	SO4/112	S		

Note: R; Rough S; Smooth M; Mucoid

3.1.2 Bacterial Gram identification

The 139 unknown bacterial colonies grown on BHI blood agar plates were subjected to Gram staining, and non-staining methods of an in-house alkaline lysis and aminopeptidase strip test (Merck KGaA, Darmstadt, Germany). The four types of cell morphology observed under light microscope were categorized as Gram positive cocci (Figure 11), Gram positive bacilli (Figure 13), Gram-negative cocci and Gram-negative bacilli (Figure 12) and are summarized in Table 3. 109 of 139 isolates (78%) were identified as Gram positive whereas another 30-isolates were identified as Gram negative (Figure 14). No lysis of bacterial cell wall observed in alkaline lysis solution for all the 109 bacteria isolate above while did not change in the color of bacterial suspension detected by the strip of aminopeptidase test indicating a Gram-positive reaction. The detailed results on the bacterial cell wall differentiation are presented in Table 4.

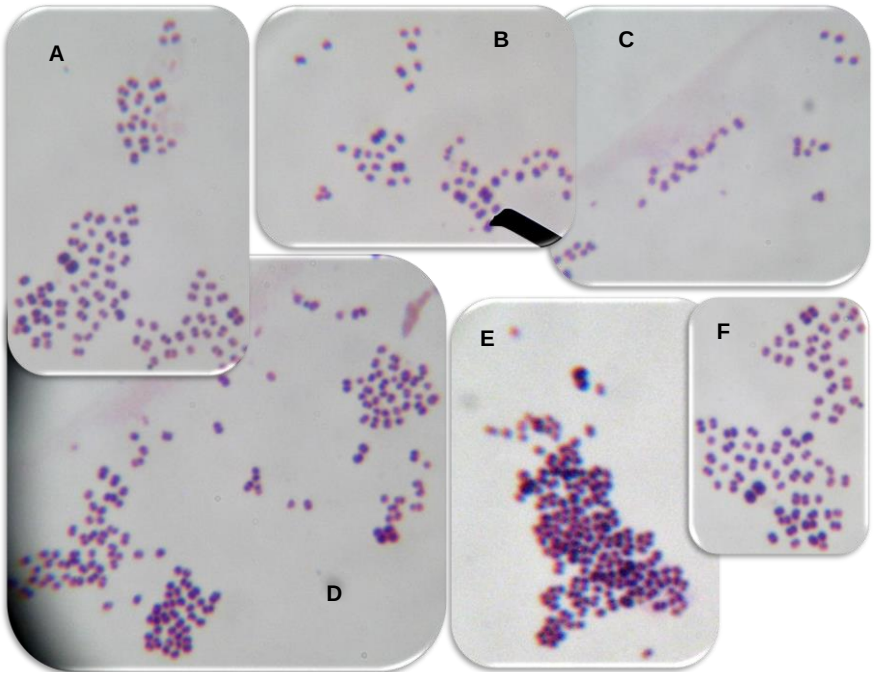

Figure 11: Representative of Gram positive cocci of unknown soil bacteria.

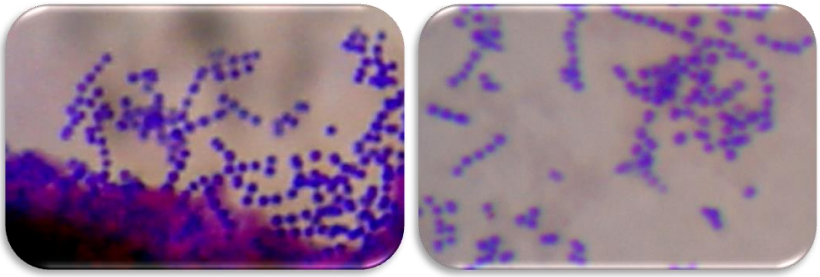

Figure 12: Representative of Gram positive (streptococci of unknown soil bacteria).

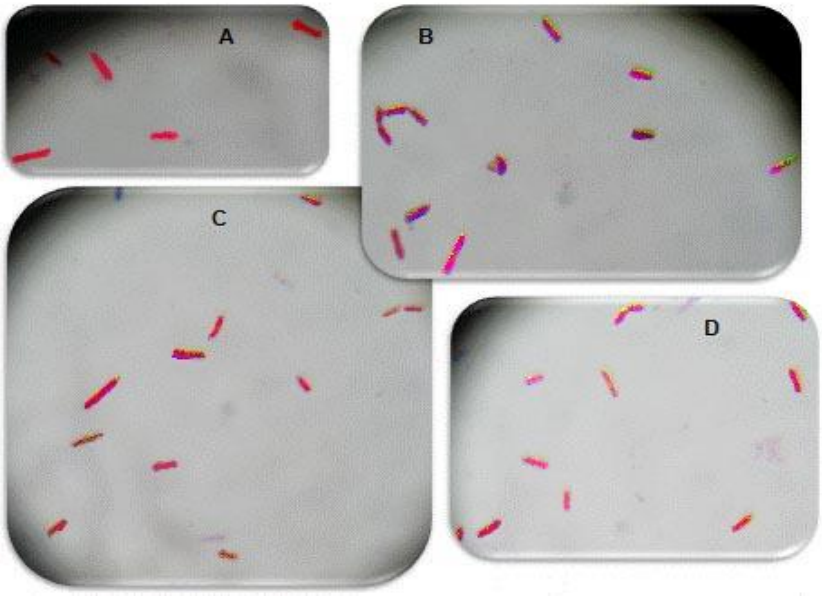

Figure 13: Gram negative bacilli shape of soil bacteria.

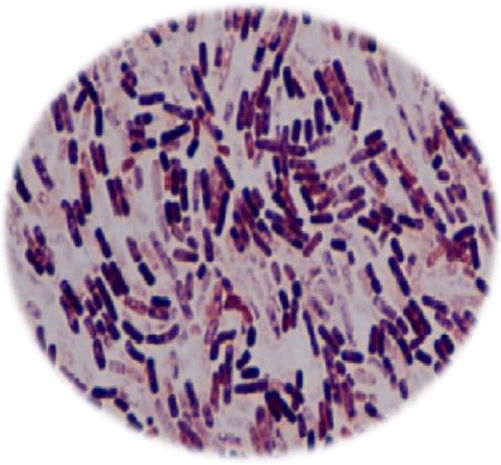

Figure 14: Representative of Gram positive bacilli of unknown soil bacteria

Table 3: Staining characteristics among 139 unknown soil bacterial isolatedfrom agricultural soil

Colony No.	Bacterial shape (cocci/rod)	Gram staining (+/-)		Colony No.	Bacterial shape (cocci/rod)	Gram staining (+/-)
SO4/1	bacilli	+		SO4/59	cocci	+
SO4/2	cocci	+		SO4/61	bacilli	—
SO4/3	cocci	+		SO4/63	bacilli	—
SO4/4	cocci	+		SO4/66	cocci	—
SO4/5	bacilli	—		SO4/67	bacilli	+
SO4/6	cocci	—		SO4/68	bacilli	+
SO4/7	cocci	+		SO4/69	bacilli	+
SO4/8	cocci	+		SO4/70	bacilli	+
SO4/9	bacilli	—		SO4/71	bacilli	+
SO4/10	cocci	—		SO4/72	cocci	+
SO4/11	cocci	—		SO4/73	bacilli	+
SO4/12	bacilli	+		SO4/74	bacilli	+
SO4/13	streptococci	+		SO4/75	bacilli	+
SO4/14	bacilli	+		SO4/76	bacilli	+
SO4/15	cocci	+		SO4/77	bacilli	+
SO4/16	streptococci	+		SO4/78	bacilli	+
SO4/17	cocci	+		SO4/79	bacilli	+
SO4/18	bacilli	+		SO4/80	cocci	+
SO4/19	bacilli	+		SO4/81	cocci	+
SO4/20	cocci	+		SO4/82	cocci	+
SO4/21	cocci	+		SO4/83	cocci	+
SO4/22	bacilli	+		SO4/84	cocci	+
SO4/23	bacilli	—		SO4/85	cocci	+
SO4/24	cocci	+		SO4/86	cocci	+
SO4/25	bacilli	+		SO4/87	bacilli	+
SO4/26	cocci	+		SO4/88	bacilli	—
SO4/27	cocci	+		SO4/89	bacilli	+
SO4/28	cocci	+		SO4/90	cocci	+
SO4/29	cocci	+		SO4/91	cocci	+
SO4/30	cocci	+		SO4/92	bacilli	+
SO4/31	bacilli	+		SO4/93	cocci	+
SO4/32	cocci	+		SO4/94	cocci	—
SO4/33	bacilli	+		SO4/95	bacilli	+
SO4/34	cocci	—		SO4/96	cocci	+
SO4/35	cocci	+		SO4/97	cocci	+
SO4/36	bacilli	+		SO4/98	cocci	+
SO4/37	cocci	+		SO4/99	bacilli	+
SO4/38	bacilli	—		SO4/100	cooci	—
SO4/39	bacilli	+		SO4/101	cocci	+
SO4/40	cocci	+		SO4/102	cocci	+
SO4/42	cocci	+		SO4/103	bacilli	+
SO4/43	cocci	+		SO4/104	bacilli	+
SO4/44	bacilli	+		SO4/105	bacilli	—
SO4/45	cocci	+		SO4/106	bacilli	+

Continued.....

SO4/107	cocci	+		SO4/133	bacilli	+
SO4/108	bacilli	+		SO4/134	bacilli	+
SO4/109	cocci	+		SO4/135	cocci	—
SO4/110	cocci	+		SO4/136	cocci	+
SO4/111	bacilli	+		SO4/137	bacilli	+
SO4/112	bacilli	+		SO4/138	bacilli	+
SO4/113	cocci	+		SO4/139	bacilli	+
SO4/114	bacilli	+		SO4/140	bacilli	+
SO4/115	bacilli	—		SO4/141	bacilli	—
SO4/116	cocci	+		SO4/142	cocci	+
SO4/117	cocci	+		SO4/143	cocci	—
SO4/118	cocci	—		SO4/144	cocci	+
SO4/119	bacilli	—		SO4/145	cocci	+
SO4/120	cocci	+		SO4/146	cocci	—
SO4/121	bacilli	+		SO4/147	bacilli	+
SO4/122	bacilli	—		SO4/148	bacilli	—
SO4/123	cocci	+		SO4/149	bacilli	+
SO4/124	cocci	+		SO4/150	bacilli	+
SO4/125	cocci	—		SO4/151	bacilli	—
SO4/126	cocci	—		SO4/152	bacilli	+
SO4/127	bacilli	+		SO4/153	cooci	+
SO4/128	cocci	—		SO4/154	bacilli	+
SO4/129	bacilli	+		SO4/155	bacilli	+
SO4/130	cocci	—		SO4/156	cocci	—
SO4/131	cocci	+		SO4/157	cocci	+
SO4/132	bacilli	+				

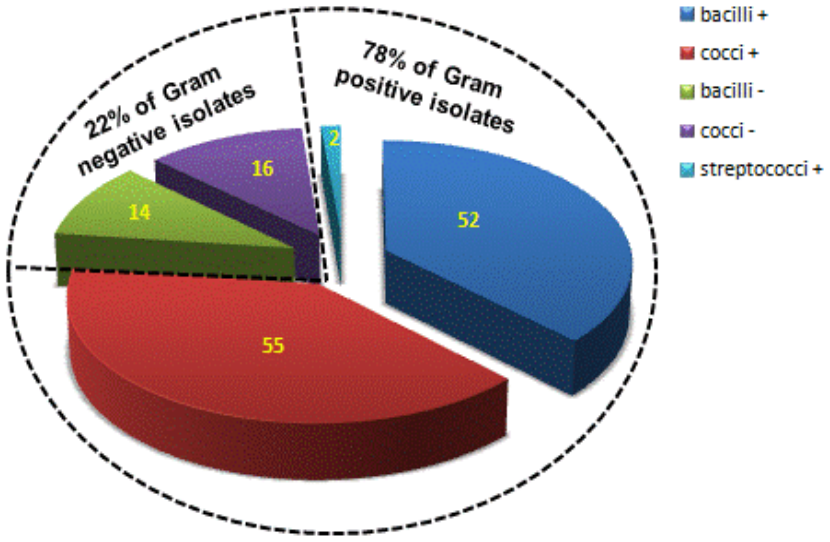

Figure 15: Pie chart showing the distribution figure of cell shaped among 139 unknown isolates with 78% of Gram positive and 22% of Gram negative isolates.

Table 4: Bacterial cell wall differentiation among 139 unknown soil bacterial

Colony No.	Gram Staining (+/-)	Aminopeptidase Strip Test (+/-)	Cell Lysis Ability (+/-)		Colony No.	Gram Staining (+/-)	Aminopeptidase Strip Test (+/-)	Cell Lysis Ability (+/-)
SO4/1	+	–	–		SO4/45	+	–	–
SO4/2	+	–	–		SO4/59	+	–	–
SO4/3	+	–	–		SO4/61	–	+	+
SO4/4	+	–	–		SO4/63	–	+	+
SO4/5	–	+	+		SO4/66	–	+	+
SO4/6	–	+	+		SO4/67	+	–	–
SO4/7	+	–	–		SO4/68	+	–	–
SO4/8	+	–	–		SO4/69	+	–	–
SO4/9	–	+	+		SO4/70	+	–	–
SO4/10	–	+	+		SO4/71	+	–	–
SO4/11	–	+	+		SO4/72	+	–	–
SO4/12	+	–	–		SO4/73	+	–	–
SO4/13	+	–	–		SO4/74	+	–	–
SO4/14	+	–	–		SO4/75	+	–	–
SO4/15	+	–	–		SO4/76	+	–	–
SO4/16	+	–	–		SO4/77	+	–	–
SO4/17	+	–	–		SO4/78	+	–	–
SO4/18	+	–	–		SO4/79	+	–	–
SO4/19	+	–	–		SO4/80	+	–	–
SO4/20	+	–	–		SO4/81	+	–	–
SO4/21	+	–	–		SO4/82	+	–	–
SO4/22	+	–	–		SO4/83	+	–	–
SO4/23	–	+	+		SO4/84	+	–	–
SO4/24	+	–	–		SO4/85	+	–	–
SO4/25	+	–	–		SO4/86	+	–	–
SO4/26	+	–	–		SO4/87	+	–	–
SO4/27	+	–	–		SO4/88	–	+	+
SO4/28	+	–	–		SO4/89	+	–	–
SO4/29	+	–	–		SO4/90	+	–	–
SO4/30	+	–	–		SO4/91	+	–	–
SO4/31	+	–	–		SO4/92	+	–	–
SO4/32	+	–	–		SO4/93	+	–	–
SO4/33	+	–	–		SO4/94	+	–	–
SO4/34	–	+	+		SO4/95	+	–	–
SO4/35	+	–	–		SO4/96	+	–	–
SO4/36	+	–	–		SO4/97	+	–	–
SO4/37	+	–	–		SO4/98	+	–	–
SO4/38	–	+	+		SO4/99	+	–	–
SO4/39	+	–	–		SO4/100	–	+	+
SO4/40	+	–	–		SO4/101	+	–	–
SO4/42	+	–	–		SO4/102	+	–	–
SO4/43	+	–	–		SO4/103	+	–	–
SO4/44	+	–	–		SO4/104	+	–	–

Continued…

Colony No.	Gram Staining (+/-)	Aminopeptidase Strip Test (+/-)	Cell Lysis Ability (+/-)		Colony No.	Gram Staining (+/-)	Aminopeptidase Strip Test (+/-)	Cell Lysis Ability (+/-)
SO4/106	+	–	–		SO4/132	+	–	–
SO4/107	+	–	–		SO4/133	+	–	–
SO4/108	+	–	–		SO4/134	+	–	–
SO4/109	+	–	–		SO4/135	–	+	+
SO4/110	+	–	–		SO4/136	+	–	–
SO4/111	+	–	–		SO4/137	+	–	–
SO4/112	+	–	–		SO4/138	+	–	–
SO4/113	+	–	–		SO4/139	+	–	–
SO4/114	+	–	–		SO4/140	+	–	–
SO4/115	–	+	+		SO4/141	–	+	+
SO4/116	+	–	–		SO4/142	+	–	–
SO4/117	+	–	–		SO4/143	–	+	+
SO4/118	–	+	+		SO4/144	+	–	–
SO4/119	–	+	+		SO4/145	+	–	–
SO4/120	+	–	–		SO4/146	–	+	+
SO4/121	+	–	–		SO4/147	+	–	–
SO4/122	–	+	+		SO4/148	–	+	+
SO4/123	+	–	–		SO4/149	+	–	–
SO4/124	+	–	–		SO4/150	+	–	–
SO4/125	–	+	+		SO4/151	–	+	+
SO4/126	–	+	+		SO4/152	+	–	+
SO4/127	+	–	–		SO4/153	+	–	–
SO4/128	–	+	+		SO4/154	+	–	+
SO4/129	+	–	–		SO4/155	+	–	–
SO4/130	–	+	+		SO4/156	–	+	+
SO4/131	+	–	–		SO4/157	+	–	–

3.2 Preliminary screening of biosurfactant-producing bacteria

All hemolytic colonies obtained from the primary culture plate (Figure 16) were further subcultured on the secondary culture plate (a grid BHI blood agar plate as shown in Figure 17 in order to screen the biosurfactant-producing bacteria which indicated with the inhibition zone surrounding the colonies. The colonies were purified on the tertiary culture plate of BHI blood medium (Figure 18) for further characterization such as for drop collapsing test, plasmid detection, mice lethality and antibiotic test. No α-hemolytic colony was observed in this study.

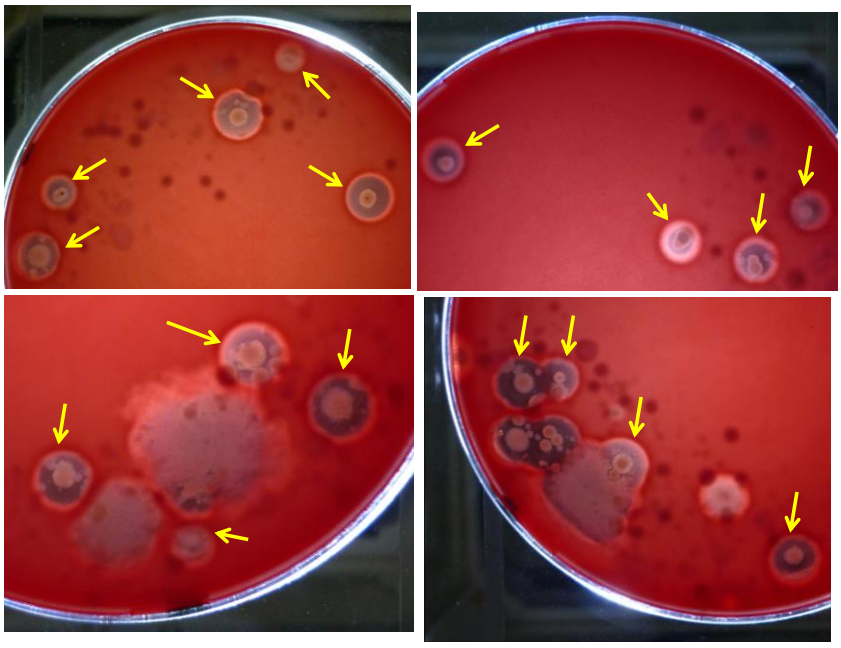

Figure 16: Demonstration of haemolytic colonies (yellow arrow) on BHI blood agar medium.

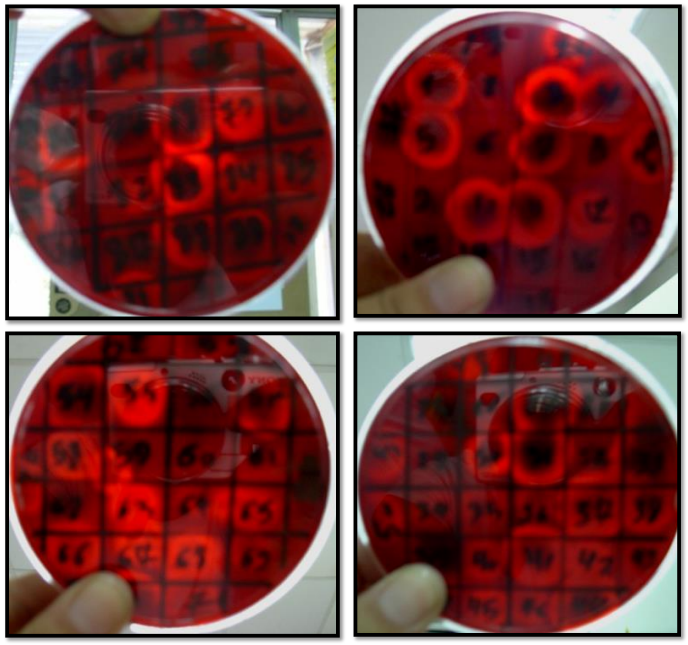

Figure 17: Screening of haemolytic activity of soil bacterial isolates on BHI blood agar medium.

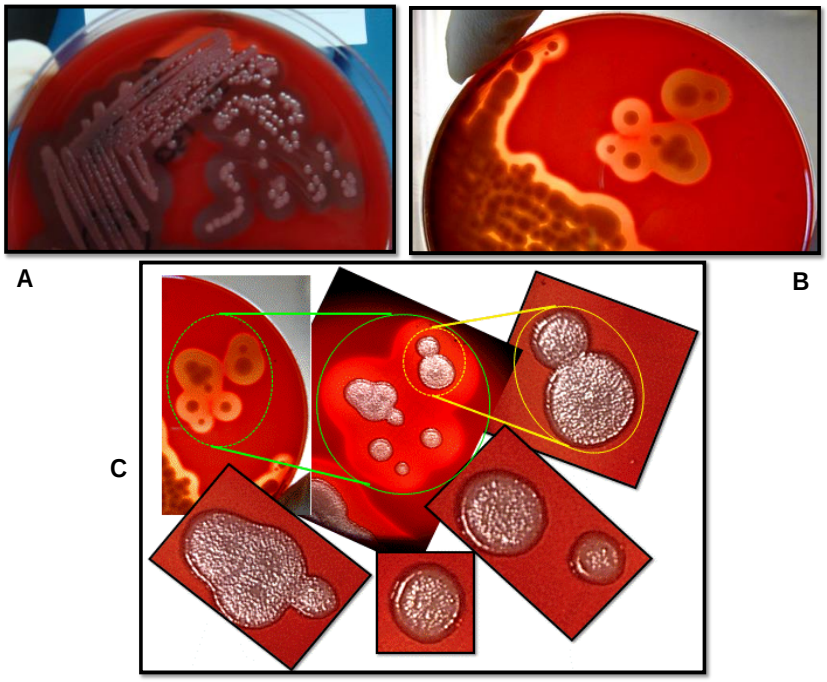

Figure 18: Single colony streaking on BHI blood agar plate showing a strong haemolytic activity of representative colony of unknown bacteria isolated and purified from agricultural soil: A; Front view, B; Back view, C; Close-up view purified colonies.

Screening for biosurfactant-producing bacteria was based on haemolytic and drop collapsing test as presented in Table 5. A drop collapsing test with emulsification activity is a simple method, sensitive, easy to perform and reproducible (Figure 19). 68 of 139 (49%) of the total isolates showed β-haemolytic activity and 71 of 139 (51%) were non haemolytic (Figure 20). 87% of the isolates were showing biosurfactant positive when tested against drop collapsing test (Figure 21). Among the 68 haemolytic isolates, 66 were biosurfactant positive ($HA^{+}CD^{+}$) and the other 2 were nonbiosurfactant producer ($HA^{+}CD^{-}$). Among 71 of the total nonhaemolytic isolates, 54 were biosurfactant positive ($HA^{-}CD^{+}$) and others 17 were nonbiosurfactant producer ($HA^{-}CD^{-}$) (Figure 22).

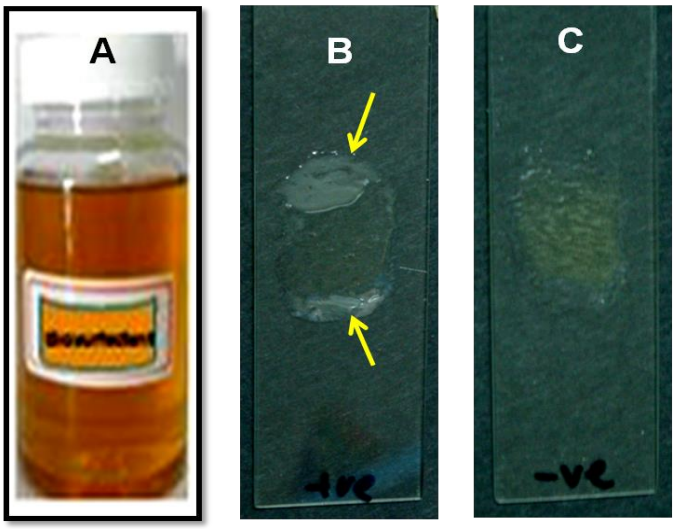

Figure 19: A; Cell-free supernatant containing biosurfactant activity (bacterial filtrate) of unknown bacteria isolated from agricultural soil. B; Positive test of drop collapsing test (yellow arrow). C; Negative test of drop collapsing test.

Table 5: Preliminary biosurfactant screening from IMO4 agricultural soil

Colony No.	Biosurfactant Activity		Colony No.	Biosurfactant Activity		Colony No.	Biosurfactant Activity	
	HA Test (+/-)	DC Test (+/-)		HA Test (+/-)	DC Test (+/-)		HA Test (+/-)	DC Test (+/-)
SO4/1	+	+	SO4/63	-	+	SO4/111	+	+
SO4/2	+	+	SO4/66	-	+	SO4/112	+	+
SO4/3	-	+	SO4/67	+	+	SO4/113	+	+
SO4/4	+	+	SO4/68	-	+	SO4/114	+	+
SO4/5	+	+	SO4/69	+	-	SO4/115	-	+
SO4/6	+	+	SO4/70	+	+	SO4/116	+	+
SO4/7	+	+	SO4/71	+	+	SO4/117	+	+
SO4/8	+	+	SO4/72	-	+	SO4/118	-	-
SO4/9	+	+	SO4/73	+	+	SO4/119	-	+
SO4/10	+	+	SO4/74	-	+	SO4/120	+	+
SO4/11	-	+	SO4/75	-	-	SO4/121	+	+
SO4/12	+	+	SO4/76	+	+	SO4/122	-	+
SO4/13	-	-	SO4/77	+	+	SO4/123	+	+
SO4/14	+	+	SO4/78	+	+	SO4/124	+	+
SO4/15	+	+	SO4/79	-	+	SO4/125	-	+
SO4/16	-	+	SO4/80	-	+	SO4/126	-	+
SO4/17	+	+	SO4/81	-	+	SO4/127	-	+
SO4/18	+	+	SO4/82	-	+	SO4/128	-	+
SO4/19	-	+	SO4/83	-	+	SO4/129	+	+
SO4/20	-	+	SO4/84	-	-	SO4/130	-	+
SO4/21	+	+	SO4/85	-	+	SO4/131	+	+
SO4/22	+	+	SO4/86	+	+	SO4/132	+	+
SO4/23	-	-	SO4/87	-	+	SO4/133	+	+
SO4/24	+	+	SO4/88	-	+	SO4/134	+	+
SO4/25	-	-	SO4/89	-	+	SO4/135	-	+
SO4/26	-	+	SO4/90	+	+	SO4/136	+	+
SO4/27	-	-	SO4/91	-	+	SO4/137	+	+
SO4/28	-	+	SO4/92	+	+	SO4/138	+	+
SO4/29	-	-	SO4/93	+	+	SO4/139	-	+
SO4/30	-	+	SO4/94	-	+	SO4/140	-	+
SO4/31	+	+	SO4/95	-	+	SO4/141	-	+
SO4/32	+	+	SO4/96	+	+	SO4/142	+	+
SO4/33	-	-	SO4/97	+	+	SO4/143	-	+
SO4/34	-	-	SO4/98	-	+	SO4/144	+	+
SO4/35	-	-	SO4/99	+	+	SO4/145	+	+
SO4/36	-	-	SO4/100	-	+	SO4/146	-	+
SO4/37	+	+	SO4/101	-	+	SO4/147	-	+
SO4/38	-	-	SO4/102	-	+	SO4/148	-	+
SO4/39	+	+	SO4/103	+	+	SO4/149	+	+
SO4/40	-	+	SO4/104	+	-	SO4/150	+	+
SO4/42	-	-	SO4/105	-	+	SO4/151	-	+
SO4/43	+	+	SO4/106	+	+	SO4/152	-	-
SO4/44	-	+	SO4/107	+	+	SO4/153	-	+
SO4/45	-	+	SO4/108	+	+	SO4/154	+	+
SO4/59	-	+	SO4/109	-	+	SO4/155	+	+
SO4/61	-	-	SO4/110	-	+	SO4/156	-	-
						SO4/157	+	+

HA = β - Hemolytic

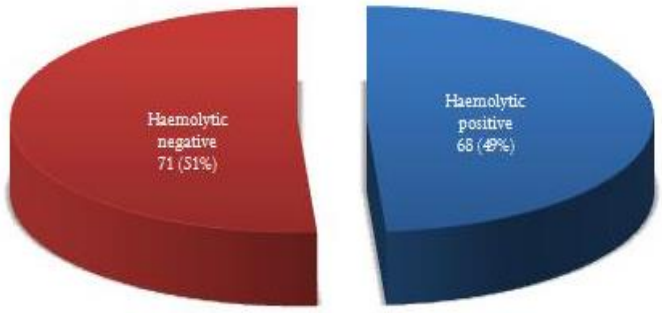

Figure 20: Pie chart showing the occurrence rate of haemolytic and nonhaemolytic of 139 unknown bacterial isolates tested on BHI agar medium.

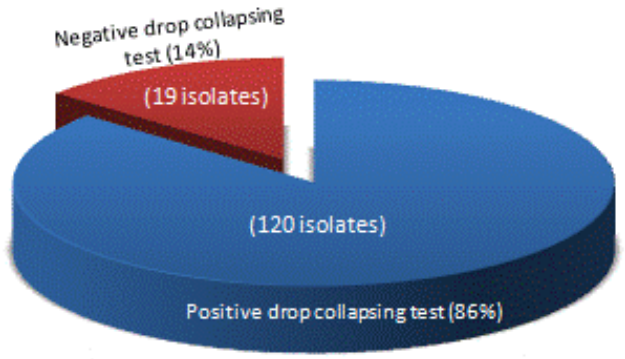

Figure 21: Pie chart showing the drop collapsing activity against 139 unknown soil bacterial isolates.

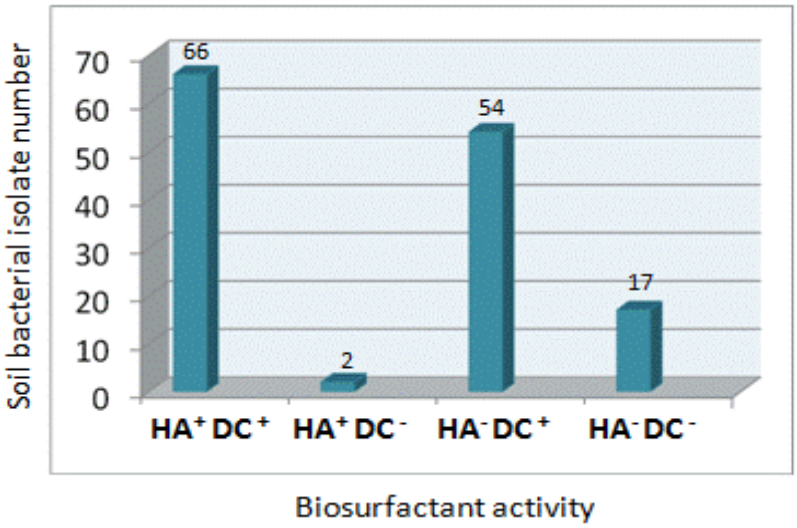

Figure 22: Bar chart showing the biosurfactant activity tested against 139 unknown soil bacterial isolates. HA^{+}; haemolytic positive. CD^{+}; positive drop

collapsing test; HA^-; haemolytic negative. CD^-; negative for drop collapsing test.

3.3 Antibiotic test

This was carried out to ascertain the antibiotic resistance pattern among 139 unknown soil bacterial isolates. However one of the isolate (SO4/156) was not carried out due to it was not survive. Figure 23 below shows the zone inhibition of representative soil bacteria. Ten (10) types of antibiotic discs were used against 138 soil bacterial isolates. All the results were recorded as shown in Table 6. 138 isolates (100%) were sensitivity to Vancomycin (Va). 135 of 138 (97.8%) of the isolates were sensitivity to gentamicin (Gm) and neomycin (Ne), followed by 133 isolates (96.4%) were sensitivity to Chloramphenicol (CM) & Erythromycin (Em), 131 isolates sensitive to Sulphonamides (S), 81 isolates (58.7%) sensitivity to Ampicillin (Am), 77 isolates (55.8%) sensitivity to Oxacillin (Ox) and Trimethoprim Sulphametaxzole (Sxt) and lastly only 39 isolates (29.3%) were sensitivity to Trimethoprim (SH) (Figure 7).

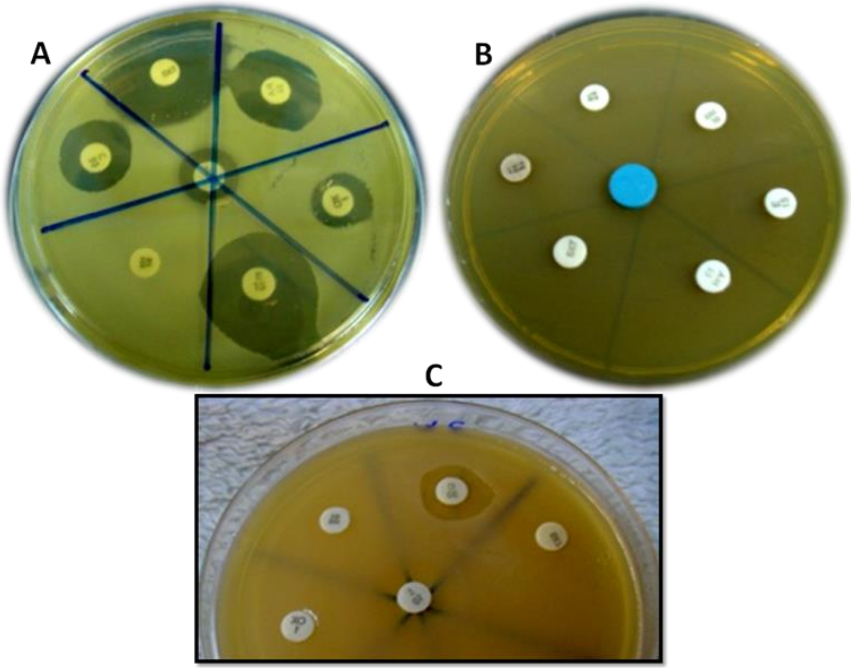

Figure 23: Antibiotic susceptibility plates showing bacterial growth among the tested antibiotics: A and C- Post incubation antibiotic plates showing clear zone inhibition; B- Prior to incubation.

Table 6: Antibiotic sensitivity test against 138 soil bacterial isolates

Colony No.	Am (10)	Cm (30)	Em (15)	Gm (10)	Ne (50)	OX (1)	S (10)	SH (10)	SXT (25)	VA (30)	Antibiogram Patterns
SO4/1	S	S	S	S	S	S	S	S	S	S	SSSSSSSSSS
SO4/2	S	S	S	S	S	S	S	S	S	S	SSSSSSSSSS
SO4/3	S	S	S	S	S	S	S	S	S	S	SSSSSSSSSS
SO4/4	R	S	S	S	S	S	S	R	R	S	RSSSSSSRRS
SO4/5	S	S	S	S	S	S	S	S	S	S	SSSSSSSSSS
SO4/6	S	S	S	S	S	S	S	S	S	S	SSSSSSSSSS
SO4/7	S	S	S	S	S	S	S	S	S	S	SSSSSSSSSS
SO4/8	S	S	S	S	S	S	S	S	S	S	SSSSSSSSSS
SO4/9	S	S	S	S	S	S	S	S	S	S	SSSSSSSSSS
SO4/10	S	S	S	S	S	S	S	S	S	S	SSSSSSSSSS
SO4/11	R	S	S	S	S	S	S	S	R	S	RSSSSSSSRS
SO4/12	S	S	S	S	S	S	S	S	S	S	SSSSSSSSSS
SO4/13	S	S	S	S	S	S	S	S	S	S	SSSSSSSSSS
SO4/14	S	S	S	S	S	S	S	S	S	S	SSSSSSSSSS
SO4/15	S	S	S	S	S	S	S	S	S	S	SSSSSSSSSS
SO4/16	S	S	S	S	S	S	S	S	S	S	SSSSSSSSSS
SO4/17	S	S	S	S	S	S	S	S	S	S	SSSSSSSSSS
SO4/18	S	S	S	S	S	S	S	S	S	S	SSSSSSSSSS
SO4/19	S	S	S	S	S	S	S	S	S	S	SSSSSSSSSS
SO4/20	R	S	S	S	S	S	S	R	R	S	RSSSSSSRRS
SO4/21	S	S	S	S	S	S	S	S	S	S	SSSSSSSSSS
SO4/22	S	S	S	S	S	S	S	S	S	S	SSSSSSSSSS
SO4/23	R	S	S	S	S	S	S	S	R	S	RSSSSSSSRS
SO4/24	S	S	S	S	S	S	S	S	S	S	SSSSSSSSSS
SO4/25	S	S	S	S	S	S	S	S	S	S	SSSSSSSSSS
SO4/26	S	S	S	S	S	S	S	S	S	S	SSSSSSSSSS
SO4/27	R	S	S	S	S	S	S	S	S	S	RSSSSSSSSS
SO4/28	S	S	S	S	S	S	S	S	S	S	SSSSSSSSSS
SO4/29	R	S	S	S	S	S	S	R	R	S	RSSSSSSRRS
SO4/30	S	S	S	S	S	R	S	R	S	S	SSSSSRSRSS
SO4/31	S	S	S	S	S	R	S	R	S	S	SSSSSRSRSS
SO4/32	S	S	S	S	S	S	S	S	S	S	SSSSSSSSSS
SO4/33	S	S	S	S	S	S	S	R	R	S	SSSSSSSRRS
SO4/34	S	S	S	S	S	R	S	R	S	S	SSSSSRSRSS
SO4/35	R	S	S	S	S	S	S	R	S	S	RSSSSSSRSS
SO4/36	S	S	S	S	S	R	S	R	R	S	SSSSSRSRRS
SO4/37	S	S	S	S	S	R	S	S	S	S	SSSSSRSSSS
SO4/38	S	S	S	S	S	R	S	S	S	S	SSSSSRSSSS
SO4/39	R	S	S	S	S	R	S	R	S	S	RSSSSRSRSS

Continued....

Colony No.	Am (10)	Cm (30)	Em (15)	Gm (10)	Ne (50)	OX (1)	S (10)	SH (10)	SXT (25)	VA (30)	Antibiogram Patterns
SO4/40	R	S	S	S	S	R	S	S	S	S	RSSSSRSSSS
SO4/42	R	S	S	S	S	S	S	S	S	S	RSSSSSSSSS
SO4/43	S	S	S	S	S	S	S	S	S	S	SSSSSSSSSS
SO4/44	S	S	S	S	S	S	S	S	S	S	SSSSSSSSSS
SO4/45	S	S	S	S	S	S	S	S	S	S	SSSSSSSSSS
SO4/59	R	S	S	S	S	S	S	S	R	S	RSSSSSSSRS
SO4/61	R	S	S	S	S	R	S	R	R	S	RSSSSRSRRS
SO4/63	R	S	S	S	S	R	S	R	R	S	RSSSSRSRRS
SO4/66	R	S	S	S	S	R	S	R	R	S	RSSSSRSRRS
SO4/67	R	S	S	S	S	R	S	R	R	S	RSSSSRSRRS
SO4/68	S	S	S	S	S	S	S	R	S	S	SSSSSSSRSS
SO4/69	R	S	S	S	S	R	S	R	R	S	RSSSSRSRRS
SO4/70	R	R	S	S	R	R	S	R	R	S	RRSSRRSRRS
SO4/71	R	S	S	R	S	R	S	R	R	S	RSSRSRSRRS
SO4/72	R	S	S	S	S	R	S	R	S	S	RSSSSRSRSS
SO4/73	R	S	S	S	R	S	S	R	R	S	RSSSRSSRRS
SO4/74	S	S	S	S	S	S	S	R	S	S	SSSSSSSRSS
SO4/75	S	S	S	S	S	S	S	R	S	S	SSSSSSSRSS
SO4/76	R	S	S	S	S	S	S	R	S	S	RSSSSSSRSS
SO4/77	R	S	S	S	S	R	S	R	S	S	RSSSSRSRSS
SO4/78	S	S	S	S	S	S	S	R	S	S	SSSSSSSRSS
SO4/79	S	S	S	S	S	R	S	R	S	S	SSSSSRSRSS
SO4/80	S	S	S	S	S	R	S	R	R	S	SSSSSRSRRS
SO4/81	S	S	S	S	S	R	S	R	S	S	SSSSSRSRSS
SO4/82	S	S	S	S	S	R	S	R	S	S	SSSSSRSRSS
SO4/83	S	S	S	S	S	R	S	R	R	S	SSSSSRSRRS
SO4/84	S	S	S	R	S	R	S	R	R	S	SSSRSRSRRS
SO4/85	S	S	S	S	S	S	R	R	S	S	SSSSSSRRSS
SO4/86	S	S	S	S	S	S	S	R	S	S	SSSSSSSRSS
SO4/87	S	S	S	S	S	R	S	R	S	S	SSSSSRSRSS
SO4/88	S	S	S	S	S	S	S	R	R	S	SSSSSSSRRS
SO4/89	R	S	R	S	S	R	S	R	S	S	RSRSSRSRSS
SO4/90	R	S	S	S	S	R	S	R	S	S	RSSSSRSRSS
SO4/91	R	S	S	S	S	R	S	R	R	S	RSSSSRSRRS
SO4/92	S	S	S	S	S	R	S	R	R	S	SSSSSRSRRS
SO4/93	R	S	S	S	S	R	S	R	R	S	RSSSSRSRRS
SO4/94	S	S	R	S	S	R	S	R	R	S	SSRSSRSRRS
SO4/95	S	S	S	S	S	R	S	R	S	S	SSSSSRSRSS

Continued....

Colony No.	Am (10)	Cm (30)	Em (15)	Gm (10)	Ne (50)	OX (1)	S (10)	SH (10)	SXT (25)	VA (30)	Antibiogram Patterns
SO4/96	S	S	S	S	S	R	S	R	R	S	SSSSSRSRRS
SO4/97	R	R	R	S	S	R	R	R	R	S	RRRSSRRRRS
SO4/98	R	S	R	S	S	S	S	R	S	S	RSRSSSSRSS
SO4/99	R	R	S	R	R	S	S	R	R	S	RRSRRSSRRS
SO4/100	S	S	S	S	S	S	S	R	S	S	SSSSSSSRSS
SO4/101	S	S	S	S	S	S	S	R	R	S	SSSSSSSRRS
SO4/102	S	S	S	S	S	S	S	R	R	S	SSSSSSSRRS
SO4/103	R	S	S	S	S	S	S	R	R	S	RSSSSSSRRS
SO4/104	R	S	S	S	S	S	S	R	R	S	RSSSSSSRRS
SO4/105	R	S	S	S	S	S	S	R	R	S	RSSSSSSRRS
SO4/106	S	S	S	S	S	S	S	R	S	S	SSSSSSSRSS
SO4/107	R	S	S	S	S	S	S	R	R	S	RSSSSSSRRS
SO4/108	R	S	S	S	S	S	S	S	R	S	RSSSSSSSRS
SO4/109	S	S	S	S	S	S	S	S	S	S	SSSSSSSSSS
SO4/110	S	S	S	S	S	S	S	R	S	S	SSSSSSSRSS
SO4/111	S	S	S	S	S	S	R	R	S	S	SSSSSSRRSS
SO4/112	S	S	S	S	S	S	R	R	R	S	SSSSSSRRRS
SO4/113	R	S	S	S	S	S	S	R	R	S	RSSSSSSRRS
SO4/114	S	S	S	S	S	S	S	R	S	S	SSSSSSSRSS
SO4/115	S	S	S	S	S	S	S	R	S	S	SSSSSSSRSS
SO4/116	S	S	S	S	S	S	S	R	R	S	SSSSSSSRRS
SO4/117	S	S	S	S	S	R	S	R	R	S	SSSSSRSRRS
SO4/118	R	S	S	S	S	R	S	R	R	S	RSSSSRSRRS
SO4/119	R	S	S	S	S	R	S	R	R	S	RSSSSRSRRS
SO4/120	R	S	S	S	S	R	S	R	R	S	RSSSSRSRRS
SO4/121	R	S	S	S	S	R	S	R	R	S	RSSSSRSRRS
SO4/122	S	S	S	S	S	R	S	R	R	S	SSSSSRSRRS
SO4/123	R	S	S	S	S	R	S	R	R	S	RSSSSRSRRS
SO4/124	R	S	S	S	S	R	S	R	R	S	RSSSSRSRRS
SO4/125	R	S	S	S	S	R	S	R	R	S	RSSSSRSRRS
SO4/126	S	S	S	S	S	R	R	R	R	S	SSSSSRRRRS
SO4/127	S	S	S	S	S	S	S	R	R	S	SSSSSSSRRS
SO4/128	R	S	S	S	S	S	S	R	S	S	RSSSSSSRSS
SO4/129	S	S	S	S	S	S	S	R	S	S	SSSSSSSRSS
SO4/130	R	S	S	S	S	S	S	R	R	S	RSSSSSSRRS

Continued....

Colony No.	Am (10)	Cm (30)	Em (15)	Gm (10)	Ne (50)	OX (1)	S (10)	SH (10)	SXT (25)	VA (30)	Antibiogram Patterns
SO4/131	S	S	S	S	S	S	S	R	S	S	SSSSSSSRSS
SO4/132	S	R	S	S	S	R	R	R	R	S	SRSSSRRRRS
SO4/133	S	S	S	S	S	S	S	R	S	S	SSSSSSSRSS
SO4/134	R	S	S	S	S	R	S	R	S	S	RSSSSRSRSS
SO4/135	S	S	S	S	S	R	S	R	S	S	SSSSSRSRSS
SO4/136	R	S	S	S	S	R	S	R	R	S	RSSSSRSRRS
SO4/137	R	R	S	S	S	R	S	R	S	S	RRSSSRSRSS
SO4/138	R	S	S	S	S	R	S	R	R	S	RSSSSRSRRS
SO4/139	S	S	S	S	S	R	S	R	S	S	SSSSSRSRSS
SO4/140	R	S	S	S	S	R	S	R	S	S	RSSSSRSRSS
SO4/141	R	S	S	S	S	S	S	R	S	S	RSSSSSSRSS
SO4/142	R	S	S	S	S	R	S	R	R	S	RSSSSRSRRS
SO4/143	R	S	S	S	S	R	S	R	S	S	RSSSSRSRSS
SO4/144	R	S	S	S	S	S	S	R	S	S	RSSSSSSRSS
SO4/145	S	S	S	S	S	S	S	R	S	S	SSSSSSSRSS
SO4/146	S	S	R	S	S	S	S	R	R	S	SSRSSSSRRS
SO4/147	S	S	S	S	S	R	S	R	S	S	SSSSSRSRSS
SO4/148	S	S	S	S	S	R	S	R	S	S	SSSSSRSRSS
SO4/149	R	S	S	S	S	R	S	R	R	S	RSSSSRSRRS
SO4/150	S	S	S	S	S	R	S	R	R	S	SSSSSRSRRS
SO4/151	S	S	S	S	S	S	S	R	S	S	SSSSSSSRSS
SO4/152	R	S	S	S	S	R	R	R	R	S	RSSSSRRRRS
SO4/153	S	S	S	S	S	S	S	S	S	S	SSSSSSSSSS
SO4/154	R	S	S	S	S	R	S	R	R	S	RSSSSRSRRS
SO4/155	R	S	S	S	S	R	S	R	R	S	RSSSSRSRRS
SO4/156	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	NA
SO4/157	R	S	S	S	S	R	S	R	R	S	RSSSSRSRRS
E. coli ATCC 25922	S	S	S	S	S	S	S	S	S	S	SSSSSSSSSS
S. aureus ATCC 25923	-	-	-	-	-	-	-	-	-	S	S

ND; not done
NA; not available

Table 7: Antibiotic susceptibility of the bacterial isolates

Antibiotic test	Number of isolates sensitive to tested antibiotics	% sensitivity
Ampicillin(Am)	81	58.7
Chloramphenicol (Cm)	133	96.4
Erythromycin(Em)	133	96.4
Gentamicin (Gm)	135	97.8
Neomycin (Ne)	135	97.8
Oxacillin (Ox)	77	55.8
Sulphonamides(S)	131	94.9
Trimethoprim (SH)	39	28.3
Trimethoprim Sulphametaxazole (Sxt)	77	55.8
Vancomycin (Va)	138	100

The twenty nine (29) antibiogram patterns obtained from this experiment were shown in Table 8. Twenty nine (21%) isolates sensitive to all 10 antibiotics tested. *E. coli* ATCC 25922 was inhibited by all the antibiotics used in this study, at the respective concentrations tested, whereas the *S. aureus* ATCC 25923 was used for vancomycin sensitive control bacteria against all the unknown bacterial isolates. 29 isolates were found sensitive to all 10 antibiotic tested and 21 of 138 isolate were sensitive to 9 antibiotics tested, followed with 28 isolates sensitive to 8 antibiotics, 26 isolates sensitive to 7 antibiotics, 28 isolates sensitive to 6 antibiotics, 3 isolates sensitive to 5 antibiotics, 2 isolates sensitive to 4 antibiotics, 1 isolate sensitive to 3 antibiotics and resistant to 7 types of antibiotics (Table 8).

Table 8: Antibiotic resistance, sensitivity and the antibiogram of bacterial isolates from formulated soil level IMO4

Number	Antibiogram patterns	Total number of bacterial isolates	Percentage (%)
1	SSSSSSSSS	29	21
2	RSSSSRSRRS	22	15.9
3	SSSSSSSRSS	15	10.9
4	SSSSSRSRSS	13	9.4
5	RSSSSSSRRS	10	7.3
6	SSSSSRSRRS	8	5.8
7	SSSSSSSRRS	6	4.4
8	RSSSSRSRSS	5	3.6
9	RSSSSSSSS	4	2.9
10	RSSSSSSRS	3	2.2
11	RSSSSSSRSS	3	2.2
12	SSSSSRSSSS	2	1.5
13	SSSSSSRRSS	2	1.5
14	RSSSSRSSSS	1	0.7
15	RRSSRRSRRS	1	0.7
16	RSSRSRSRRS	1	0.7
17	RSSSRSSRRS	1	0.7
18	SSSRSRSRRS	1	0.7
19	RSRSSRSRSS	1	0.7
20	SSRSSRSRRS	1	0.7
21	RRRSSRRRRS	1	0.7
22	RSRSSSSRSS	1	0.7
23	RRSRRSSRRS	1	0.7
24	SSSSSSRRRS	1	0.7
25	SSSSSRRRRS	1	0.7
26	SRSSSRRRRS	1	0.7
27	RRSSSRSRSS	1	0.7
28	SSRSSSSRRS	1	0.7
29	RSSSSRRRRS	1	0.7
Total isolates		**138**	

3.4 Presence of plasmid in soil bacterial isolates

No plasmid band was detected in agarose gel from all the 138 isolates using all the extraction techniques in Section 2.18 (Figure 24 and 25).

Figure 24: 0.7% Agarose gel electrophoresis of plasmid DNA analysis showing chromosomal DNA of representative soil bacterial isolates alongside with the DNA molecular weight marker:

Lane 1: Unknown Gram positive cocci bacteria

Lane 2: Unknown Gram positive bacilli bacteria

Lane 3: Unknown Gram negative cocci bacteria

Lane 4: Unknown Gram negative bacilli bacteria

Lane 5: *P. haemolyticus* ATCC BAA-2268 strain 139SI chromosome (CHR)

Lane 6: Supercoil DNA marker/ladder

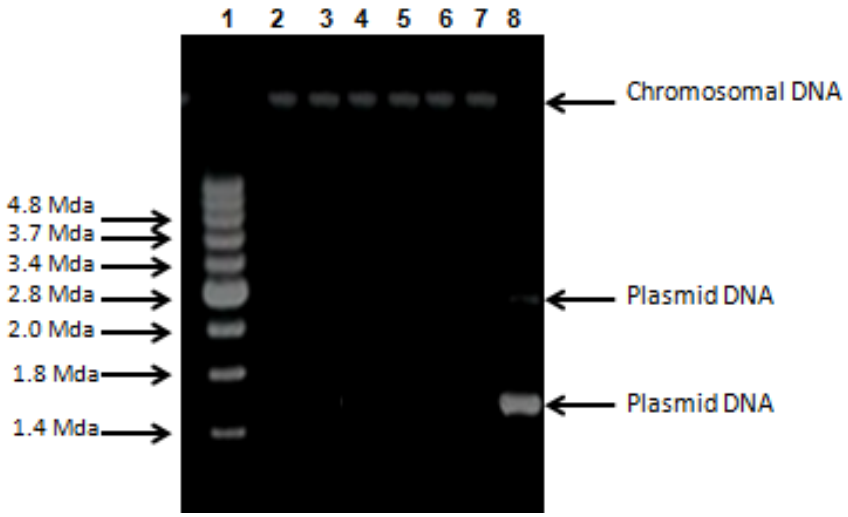

Figure 25: 0.7% Agarose gel electrophoresis of plasmid DNA analysis showing chromosomal DNA of representative soil bacterial isolates alongside with the DNA molecular weight marker:

Lane 1: *E. coli* plasmid DNA marker

Lane 2: Unknown Gram positive cocci bacteria

Lane 3: Unknown Gram positive bacilli bacteria

Lane 4: Unknown Gram negative cocci bacteria

Lane 5: Unknown Gram negative bacilli bacteria

Lane 6: *P. haemolyticus* ATCC BAA-2268 strain 139SI chromosomal DNA

Lane 7: *E. coli* chromosomal DNA

Lane 8: *E. coli* plasmid DNA (2.7 kb = 1.62 Mda)

3.5 Mouse pathogenicity studies on unknown soil bacterial isolates

This study was carried out to determine toxin production among the unknown soil bacterial isolates via mice injection. The toxicity activity determined as a lethality of the mice within 72 hours. For virulence determination, the bacterial cells of each isolates were injected intraperitoneally into the mice. For toxigenicity determination the cell-free supernatant of the isolates were injected intraperitoneally. Positive control used was *P. multocida* PMB202 virulent strain. Negative controls were saline solution (NaCl, 0.85%) and non-virulent strains of *E. coli* ATCC 225922 and *P. haemolyticus* ATCC BAA-2268 strain 139SI.

3.5.1 Virulence of soil bacterial isolates in mice

Groups of six ICR mice (provided by the Central Animal House, University Hospital) were injected with 10^9 CFU of overnight grown soil bacterial cultures. It was found that all soil bacterial isolates were non-virulent to the mice tested.

3.5.2 Toxicity of cell-free supernatant in mice

Bacterial cell-free supernatant were injected in mice intraperitoneally to determine their toxicity. It was found that all isolates from agricultural soil produced extracellular of non-toxigenic compounds. None of the mice were affected by the bacterial isolates compared to the controls.