International Research Journal of Environment Sciences E-ISSN 2319–1414
Vol. **5(9),** 22-26, September **(2016)** *Int. Res. J. Environment Sci.*

Determining the Presence of *Bdellovibrio* spps in Soils from Parts of Benue State, Nigeria

Sar T. Terdzungwe[1*], Umeh U. Ebele[1], Amali Okwoli[1] and Akosu D. Daniel[2]
[1]Department of Biological Sciences, University of Agriculture, Makurdi, Nigeria
[2]Department of Biological Sciences, University of Mkar, Mkar, Gboko, Nigeria
terdzungwe@yahoo.com

Available online at: www.isca.in, www.isca.me
Received 10th June 2016, revised 20th August 2016, accepted 16th September 2016

Abstract

Bdellovibrio are Gram-negative bacteria that are predatory on other Gram–negative bacterial cells. To investigate the occurrence of Bdellovibrio in some Nigerian soils, 81 soil samples of three types; Loamy, Sandy and Swampy from three locations in Benue State, Gboko, Katsina-Ala and Makurdi were characterised, suspended in sterile distilled water, membrane filtered, cultured and incubated at 35°C for between 36 to 120 hours, on three host bacteria namely E. Coli, Salmonella and Shigella respectively. A total of 409 Plaque Forming Units (PFUs) were observed, all on soil sample cultures from sites in Makurdi. In the distribution of PFUs on various soil types, the highest occurrence of 100 (24.4%) PFUs were observed from loamy soil cultures, while the least total occurrence of 46 (11.25%) PFUs were from Swampy soil sites. However, cultures from Makurdi Swampy soils showed occurrences of between 60 to 80 PFUs. With regards to host preferences, the highest occurrence of 286 (69.92%) PFUs, were observed on S. typhi, while Shigella and E. coli had 73 (17.85%) and 50 (12.22%) PFUs respectively. While the presence of the Bdellovibrio spps in soil niches of Nigeria has been established, the unpredictable occurrence and distribution on soils in Benue State warrants further investigation to determine if a specific pattern exists or other factors could be responsible for the observed distribution, for effective future exploitation.

Keywords: *Bdellovibrio,* Plaque Forming Units, Prey bacteria, Soils, Benue, Nigeria.

Introduction

Bdellovibrio are extremely small, vibroid, Gram-negative bacteria that have size ranges of between 0.3 -0.5 *µm* by 1.4 -2.5 *µm*. They are highly motile, with single sheathed polar flagellae which exhibit characteristic dampened waveforms. They are predatory, attacking and preying on other Gram-negative bacteria, including animal and plant pathogens. Using an array of degradative enzymes, they create pores in the host cell walls, access the periplasm, use prey cytoplasmic contents as nutrients for growth and reproduction, and finally burst the host cell envelopes[1].

Bdellovibrio species attack a wide range of Gram-negative bacterial species in nature and have aroused interest because their unique lifestyle has potential for applications in controlling some human infections, especially as they have no known or demonstrable effects on cells other than prokaryotes, and can thus be safely employed in control of infections of eukaryotic tissue, such as wound infections and dental/oral biofilms[2].

Materials and Methods

Study Area: The study was carried out in 3 locations of Benue State, north-central Nigeria; Gboko, Katsina-Ala and Makurdi respectively. Benue State has the following coordinates; latitude 7.333^{0} and longitude 8.75^{0}, and covers an area of about 34, 059 km^{2}. Makurdi town is the capital. Other major towns in the state are Adikpo, Gboko, Katsina-ala, Otukpa, Otukpo and Vandeikya among others. The climate is typically tropical, with two seasons; rainy or wet season, between April to October, and Dry (Harmattan) season, between November to March. Mean annual temperatures fluctuate between 23 and 31^{0}C. The state has rich soil deposits and is a rich agricultural region[3].

Soil Samples Collection: Three sites each, in three locations of Benue state, Gboko, Katsina-Ala and Makurdi were selected for determining *Bdellovibrio* distribution in local soils. Eighty-one Loamy, Sandy and Swampy soil samples, each in triplicate, were collected at three different periods of each sampling day: morning, afternoon and evening, at average depths of about 30cm from the surface and investigated for *Bdellovibrio* occurrence. Each sample was immediately sent to the laboratory for analysis after collection.

Protocol for Isolation of *Bdellovibrio* from Soil Samples: Isolations from Soil were carried out using the method of Varon and Shilo[4]; with a Soil Auger, soil samples, each weighing about 100g, were collected at a depth of between 10 to 30 cm from the earth's surface. Each sample was suspended in 100 ml distilled water and agitated on a Rotary Shaker for 30 minutes to ensure loosening of soil clumps and uniform distribution of organisms. The soil suspensions were triple-filtered using Watman No.1 - (9 mm diameter) - filter paper to remove large soil particles and other aggregated materials. The filtrates

obtained were re-filtered using membrane filters of pore size 0.45 *µm*. Thus *Bdellovibrio*, usually smaller than this diameter, passed through the membrane filters, while larger organisms were retained. Each filtrate was diluted five-fold and one tenth ml of 10^{-5} dilution mixed with 48 hour nutrient broth cultures of host organisms *E. coli*, *S. typhi* and *Shigella* respectively. The mixture of filtrate *(Bdellovibrio)*, and respective host bacterium was mixed with 25 ml of 50% (soft) Nutrient Agar, and poured over previously solidified Nutrient agar in Petri dishes. The cultured plates were incubated for 3 – 5 days at $37^{O}C$ and observed for *Bdellovibrio* plaques, reported as Plaque Forming Units (PFUs). The number of viable organisms per ml of sample was calculated. Random plaques were examined under high power microscopic fields for small, rapidly swimming *Bdellovibrio* cells.

Determination of Physico-Chemical Parameters of Soils: Hydrogen Ion Concentration (pH): The procedure was carried out as follows: two level 2 ml scoops of soil were added to 10 ml deionised water and gently shaken for one to two minutes, after which the pH electrode was inserted and the soil pH read.

Soil Moisture Content (SMC): The procedure was carried out as follows: an empty crucible was weighed and recorded as weight (W_1). 10 g of soil samples were taken in the weighed crucible, weighed and recorded as weight two (W_2). The soil samples were heated on a hot plate for 30 minutes, allowed to cool and the weight recorded as W_3. Various samples were heated for periods of 20, 15 and 10 minutes respectively and the weights recorded as W_4, W_5 and W_6 respectively. From the results obtained, the moisture content was calculated.

Organic Matter Content (OMC): The procedure was carried out using the Dichromate method: the stored program number for soil organic matter was entered. The dial for setting wavelength was turned until the display showed 610 nm as required. 1.00 g of soil was weighed and transferred to an Erlenmeyer flask of 250 ml capacity. 10 ml of 1N Potassium Dichromate solution was then pipetted into an empty 250 ml Erlenmeyer flask. 20.0 ml of concentrated Sulphuric acid was poured into each flask. Each flask was gently swirled to mix, and the contents placed on a cooling pad for 10 minutes. One hundred ml of de-ionised water to each flask which and swirled to mix. Twenty-five ml of the soil was filtered into a 50 ml Erlenmeyer flask. The blanks were filtered using glass fibre filters. 25 ml of the blank solution was poured into a sample cell holder. The blank was placed in the cell holder, and the light aperture shield closed. 25 ml of the filtered soil solution was poured into a sample cell holder and the light aperture shield closed. The results, in per cent organics, were displayed.

Oxygen Content (OC): Each soil sample was collected in a clean Biological Oxygen Demand (BOD) glass bottle, mixed with deionised water, and allowed to overflow the bottle for 2 – 3 minutes to ensure elimination of air bubbles. The contents of two reagents - Dissolved Oxygen 1 and Dissolved Oxygen 2 powder pillow were added. The bottle was immediately corked and gently shaken to mix. An orange-brown flocculent precipitate formed, if oxygen was absent, settled slowly. After about five minutes, the bottle was again inverted several times and stood until the flocs again settled. The contents of one dissolved oxygen 3 powder pillow were added, the stopper replaced without trapping air and inverted several times to mix. The floc dissolved and a yellow colour was left if oxygen was present. 20 ml of the sample was transferred to a 250 ml Erlenmeyer flask. The solution was titrated with 0.2000 N Sodium Thiosulfate until the sample changed from colour yellow to colourless. Total Dissolved Oxygen value was then calculated[5].

Soil Temperature (ST): A thermometer was inserted into a bore made in the soil. The reading was taken with the thermometer *in situ* in the soil and recorded.

Results and Discussion

Table-1 shows the physico-chemical properties of 81 Loamy, Sandy and Swampy soil samples investigated for *Bdellovibrio* presence. Average Moisture Content (AMC) of the soils from Gboko was 15.9%; Katsina-Ala, 15.9%, while Makurdi soils had 7.12% AMC respectively. Gboko soils had an average temperature of 22.4 ^{O}C; Katsina-Ala samples 26.7 ^{O}C, while Makurdi samples averaged 27.8 ^{O}C respectively. In terms of pH, Gboko soils had an average of 8.24, Katsina-ala 9.32, while Makurdi samples had 8.25 as average pH.

Table-2 shows the occurrence and isolation of *Bdellovibrio* from soils in Benue State. A total of 359 PFUs were observed on host bacteria. 50(13.9%) plaques were formed on *E. coli*, 286(79.7%) were observed on *S. typhi,* while 73(20.3%) were on *Shigella* spp. With regards to occurrence on soil sample type, 159(44.3%) PFUs were obtained from loamy soil sites, 52(14.5%) from sandy soils and 208(57.9%) from swampy soils, all from sample sites in Makurdi. Interestingly, no plaques were observed on plates cultured from similar soil samples from sites in Gboko and Katsina-Ala.

Discussion: Several factors could have influenced the isolation of *Bdellovibrio* from soils, such as the soil moisture content, Soil depth at which soil samples were taken, nutrient availability, acidity and temperature. According to some authors, the density and composition of the flora in a soil is dramatically affected by environmental conditions[6]. Wet soils, unfavourable for aerobic microbes, may have diminished their numbers to such levels as to make them unavailable for predation by *Bdellovibrio*. This may account for scanty isolation of *Bdellovibrio* from swamps or other soils with high moisture contents. This premise however, makes the non-isolation of *Bdellovibrio* from the relatively drier soils difficult to explain, especially as the highest average moisture content of the soil samples was only 35.54%, not high enough to have prevented their occurrence. The average moisture content of the soils was also not a constant value, but fluctuated periodically, depending

on the amount of precipitation available to the soil, mostly from rainfall. However, other prevailing soil conditions at the time of sampling may have also had an impact on the organism's occurrence.

Table-1
Physico-Chemical Properties (PCPs) of Soil Samples

Soil Sample Code	**PCP** (Averages)				
	OMC (mg/L)	**MC** (%)	**Temp**(^{O}C)	**pH**	**OC**(mg/L)
GBL1	4.50	22.21	22	7.82	10.00
GBL2	3.62	9.57	22	7.22	6.90
GBL3	4.57	21.90	22	8.75	3.90
GBS1	3.96	11.95	20	8.19	2.30
GBS2	1.77	8.72	22	8.72	6.70
GBS3	2.11	8.12	22	8.81	6.10
GBW1	3.95	19.78	24	7.93	7.70
GBW2	4.63	21.54	24	8.15	15.0
GBW3	3.86	20.20	24	8.64	9.80
KAL1	3.16	8.78	28	9.42	3.70
KAL2	2.70	12.49	26	9.46	4.50
KAL3	2.99	8.04	26	9.16	7.80
KAS1	1.94	9.34	25	9.53	1.60
KAS2	3.28	12.20	27	9.46	5.00
KAS3	2.54	9.68	27	9.47	6.60
KAW1	4.65	21.91	26	8.78	5.40
KAW2	4.12	25.27	27	9.23	1.30
KAW3	4.85	35.54	28	9.36	0.60
MKL1	4.93	0.99	29	7.82	7.70
MKL2	4.57	4.53	28	7.22	4.80
MKL3	4.79	4.52	26	8.75	4.00
MKS1	2.50	0.021	26	8.19	7.70
MKS2	3.75	12.48	30	8.72	10.00
MKS3	4.32	5.03	28	8.81	5.60
MKW1	4.72	16.30	28	7.93	4.90
MKW2	4.11	10.70	30	8.15	10.50
MKW3	3.93	9.49	26	8.64	9.20

PCP = Physico-Chemical Property; OMC = Organic Matter Content; MC = Moisture Content; Temp = Temperature; pH = Hydrogen ion Concentration; OC= Oxygen Content APS = Average Particle Size; GB = Gboko; KA = Katsina-Ala; MK = Makurdi; SA1, SA2, SA3 = Sandy soils sites 1, 2 and 3; L1, L2, L3= Loamy soils sites 1, 2 and 3; W1, W2 and W3 = Swampy soils sites 1, 2 and 3**.**

Table-2
***Bdellovibrio* Isolated from Soil Samples**

Site Code	Culture Conditions			Observation(s) PFUs on Host Bacteria			Total	PFU/ml
	No. (%)	Temp(^{O}C)	Period (Hrs)	*E. coli*	*S.* typhi	*Shigella*	**No. (%)**	
MKS3	3	35	36	-	-	52(12.7	52(12.7)	5.2 x 10^{-3}
MKL1	3	35	36	-	50(12.2)	09(2.2)	59(14.4)	5.9 x 10^{-3}
MKL2	3	35	36	50(12.2)	50(12.2)	-	100(24.4)	1 x 10^{-2}
MKL3	3	35	36	-	-	-	-	-
MKW1	3	35	36	-	80(19.6)	12(2.9)	92(22.5)	9.2 x 10^{-3}
MKW2	3	35	36	-	60(14.7)	-	60(14.7)	6.0 x 10^{-3}
MKW3	3	35	36	-	46(11.2)	-	46(11.2)	4.6 x 10^{-3}
Total	-	-	-	50(12.2)	**286**(69.9)	**73**(17.8)	**409**	

MKS3 = Makurdi Sandy Soil Site 3; MKL1 = Makurdi Loamy Soil Site 1; MKL2 = Makurdi Loamy Soil Site 3; MKW1 = Makurdi Swampy Soil Site 1; MKW2 = Makurdi Swampy Soil Site 2; MKW3 = Makurdi Swampy Soil Site 3

Another crucial factor, the presence of antagonistic chemicals, such as herbicides, in the drier soils may also have inhibited *Bdellovibrio* growth. The effects of 17 commercial herbicides for activity against *Bdellovibrio* isolated from soil and its *Pseudomonas* host was studied. It was found that 11(64.71%) of the herbicides inhibited *Bdellovibrio* plaque development[7]. In this study, soil samples were obtained from farm sites, rice paddies, semi-urban areas or other sites with significant human agricultural activity, where in recent times there have been high rates of application of herbicides, insecticides or other farm chemicals, such as fertilizers, to control the occurrence of pests, weeds and to promote growth of crops[8, 9]. It is thus possible that these chemicals may have affected the rather fastidiously sensitive resident soil *Bdellovibrio*, and reduced their numbers to such levels that further dilution of the samples for their laboratory enumeration reduced them to undetectable levels.

Furthermore, the depth of 30 cm from the surface at which samples were taken may also not have been the optimum depth or niche for *Bdellovibrio* activity. High tropical temperatures could be extremely high, such that the rather temperature sensitive *Bdellovibrio* may have been driven deeper into the soils, rather than occur at surfaces.

In the tested soil samples, it was observed that the highest frequency of isolation of *Bdellovibrio* was on *S. typhi* as host. In a study to determine the presence of *Bdellovibrio* in water bodies in Benue state, Nigeria, *E. coli* was recorded as the host with the highest frequency of *Bdellovibrio* isolation[10]. This observation could be related to the survival periods of these respective organisms in these habitats. Some authors have reported that compared to *S. typhi*, *E. coli* has a longer survival period in water, and is an indicator organism in testing for animal faecal contamination of water bodies[11]. However, in soils, other authors have shown that *S. typhi* may survive for longer periods as compared to *E. coli*[12]. As observed for *E. coli* in water, the relatively longer survival of *S. typhi* in soils may have made it, by natural selection and co-evolution, the prey of choice for *Bdellovibrio* over *E. coli*. To further give credence this reasoning, survival periods in sandy soils for *S. typhi* ranging between 29 and 58 days, which survival period increased substantially to about 74 days on the addition of small quantities of manure to the soils have been reported[13]. Typically, soils investigated in this study were rich in organic matter. Of course the OM content of the soils would have had no direct influence on *Bdellovibrio*, as no really important nutrients enter into microbial cells from the environment, except, perhaps, O_2 and CO_2 [14]. Thus while relatively high OM was observed in the soils analysed, such OM would be of little direct effect on the metabolism of *Bdellovibrio* but would significantly affect the survival of *S. typhi*, and hence availability for predation by *Bdellovibrio*.

Conclusion

The presence and occurrence of *Bdellovibrio* spps in Benue soils, and by extension and implication, in Nigeria has been established. The distribution however has not been shown to be, or to confirm to any particular pattern. It is therefore advocated that further research, especially taking into consideration limitations of the present study, be carried out, to determine all factors that could influence local distribution patterns of *Bdellovibrio*.

The next level of global research of these fascinating organisms will be in the area of clinical, medical and environmental applications in disease and infection control, and remediation of environmental issues. It is therefore, pertinent that the potentials of the organisms best suited to each environmental niche and prevailing conditions be properly documented for maximal exploitation.

Acknowledgement

The authors are grateful to the School Management and laboratory staff of the University of Mkar, Mkar, Gboko, Benue State, Nigeria for permission access to the Microbiology laboratory for this work.

References

1. Kilpatrick R. (1999). Bdellovibrio. http://web.mst.edu/ ~microbio/B1022-1999/Bdellovibrio.html. 8/22/2008

2. Robson M.C., Mannari R.J., Smith P.D and Payne W.G (1999). Maintenance of wound bacterial balance. *Am. J. Surg*., 178, 399-402.

3. Free Library (2014). Benue State: facts and figures. The Free Library, Retrieved 14/03/2016 from http://www.thefreelibrary.com/Benue+State+facts+and+figures.-a0140998471.

4. Varon M. and Shilo M. (1970). Methods for separation of *Bdellovibrio* from mixed bacterial population by filtration through Millipore filters or by gradient differential centrifugation. *Rev. Int. Oceanogr. Med.,* 18-19, 145-152.

5. Hach D.R. (1993). Spectrophotometer Handbook Procedures Manual. HACH Company, Loveland, USA, 769.

6. Nester W.N, Anderson D.G, Roberts C., Pearhall N.N, Nester T.M and Hurley D. (2004). Microbiology: a human perspective. McGraw-Hill, NY, 817.

7. Wehr N.B. and Klein D.A. (1971). Herbicide effects on *Bdellovibrio bacteriovorus* parasitism of a soil pseudomonad. *Soil Biology and Biochemistry*, 3(2), 143-149.

8. Ita E.O. (1993). Inland fisheries resources of Nigeria. CIFA Occasional Paper –CIFA/OP 20, 120, Retrieved 14/03/2016 from http://www.fao.org/DOCREP/005/ T1230E/T1230E01.htm.

9. Yahaya K.M. and Olajide B.R. (2002). The impact of agriculture on environmental health in Nigeria. *Journal of Environ. Extension,* 3, 47-58.

10. Sar T.T., Umeh E.U. and Akosu D.D. (2015). Occurrence, Detection and Isolation of *Bdellovibrio* spps from Some Fresh Water Bodies in Benue State, Nigeria. *Microbiology Journal,* 5(1), 21-27, DOI: 10.3923/mj.2015.21.27.

11. Ochei J. and Kolhatkar A. (2000). Medical laboratory science, Theroy and Practice. Tata McGraw-Hill New Delhi, 1338.

12. Ongeng D., Muyanja C., Geeraerd A.H., Springael D. and Ryckeboer J. (2011). Survival of *Escherichia coli* O157:H7 and *Salmonella enterica* serovar Typhimurium in manure and manure-amended soil under tropical climatic conditions in Sub-Saharan Africa. *Journal of Applied Microbiology,* 110(4), 1007-1022, doi:10.1111/j.1365-2672.2011.04956.x http://caes.mak.ac.ug/Publications /2011/D-Ongeng-et-al-Survival-of-Escherichia-coli-O157-H7-and-Salmonella-enterica-serovar-Typhimurium-in-manure-and-manure-amended-soil-under-tropical-climatic-conditions-in-Sub-Saharan-Africa.pdf 1364 -5072.

13. Mitscherlich E. and Marth E.H. (2012). Microbial Survival in the Environment: Bacteria and Rickettsiae Important in Human and Animal Health. Springer-Verlag, Berlin, Heidelberg, 803, ISBN 978-3-642-69976-4.

14. Tilton R.C. (1999). Pre-test Microbiology. 9th edition, McGraw-Hill, NY, 189.