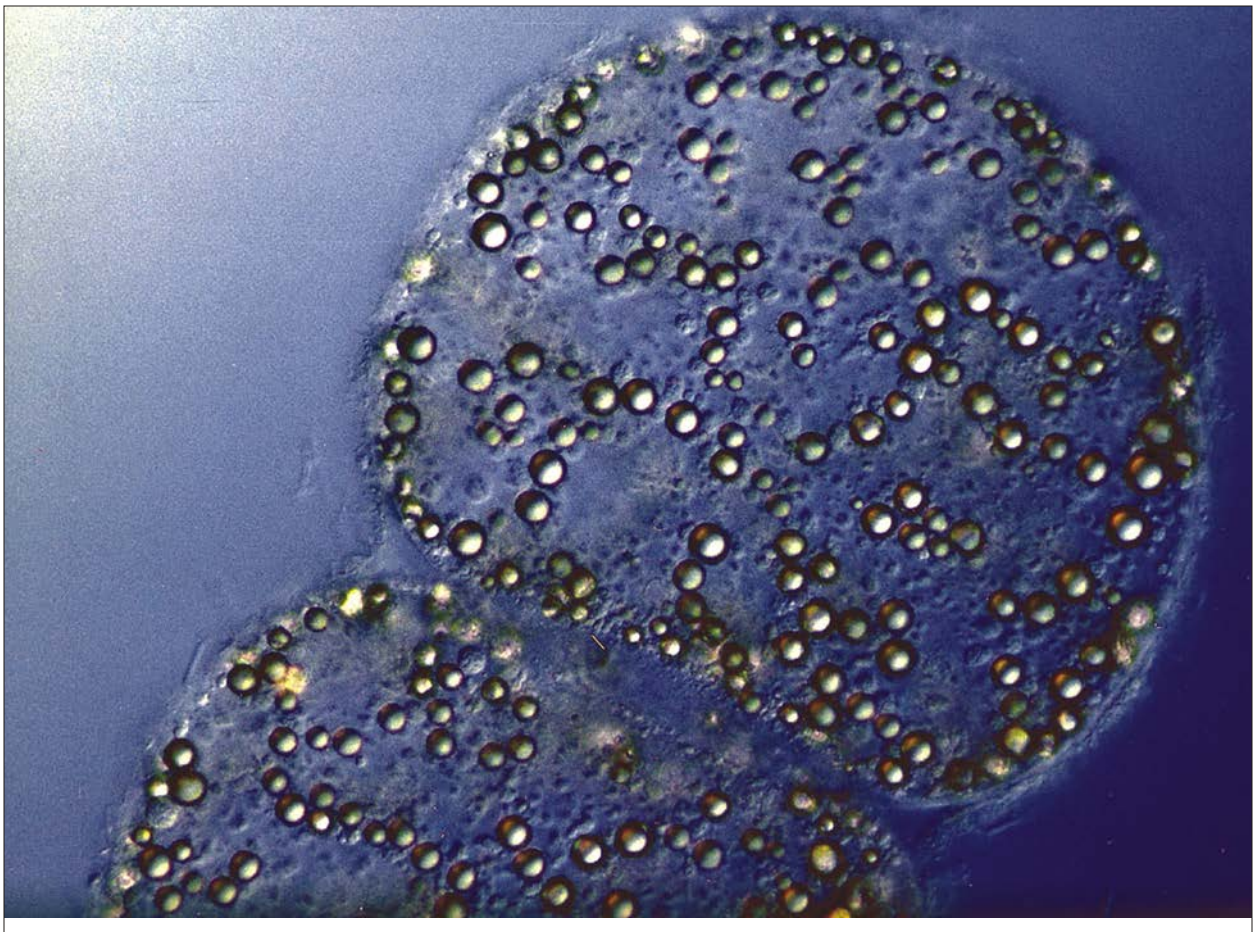

Microbiology

Journal

ISSN 2153-0696

Academic
Journals Inc.

www.academicjournals.com

Microbiology Journal 5 (1): 21-27, 2015
ISSN 2153-0696 / DOI: 10.3923/mj.2015.21.27

Occurrence, Detection and Isolation of *Bdellovibrio* spps. from some Fresh Water Bodies in Benue State, Nigeria

[1]T.T. Sar, [1]E.U. Umeh and [2]D.D. Akosu
[1]Department of Biological Sciences, University of Agriculture, Makurdi, Nigeria
[2]Department of Biological Sciences, University of Mkar, Mkar, Gboko, Nigeria

Corresponding Author: T.T. Sar, Department of Biological Sciences, University of Agriculture, Makurdi, Nigeria

ABSTRACT

One hundred and thirty-five water samples were collected from five fresh water bodies in three locations in Benue State, Nigeria and screened for the occurrence of *Bdellovibrio* spp. using the double layer agar technique. *E. coli*, *S. typhi* and *Shigella* spp. were used as prey bacteria. All water bodies sampled had average temperatures ranges of 26-27°C. pH values ranged between 7 and 8. Total hardness values ranged between 48 and 85 mg L^{-1}, while TDO values were between 5.0 and 15.3 mg L^{-1}. A total of 53 *Bdellovibrio* PFUs occurred with the following frequencies on respective host prey; 42 (79.2%) on *E. coli*, 10 (18.86%) on *S. typhi* and 1 (1.88%) on *Shigella* spp. More research on *Bdellovibrio* occurrence in other niches in Nigeria is advocated to better understand the dynamics of these unique bacteria in a bid to harness its potential as control for Gram negative infections.

Key words: *Bdellovibrio*, plaque forming units, prey bacteria, fresh water bodies, Nigeria

INTRODUCTION

Bdellovibrio are gram negative, vibroid and extremely small bacteria that range in size from 0.3-0.5 by 1.4-2.5 μm. They are highly motile predatory bacteria, with single sheathed polar flagella, which exhibit characteristic dampened waveforms. They attack and utilise the cellular contents of other gram-negative bacteria, which include those that are 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, which invariably leads to host cell deaths (Sockett, 2009).

The organisms are of interest to microbiologists firstly because they have potential of being harnessed as living controls of many pathogenic gram-negative micro-organisms and secondly because studies on their degradative enzymes shed light on those targets in prey cells that can also be targeted by other artificial or manmade antimicrobial agents (Lambert *et al*., 2006).

Since, the discovery of the potentials of the *Bdellovibrio* as bio-control agents, research on the organism has mostly been outside tropical environments. Search through literature reveals the dearth of local findings on the organism from within the West African sub region and especially Nigeria. Furthermore, evidence of the presence of *Bdellovibrio* in tropical Africa is not available.

This study will potentially add to the existing database of information on *Bdellovibrio* thereby, helping to bring a step closer the eventual widespread applications of the bacteria for treatment of infections in man.

This study is therefore, one of the pioneer efforts in documenting information on the *Bdellovibrio* in Nigeria and will help to bridge the gap between research findings on *Bdellovibrio* in other areas and in this region.

MATERIALS AND METHODS

Sample sites were Makurdi, Gboko and Katsna-Ala, all in Benue State, Nigeria. Makurdi is located on longitude 8°31' E and latitude 7°45' N and is 90 m above sea level. Gboko is located between latitude 6°30" and 8°10" North of the equator and longitude 8' and 10" East of the Greenwich meridian and Katsina-Ala has an area of 2,402 km^2. It is located between latitude 7°10" N of the equator and longitude 9°16" E of the Greenwich meridian. The town is located on low altitudes of between 250-300 m above sea level (Agisui and Ogbu, 2005).

Isolating *Bdellovibrio* from water samples: The isolation of *Bdellovibrio* from water samples was carried out as described by Williams *et al*. (1995). Fresh water samples were collected from River Benue, River Katsina-ala and other smaller streams in Makurdi, Gboko and Katsina-ala. The water samples were filtered; first with Whatman filter paper No. 1 and then with 0.45 μm pore size, membrane filters. This ensured that *Bdellovibrio*, which are usually smaller than this diameter, passed through the membrane filters, while larger micro-organisms were retained.

One tenth millilitre of the filtrate was used to seed *E. coli* cultures on *Bdellovibrio* Agar plates. The plates were examined for the presence of plaques after incubation for 3-7 days at 35°C. Numbers of organisms per mL of each water sample were calculated as Plaque Forming Units (PFUs).

Determination of physico-chemical parameters of sampled water bodies: Temperature (T°C), pH, Total Hardness (TH) and Total Dissolved Oxygen (TDO) of the water samples were determined according to the methods described by Sharma (2007).

RESULTS

***Bdellovibrio* from water samples:** A total of one hundred and thirty-five water samples were collected from five fresh water bodies in the three locations of Benue state *viz* Katsina-Ala, Gboko and Makurdi. Three one-litre volume samples were taken from each of the water bodies at three different periods; morning, afternoon and evening, in 1 L containers and immediately transported to the laboratory for analysis. The temperatures of the water bodies were taken at the points of sample collection and the means determined.

Table 1 shows the physico-chemical properties of water bodies sampled in the three locations. The water samples from Makurdi, Gboko and Katsina-Ala had average temperatures of 26.6, 26 and 27.6°C and Standard Deviation (SD) values of±0.8, 0 and ±1.36°C respectively. Similarly, pH values of Makurdi water bodies averaged 8.104 (SD = ±0.609). Gboko water bodies had mean pH values of 8.012 (SD = ±0.51) and pH of Katsina-ala water bodies had a mean of 7.994 (SD = ±0.24).

Total Dissolved Oxygen (TDO) values, a measure of the oxygen availability for aquatic life, of the Makurdi samples had a mean of 9.16 mg L^{-1} (SD = ±2.32). TDO for Gboko water bodies ranged between 5.0 and 15.3 mg L^{-1} with an average of 9.94 mg L^{-1} (SD = ±3.36). Katsina-ala waters showed TDO range of between 5.6 and 9.7 mg L^{-1}, with a mean of 7.46 mg L^{-1}, (SD = ±1.38). Katsina-ala water bodies thus had the lowest mean TDO values of the water bodies sampled.

Table 1: Physico-chemical parameters of sampled water bodies

Water body codes	Sites	Depth (cm)	Period of collection			Colour of water sample	Odour	Mean values			
			1st (am)	2nd (pm)	3rd (pm)			T (°C)	pH	TDO	TH (mg L^{-1})
MK1	MKD	40	7.05	1.00	6.11	Black	Putrid	26	7.83	9.5	77
MK2	MKD	50	6.23	1.08	6.15	Cloudy	-	26	8.24	9.6	49
MK3	MKD	40	6.32	1.12	6.30	Muddy	-	27	8.31	6.8	99
MK4	MKD	600	6.47	1.20	6.12	Muddy	-	28	8.69	13.1	101
MK5	MKD	60	7.49	1.10	6.00	Cloudy	-	26	7.45	6.8	99
GBK1	GBK	40	5.50	1.00	6.08	Clear	-	26	8.16	5.8	88
GBK2	GBK	60	6.35	1.15	6.25	Clear	-	26	7.96	11.0	47
GBK3	GBK	50	6.43	1.03	6.10	Clear	-	26	8.28	8.4	26
GBK4	GBK	30	6.20	1.30	6.14	Muddy	Musky	26	7.52	10.0	119
GBK5	GBK	50	6.06	12.50	6.13	Cloudy	-	29	8.14	15.3	56
KA1	K/Ala	30	6.47	2.00	6.00	Muddy	-	26	7.69	6.5	67
KA2	K/Ala	30	6.56	1.07	5.49	Cloudy	-	28	8.04	9.7	68
KA3	K/Ala	30	7.07	1.13	6.14	Muddy	-	26	7.55	7.7	44
KA4	K/Ala	600	7.17	12.56	6.10	Muddy	-	26	8.25	5.6	45
KA5	K/Ala	70	6.36	1.12	6.12	Clear	-	29	8.24	7.8	20
Average total		118.66	-	-	-	-	-	26.73	8.02	26.72	67

MK1: Abbattoir stream, MK2: Idye stream, MK3: Urudu stream, MK4: River Benue, MK5: Demekpe stream, GBK1: Ahungwa stream, GBK2: Kontien stream, GBK3: Uwev stream, GBK4: Nguembi stream, GBK5: Konshisha stream, KA1A: Buan stream, KA2A: Ankyegh-hungur stream, KA3A: Gbor-aya stream, KA4A: River katsina-ala, KA5A: Ahungwa stream, -: Negative/no odour, TDO: Total dissolved oxygen, pH: Hydrogen ion concentration and TH: Total hardness

Table 2: *Bdellovibrio* isolation from water bodies

Water body codes	Incubation		Plaque formation (host range)			PFU 0.1 mL^{-1}/description			Approx. PFU mL^{-1}		
	Days (h)	Temp (°C)	*E. coli*	*S. typhi*	*Shigella* spp.	*E. coli*	*S. typhi*	*Shigella* spp.	*E. coli*	*S. typhi*	*Shigella* spp.
MK1	96	35	-	-	-	-	-	-	-	-	-
MK2	96	35	-	-	-	-	-	-	-	-	-
MK3	48	35	+	-	-	2 (C)	-	-	$2x10^6$	-	-
MK4	96	35	-	-	-	-	-	-	-	-	-
MK5	96	35	+	-	+	3 (D)	-	1 (C)	$3x10^5$	-	$1x10^5$
GBK1	96	35	-	-	-	-	-	-	-	-	-
GBK2	96	35	+	-	-	31 (D)	-	-	$3.1x10^6$	-	-
GBK3	96	35	-	-	-	-	-	-	-	-	-
GBK4	96	35	-	-	-	-	-	-	-	-	-
GBK5	48	35	+	+	-	3 (C)	10 (D)	-	$3x10^6$	-	$1x10^7$
KA1	96	35	+	+	-	1 (C)	-	-	$1x10^7$	-	-
KA2	96	35	-	-	-	-	-	-	-	-	-
KA3	96	35	-	-	-	-	-	-	-	-	-
KA4	48	35	+	-	-	2 (C)	-	-	$2x10^6$	-	-
KA5	96	35	-	-	-	-	-	-	-	-	-

MK1: Abbattoir stream, MK2: Idye stream, MK3: Urudu stream, MK4: River Benue, MK5: Demekpe stream, GBK1: Ahungwa stream, GBK2: Kontien stream, GBK3: Uwev stream, GBK4: Nguembi stream, GBK5: Konshisha stream, KA1: Buan stream, KA2: Ankyegh-hungur stream, KA3: Gbor-aya stream, KA4: River katsina-ala, KA5: Ahungwa stream, -: No plaque seen, +: Plaque seen, PFU: Plaque forming units, -:No plaque observed, (C) Confluent plaque growth, (D) Single/Isolated plaques

Total Hardness (TH) values, showed that Makurdi water bodies had a mean TH of 85 mg L^{-1} (SD = ±20.04). Gboko bodies had a mean TH value of 67.2 mg L^{-1} (SD = ±32.72). Katsina-ala water bodies had a mean TH value of 48.8 mg L^{-1} (SD = ±17.70). Thus, TH values were lowest for the water samples from Katsina-Ala, suggesting that these water bodies less amounts of dissolved salts and other solutes in them, than the samples from Gboko and Makurdi (Table 1).

Table 2 shows results of cultures from the water samples. Two samples from Makurdi water bodies showed plaque formation; MK3 cultured on *E. coli* and MK5 on *E. coli* and *Shigella* respectively. Similarly, one sample from Gboko, GBK5 showed plaque formation on *E. coli* host.

Table 3: Frequencies of *Bdellovibrio* plaque occurrence on respective host bacteria and water samples

Water body codes	Incubation period		Plaque formation host range			Total PFU/Description on host			
	Time (h)	Temp (°C)	*E. coli*	*S. typhi*	*Shigella* spp.	*E. coli*	*S. typhi*	*Shigella* spp.	Total (%)
MK3	48	35	+	-	-	2 (C)	-	-	02 (3.77)
MK5	96	35	+	-	+	3 (D)	-	1 (D)	04 (7.54)
GBK2	96	35	+	-	-	31 (D)	-	-	31 (58.49)
GBK5	48	35	-	+	-	3 (C)	10 (D)	-	13 (24.53)
KA1	96	35	-	+	-	1 (C)	-	-	01 (1.89)
KA4	48	35	+	-	-	2 (C)	-	-	02 (3.77)
Total PFU (%)						42 (79.25)	10 (18.87)	1 (1.89)	53 (100)

MK1: Abbattoir stream, MK2: Idye stream, MK3: Urudu stream, MK4: River Benue, MK5: Demekpe stream, GBK1: Ahungwa stream, GBK2: Kontien stream, GBK3: Uwev stream, GBK4: Nguembi stream, GBK5: Konshisha stream, KA1: Buan stream, KA2: Ankyegh-Hungur stream, KA3: Gbor-aya stream, KA4: River katsina-ala, KA5: Ahungwa stream, PFU: Plaque forming unit, +: Plaque observed, -: No plaque observed, (C): Confluent plaque growth, (D): Single/isolated Plaques

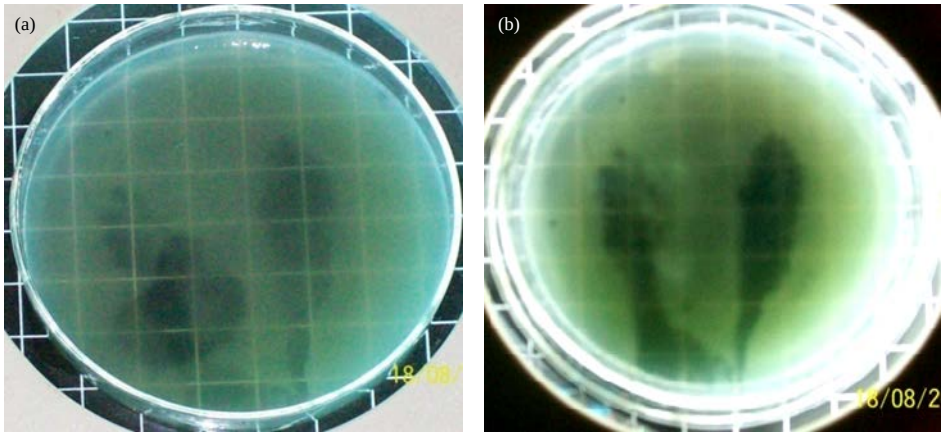

Fig. 1(a-b): Typical *Bdellovibrio* plaques on *E. coli* after 72 h at 37°C from (a) KA4 and (b) MK3, respectively

Two samples from Katsina-ala, KA1 and KA4 showed plaque formation on *S. typhi* and *E. coli* hosts respectively.

Table 3 shows the frequencies of occurrence of *Bdellovibrio* plaques on respective hosts. GBK2 had the highest frequency of 31 PFU, all of which occurred on *E. coli* host, representing 58.9% total PFUs. The least occurrence of 1 PFU was from sample KA1, represented 1.89% of total PFU and occurred on *S. typhi* host.

Figure 1 shows typical appearances of plaques formed after 72 h of incubation at 37°C. *Bdellovibrio* plaques typically continued expanding in size as the predator devoured increasing areas of host bacteria lawns.

DISCUSSION

The isolation of *Bdellovibrio* from water bodies in Benue state agrees with the reports of various authors that *Bdellovibrio* occur in diverse habitats including fresh and marine water, soil, sewage and even intestines of animals and man (Jurkevitch, 2006; Williams *et al*., 1995). In Nigeria and particularly in Benue state, animal and human wastes are routinely and indiscriminately dropped in soils and on farmlands from where they are washed into water bodies in raw untreated form (Houmsou *et al*., 2010). Such wastes could be the origins of the isolates from water bodies.

Williams *et al*. (1995) carried out an investigation on the distribution of *Bdellovibrio* over a wide area of the Chesapeake Bay. They recovered *Bdellovibrios* from five aquatic habitats, which included water, sediments, Oyster shell surface biofilms, zooplankton and plants over a wide range of physical parameters such as salinity and temperature levels. In their work, they persistently recovered *Bdellovibrio* from biofilms irrespective of temperature and salinity levels of the host environment. Similarly, in this study, *Bdellovibrio* have been isolated across various physico-chemical parameters.

Even though the optimal growth temperatures for *Bdellovibrio* have been reported as ranging between 28-30°C, by various authors (Jurkevitch, 2006; Kadouri and O'Toole, 2005; Lambert *et al*., 2006), in this study however, they have been isolated at incubation temperatures of 35°C. This could be as a result of the higher tropical environmental temperatures in which prevail in Benue State, where they occur, which may have caused the organisms to adapt to the prevailing temperatures, as opposed to the lower ambient temperatures in temperate climes where much of the work on the organism has been carried out. In such environments, 25°C has been reported as the optimal growth temperature for the organism. Some investigators have reported temperatures of between 19-37°C as ranges for activity of *Bdellovibrio*, which is in line with temperatures used to isolate the organisms in this work (Fratamico and Cooke, 1996).

Each microorganism has an optimum pH at which it grows best. The hydrogen ion concentration (pH) of an organism's environment exerts the greatest influence the activity of enzymes with which an organism is able to synthesize new protoplasm. In this study, *Bdellovibrio* were isolated at varied pH ranges, this is in agreement with previous reports that the minimum and maximum pH ranges for growth of microorganisms, remain true only when other environmental factors remain constant. If for example, the composition of the medium, incubation temperatures or osmotic pressure are varied, the pH requirements also change (Benson, 2002). In this study, the other environmental parameters, such as temperature, TH and TDO at which *Bdellovibrio* were isolated varied from one water sample to another, which no doubt must have had influence on the observed pH values at which they were shown to have occurred.

E. coli is found in faecally contaminated water bodies and is indeed used as an indicator organism of faecal pollution in such water bodies (Sharma, 2007). In Nigeria and especially in rural and semi-rural areas of Benue State, proper toilet and other sanitary facilities are often lacking, leading to dropping of human and animal wastes in water bodies. This situation has probably made *E. coli* to be widely available in these water bodies for predation by *Bdellovibrio*, which may have led to a co-evolutionary process of these two organisms, leading to the observed high rate of predation on *E. coli*. Other gram negative hosts were also predated on, though to a lesser degree, agreeing with reports that *Bdellovibrio* generally predates on gram-negative bacteria, though they seem to show some specificity for hosts, depending on both the host and *Bdellovibrio* strains (Jurkevitch, 2006; Kadouri and O'Toole, 2005).

The isolation of *Bdellovibrio* which are strict aerobes from water bodies with very low TDO values in this study was unexpected, though not unusual. Low oxygen environments have been stated to have no significant influence on the occurrence of the organism (Schoeffield *et al*., 1996). Moreover, *Bdellovibrio* can survive anoxic periods as attack phase organisms or Bdelloplasts (Jurkevitch, 2006). Halo-tolerant species have been shown to grow under microaerobic conditions, such as during spells of low oxygen tensions in water and soils. It is, therefore, concluded that the range of survival of *Bdellovibrio* may not be limited to permanently aerobic environments (Schoeffield *et al*., 1996).

The observed TH values may also have influenced the occurrence and growth of *Bdellovibrio*. Bacteria can be profoundly affected by the osmotic potential of the environment. When the medium surrounding an organism is hypotonic (low solute content), a resultant higher osmotic pressure occurs in the cell. Except for some marine forms, this situation is not harmful to most bacteria. The cell wall structure of most bacteria is so strong and rigid that even slight cellular swelling is generally unapparent. In the reverse situation, however, when bacteria are placed in a hypertonic solution (high solute content), their growth may be considerably inhibited (Benson, 2002).

There are several reasons why *Bdellovibrio* were not isolated from every water sample. Firstly, the organism is reported to be particularly sensitive to pollution (Varon and Shilo, 1981; Markelova, 2002). In Nigeria, poor regulation of wastes disposal invariably leads to their being indiscriminately dumped into water bodies, by individuals, households and industries. Such wastes could well have high levels of substances antagonistic to *Bdellovibrio* growth. Lambina *et al*. (1974) observed that though a higher total bacterial load can promote *Bdellovibrio* growth, the bacterium is sensitive to the presence of environmental pollutants such as heavy metals, detergents, pesticides and the concentrations of *Bdellovibrio* isolated from water bodies correlated with (levels of) water pollution.

Secondly, lack of suitable laboratory prey could have led to their non-detection in some samples. In this study, certain predetermined hosts were chosen for *Bdellovibrio* isolation. However, not all these prey may have been suitable for predation and isolation of all strains of *Bdellovibrio* occurring in all samples from every niche. However, the number of *Bdellovibrio* plaques detected in any environmental sample is dependent on the host chosen, the processing of the sample and the isolation protocol. *Bdellovibrio* show variation in host range and no single bacterial species can potentially support the growth of all isolates (Jurkevitch, 2006).

Finally, the lack of suitable biofilms in the sampled water bodies no doubt would have affected the occurrence of *Bdellovibrio* in these aquatic habitats. Most of the water bodies sampled were small and fast flowing, which meant that they would probably have a dearth of suitable biofilms and hosts for *Bdellovibrio* growth and predation. Most *Bdellovibrio* are members of bio-films, preferring the submerged communities to planktonic existence. Continuous flow of water in streams and all but the largest rivers prevents the development of significant planktonic communities (Willey *et al*., 2008).

It is known that compared to *S. typhi, E. coli* has a longer survival period in water and is indeed used as an indicator organism in testing for the contact of animal faecal contamination of water bodies (Ochei and Kolhatkar, 2000). It is thus possible that the shorter survival period of *S. typhi* in water and hence, its relative unavailability may have influenced the selection of *E. coli* in water over *S. typhi* as prey by *Bdellovibrio*.

CONCLUSION

The occurrence of *Bdellovibrio* spp has been demonstrated in some local fresh water bodies in Nigeria. This is not altogether surprising or unexpected, given the reported wide distribution of this predator.

However, a large amount of work still remains to be done in determining phylogenetic relationships of species isolated locally with those previously isolated elsewhere. The challenge also remains in research on *Bdellovibrio* in Nigeria catching up with the more advanced findings on the organism in other parts of the world, especially as it has been shown that its prey range includes

some of the more common gram negative pathogens, like *S. typhi* and *Shigellae*, plaguing Nigerian health care institutions with morbid illnesses.

ACKNOWLEDGMENTS

The authors acknowledge all, especially the staff of the Benue State Urban Development Laboratory, Makurdi, for their assistance in this research.

REFERENCES

Agisui, E.T. and J.A. Ogbu, 2005. Benue state: The making of the food basket of the nation. Benue State Ministry of Information and Orientation, Makurdi, Nigeria, pp: 40.

Benson, H.J., 2002. Microbiological Applications: Laboratory Manual in General Microbiology. 8th Edn., McGraw-Hill, New York, pp: 455.

Fratamico, P.M. and P.H. Cooke, 1996. Isolation of bdellovibrios that prey on escherichia coli o157: H7 and *Salmonella* species and application for removal of prey from stainless steel surfaces. J. Food Safety, 16: 161-173.

Houmsou, R.S., E.U. Amuta and T.T. Sar, 2010. Impact of urbanization on parasitic infections in developing countries. Rev. Infect., 1: 38-41.

Jurkevitch, E., 2006. The Genus *Bdellovibrio*. In: The Prokaryotes: A Handbook on the Biology of Bacteria, Dworkin, M., S. Falkow, E. Rosenberg, K.H. Schleifer and E. Stackebrandt (Eds.). 3rd Edn., Springer, New York, pp: 12-30.

Kadouri, D. and G.A. O'Toole, 2005. Susceptibility of biofilms to *Bdellovibrio bacteriovorus* attack. Applied Environ. Microbiol., 71: 4044-4051.

Lambert, C., K.A. Morehouse, C.Y. Chang and R.E. Sockett, 2006. *Bdellovibrio*: Growth and development during the predatory cycle. Curr. Opin. Microbiol., 9: 639-644.

Lambina, V.A., N.A. Chuvil'skaia, L.A. Ledova, A.V. Afinogenova and I.V. Averburg, 1974. [Quantitative characteristics of the distribution of *Bdellovibrio bacteriovorus* in river water]. Mikrobiologiia, 43: 715-720, (In Russian).

Markelova, N.Y., 2002. Effect of toxic pollutants on *Bdellovibrio*. Process Biochem., 37: 1177-1181.

Ochei, J. and A. Kolhatkar, 2000. Medical Laboratory Science: Theory and Practice. Tata McGraw-Hill, New Delhi, Pages: 1338.

Schoeffield, A.J., H.N. Williams, B.F. Turng and W.A. Falkler Jr, 1996. A comparison of the survival of intraperiplasmic and attack phase bdellovibrios with reduced oxygen. Microb. Ecol., 32: 35-46.

Sharma, K., 2007. Manual of Microbiology: Tools and Techniques. 2nd Edn., Ane Books Pvt. Ltd., New Delhi, India, ISBN: 9788180521430, Pages: 424.

Sockett, R.E., 2009. Predatory lifestyle of *Bdellovibrio bacteriovorus*. Ann. Rev. Microbiol., 63: 523-539.

Varon, M. and M. Shilo, 1981. Inhibition of the predatory activity ofBdellovibrio by various environmental pollutants. Microbial Ecol., 7: 107-111.

Willey, J.M., L. Sherwood and C.J. Woolverton, 2008. Prescott, Harley and Klein s Microbiology. 7th Edn., McGraw-Hill Co., New York, USA., ISBN-13: 9780071102315, Pages: 1088.

Williams, H.N., A.J. Schoeffield, D. Guether, J. Kelley, D. Shah and W.A. Falkler Jr., 1995. Recovery of bdellovibrios from submerged surfaces and other aquatic habitats. Microb. Ecol., 29: 39-48.