Water. Environ. Sustainability. 1 (2): 6-13, 2021

Water and Environmental Sustainability
ISSN: 2710-3404
DOI: 10.52293/WES.1.2.613
H o m e p a g e: https://www.journalwes.com/

Research paper

Acute Toxicity of Organotin on Fresh Water Shrimps and its Resistance by Marine Bacteria

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A R T I C l E I N F O

Keywords:
Acute toxicity
Organotin, Shrimps
Tributyltin
Diphenyltin
Lethal dose

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Received: 11 February, 2021
Accepted: 03 March, 2021
Available online: 05 April, 2021

A B S T R A C T

This present study was aimed at investigating the acute toxicity of organotin on fresh water shrimps and its resistance to marine bacteria. 200 water shrimps were exposed to varying concentrations of Tributyltin Chloride (TBTCL) and Diphenyltin Chloride (DPTCL) for 96 hours and a probit was used to determine the lethal dose (LD50). 200g of sediment from Onne sea port Rivers State was manually polluted by TBTCL and DPTCL for 56 days. Organotin resistant bacteria were screened on mineral salt medium at different concentration of the organotin using the spread plate technique. Results from this study showed a lethal doze (LD50) of 4.24mg/l after 24 hours and 1.97mg/l after 48 hours for TBTCl on fresh water shrimps and a lethal doze of 21.05mg/l after 24 hours, 0.83mg/l after 48 hours and 0.006mg/l after 72 hours for DPTCl. The total viable count of bacteria obtained from varying concentrations of TBTCl indicates that approximately 65% of bacterial populations were resistant to 3.0mM of TBTCl and DPTCI since these isolates could grow on MSA supplemented with TBTCI and DPTCI. Statistically, there was no significant difference between the bacterial loads between the different concentrations of TBTCL. Acute toxicity effect of TBTCl and DPTCl on fresh water shrimps reveals TBTCl and DPTCl as one of the toxic substances in the marine ecosystem however; marine bacteria can be harnessed for their resistant abilities.

Introduction

Organotin compounds are synthetic persistent organometallic xenobiotics widely used in several commercial applications. It is considered as one of the most toxic contaminant entering the environment. Its effects are of great concern since it causes induction of reproductive abnormalities and destabilization of female prosobranch (Heloise *et al*., 2010). They exert well described harmful effects in brain, liver, adipose tissue, and reproductive organs as they are endocrine-disrupting chemicals as well as the total well-being of the organism (Carolina *et al* 2018). Trisubtituted organotin species have being known to possess biocidal properties and can be used as agricultural pesticides, wood preservatives and antifouling paints on ships (Sena *et al.,* 2017). Tributyltin (TBT) causes impairments in growth and development, and induces reproductive failures, shell anomalies, and gel formation. It also causes chambering and high mortality, disturbs the energy metabolism of bivalve, and inhibits the activity of

many enzymes, these effects reduce the survival of many species (Beaumont and Budd, 1984; Haggera *et al*., 2005). Tributyltin as early as the 1970s was known to be very toxic to many aquatic organisms and their high toxicity of is attributed to its effects on mitochondrial function (Blabber, 1970; Smith, 1981). The embryonic and larval stages of marine invertebrates are less tolerant to toxicants than are adults and this difference has been used to assess the biological quality of marine water and sediments (Fent and Muller, 1991).

Tributyltin is known to have toxic endpoints, e.g., acute lethal toxicity in rock shell larvae (*Thais clavigoa*) (Horiguchi *et al*., 1998). Tributyltin is known to inhibit oxidative phosphorylation, which affects cell metabolism by stimulating the production of adenosine diphosphate, and results in mitochondrial membrane malformation (Chun-Fa *et a*l., 2018; Laura *et al*., 2015).

Tributyltin affected larval development of bivalve (*C.tsubasa*) and caused sexual disturbance in gastropods (*C.nankaiensis*) at nanogram per liter levels in seawater. At a level of 1.0ng L^{-1}, TBT cause masculinization in many female gastropods (*C.nankaiensis*), and known as imposex. It also limits cell division in phytoplankton and reproduction of zooplankton tributyltin has been reported to induce shell calcification anomalies in the oyster *Crassostrea gigas* at a level of 2 ng l^{-1} and to disturb the reproduction of bivalve mollusks at 20 ng l^{-1} (Bella *et al*., 2005). Ruiz *et al*. (1995) investigated the effect of TBT exposure on veliger larval development of the bivalve (*C tsubas*) they found that TBT contributed to the demise of clam population by preventing successful and timely development of veliger larvae. Tributyltin also affects the abundance and relative growth rate of male and female whelks around marines (Gil *et al*., 2000).

However, many marine bacterial strains are resistant to toxic organontin compounds (TBT) (Dubey and Roy, 2003). Degradation is achieved by both biotic and abiotic factors. Photodecomposition by ultraviolet (UV) light is the most important abiotic degradation process. In aquatic and terrestrial ecosystems, biological processes are the most important factor effecting degradation of organotin compounds. Studies have shown that organotin degradation is mediated by microorganisms (Ebah *et al*., 2016; Jerry and Damian, 2018).

The main aim of this paper is to evaluate the toxicity of organotin compounds in the Nigerian environment and to also evaluate the resistance of this compound to marine bacteria.

Materials and Methods

Study Area

Two hundred (200) Water shrimps (*Palaemonates africana*) were collected from Elechi Creek Diobu Port Harcourt in Rivers State into a 20 liter capacity cooler. Twenty five (25) liters of water was collected with the shrimps and the cooler left open while it was transported to the laboratory for analysis. This was done to ensure the survival of these shrimps and to create an environment similar to their natural habitat.

The shrimps were brought into the laboratory and left for 24 hours for them to acclimatize. Two liters of the river water was dispensed into clean, transparent five (5) liter capacity bucket to ensure a good view during the experiment. Twenty (20) shrimps were introduced into each bucket containing varying concentrations of tributyitin chloride and diphenyltin chloride. Concentrations of 100, 10, 1.0, 0.1, 0.01 (mg/l) were used respectively for the test chemicals according to Horiguchi *et al*. (1996). Each concentration was done in duplicate and the experiment carried out for 96 hours. The number of mortality per concentration was recorded accordingly and analysis was conducted using the Probit.

Microbial Analysis

Two hundred grams (200g) sea sediments were collected from the Onne Sea Port, Rivers State, Nigeria with the aid of an Earth Man Grab. Sediments were immediately transferred to the laboratory for analysis.

Selection of the organotin resistant bacteria was conducted in a screening medium, which contained the following: 1.0g of K_2HPO_4, 1.0g of KH_2PO_4, 1.0g of $(NH4)_2SO_4$, 0.4g of $MgCl_2$, 0.125g of yeast extract and 1.0ml of glycerol per liter and 3.0mM of tributyltin chloride and diphenyltin chloride respectively. The bacterial isolates which grew on MSA with 3.0mM TBTCl and DPTCl were sub-cultured continuously on MSA with varying concentrations of TBTCl and DPTCl ranging from 5mM – 10mM. Isolates showing varying range of tolerance to TBTCl and DPTCl that is, 5mM, 7mM and 10mM were selected for further characterization.

Pure cultures of the bacterial isolates were characterized and presumptively identified on the basis of their cultural, morphological and physiological characteristics. Confirmatory biochemical reactions in the identification processes were carried out employing various tests such as Gram Staining, sugar fermentation, H_2S production, motility, coagulase, methyl red and Vogue-Proskaver's test (MR VP), oxidase, urease, citrate starch hydrolysis. The characteristics of the cultures were compared with the characteristics of known taxa using the determination schemes of Cowan (2003) and Ho *et al*. (1997).

Results

The result of the acute toxicity of TBTCl on fresh water shrimps shows that, the lethal doze (LD_{50}) was 4.24mg/l after 24 hours and 1.97mg/l after 48 hours. There was a similar occurrence for DPTCl. The lethal doze was 21.05mg/l after 24 hours, 0.83mg/l after 48 hours and 0.006mg/l after 72 hours.

This occurrence reveals that the higher the doze of TBTCL and DPTCI the shorter the time interval required to kill the organisms and the lesser the doze the longer the time interval required to kill the organisms.

The total bacterial counts after 56 days of degradation analysis varied from $42x\ 10^2$ to $64.4x10^2$cfu/g when plated on MSA only. However, the variable counts on MSA to 3mM, TBTCl and MSA + 5Mm TBTCl range from 22×10^4 to 38.5×10^2 Cfu/g and $18x10^1$ from $21.9x10^1$ Cfu/g respectively, It was observed that for all samples with increasing concentrations of TBTCl, the number of bacterial (Cfu/g) decreases significantly and the percentage of resistance bacterial was lower compared to control.

Table 1.Toxicity test of TBTCL on Shrimps for 96hs

Concentration of TBTCL (mg/L)	No of death per day				Total number left
	1	2	3	4	
100	18	2	-	-	-
10	17	10	3	-	-
1.0	7	10	3	-	-
0.1	5	9	4	1	1
0.01	3	8	7	1	1
Control	1	1	2	1	15

KEY: TBTCl-Tributyltin Chloride

Table 2.Toxicity test of DPTCL on Shrimps for 96hs

Concentration of DPTCL (mg/L)	No of deaths per day				Total number left
	1	2	3	4	
100	13	7	-	-	-
10	9	9	2-	-	-
1.0	4	8	7	1	-
0.1	2	6	5	2	5
0.01	1	8	2	4	5
Control	-	2	1	2	15

KEY: DBTCl-Diphenyltin Chloride

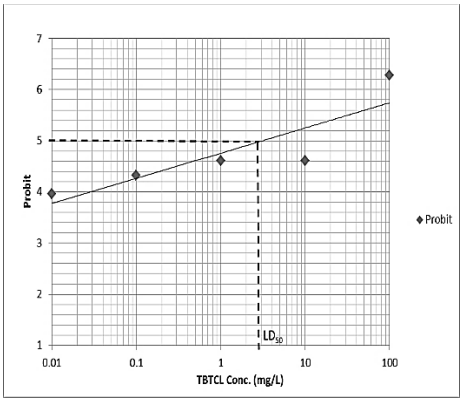

Figure 1: Acute toxicity of TBTCl on shrimps after 24 h exposure

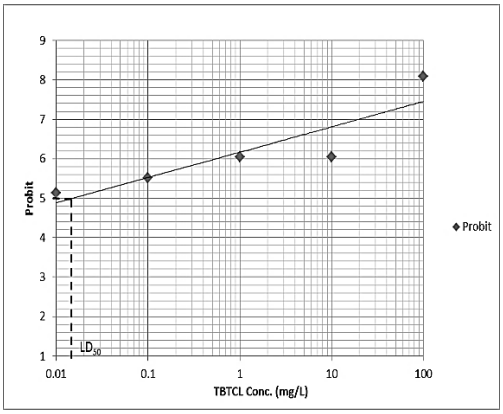

Figure 2: Acute toxicity of TBTCl on shrimps after 48 h exposure

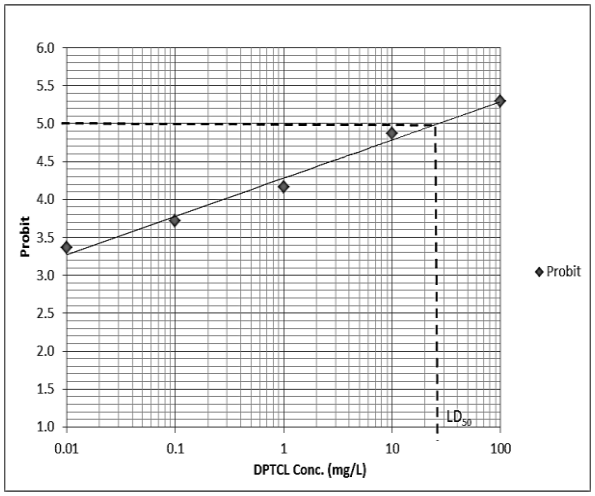

Figure 3: Acute toxicity of DPTCl on shrimps after 24 h exposure

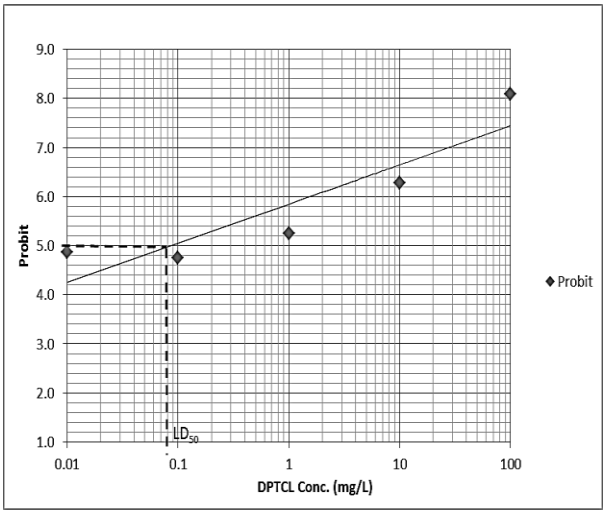

Figure 4: Acute toxicity of DPTCl on shrimps after 48 h exposure

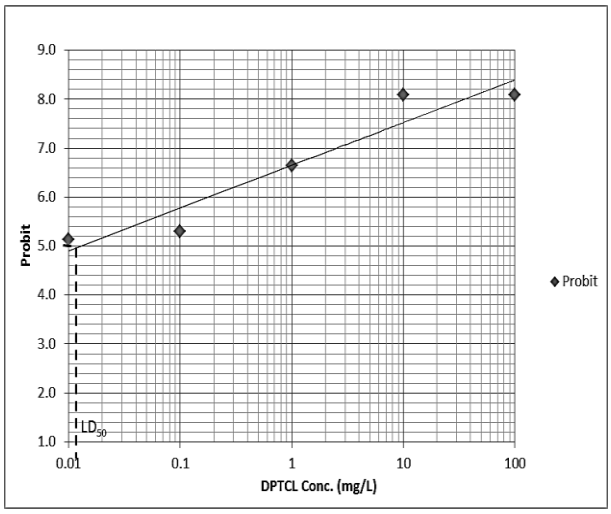

Figure 5: Acute toxicity of DPTCl on shrimps after 72 h exposure

Table 3 Total Heterotrophic Counts in varying Concentrations of Organomatal

	Vaible counts	Varying Concentration Organometal (Cfulg)			
Samples	MSA only	MSA + 3Mm	MSA + 5mM	MSA + 7mM	MSA + 10Mm
A	64. 4x10^2	34.3 X10^2	20.2 x 10^1	10.3 x10^1	3.8 x10^1
B	61.2 x10^2	38.5 x10^2	21.4 x 10^1	9.2 x 10^1	2.1 x 10^1
C	60.3 x10^2	28.7 x10^1	21.9 x 10^1	8.9 x 10^1	1.2 x 10^1
D	42.1 x 10^2	22. 8 x10^1	18.1 x 10^1	7.8 x 10^1	1.1 x 10^{12}
E	58.3 x 10^2	24.6 x 10^1	19.5 x 10^1	5.4 x 10^1	1.3 x 10^1

KEYS: MSA -Mineral Salt Agar,TBTCl-Tributyltin Chloride

Table 4.2 Biochemical Identification of Isolated Bacteria

S/N	Suspected Bacteria	Test										
		G.R	Glu.	Lact.	Man.	Suc.	Cit.	Urea.	Ind.	Vp.	Mot.	Ox.
1	*Klebsiella sp.*	-	+	+	+	+	+	+	-	+	-	-
2	*Salmonella sp.*	-	+	-	+	-	-	-	-	-	+	-
3	*Serratia sp.*	-	+	-	+	+	+	+	-	+	+	-
4	*Pseudomonas sp.*	-	-	-	-	-	+	-	-	-	+	+
5	*Providencia sp.*	-	+	-	-	-	+	-	+	-	+	-
6	*Proteus sp.*	-	+	-	-	-	+	+	-	-	+	-
7	*Escherichia sp*	-	+	+	+	-	-	-	+	-	+	-
8	*Bacillus sp.*	+	+	-	-	-	+	-	-	-	+	-
9	*Enterobacter sp.*	-	+	+	+	-	+	-	+	-	+	-

KEY: G.R = Grams Reaction; Glu. – Glucose, Lact. = Lactose, Man. = Mannitol, Suc. = Sucrose, Cit. = Citrate test, Urea. = Urease test, Ind. = Indole test, Vp. = Voges –Proskeur test, Mot. = Motility, Ox. = Oxidase test

Discussion

A 96 hour acute toxicity test conducted on freshwater shrimps reveals the high toxicity of TBTCl and DPTCl. Probit analysis showed that out of 200 shrimps that were used for the experiment, 80% of the shrimps died within 24 - 48 hours at a concentration between 100.0 mM/L to 10 mM/L of TBTCl and 50% of the shrimps died within 24 – 48 hours at a concentration of 100.0 mM to 10 mM/l of DPTCl.

This present study was interested in freshwater shrimps since most population are lovers of aquatic food, and more so organotin compounds reach humans primarily through the diet, mainly by fish and other sea food. Several findings have also showed high toxicity of organotin on marine invertebrates which corroborates the findings of this study. According to the findings of Beaumont and Budd (1984), Haggera *et al.* (2005), TBT causes impairments in growth and development and induces reproductive failures, shell anomalies and gel formation. It also cause chamberg and high mortality, disturbs the energy metabolism of bivalves and inhibits the activity of many enzymes; these reduce the survival of many species. Horiguchi *et al.* (1998) and Fent (2000) also reported a high toxicity of TBT on mollusks. Tributyltin is therefore

considered as one of the most toxic pollutants for aquatic lives known so far (Fent, 2006).

There was an obvious reduction in the total viable count from the different treatment used at the course of this present study. The reduction in the total viable count from 4.55×10^4 Cfu/g to 3.25×10^4 of TBTCl 4.47 to 3.0×10^4 cfu/g of DPTCl shows clearly that the bacterial isolates are likely to have inherent capability to utilize these chemical as their source of carbon In this present study, isolates were sub-cultured with increasing concentrations of TBTCl and DPTCl (3.0mM to 7.0mM). Out of 97 isolates growing in 3mM of these chemicals, 7 isolates showed consistent good growth in presence of 3 mM, 5 mM, 7mM and 10mM with of TBT. This observation was attributed to the fact that bacterial isolates marine are likely to possess inherent capability to resist and degrade TBTCl and DPTCl. Most of the bacterial isolates could not grow in presence of higher concentration of TBTCl (7 mM – 10 mM) probably due to cellular toxicity and inhibitory effect on metabolic process and viability of bacterial strains (Pettibone and Cooney 1999).

Singh (1987) and White (1999) have reported the range of microbial resistance up to 0.007 mM for different organotin compounds which is in agreement with this present studies. Debutylation of TBT compounds to di- and mono-butyltins is known to occur in bacterial, algae and fungi which provides one route for detoxification of tributyltin. In addition, microorganisms are capable of accumulating TBT compounds, which is another mechanism of removal of TBT from marine environment (Goddy, 2000). The studies of Fukagawa *et al.* (1994) reported that TBTCl tolerant bacterial are present in sea water and sediment and the finding of Suzuiki *et al*. (1992) reported that *Pseudomonas aeruginosa* can degrade tributyltin oxide at 2.5 ppm level. All these findings corroborate the findings of this present study.

Conclusion

The acute toxicity effect of TBTCl and DPTCl on fresh water shrimps reveals TBTCl and DPTCl as a serious threat in the marine ecosystem. Conscious effort should be made by regulatory bodies to restrict the use of this TBT based antifouling paints and such like products since they are the prime source of this chemical in the marine environment. Further studies should be carried out on the resistant abilities of marine bacteria.

References

BEAUMONT, A.R., AND BUDD, M.D. (1984). High mortality of the larvae of the common mussel at low concentration of tributyltin. *Marine pollution Bulletin*. 15:402-405.

BLABBER, S. J. M. (1970). The occurrence of penis – like outgrowth behind the right tentacle in spent females of *Nucella laillus*.*The Malacological society of London*, London.

BELLA, J., HALVERSON, A., AND GRANMO, A. (2005b). Sublethal effects of a new antifouling candidate in lumpfish (*Cyclopterus lumpus L.*) and Atlantic cod (*Gadus morhua*) larvae. *Biofouling, 2*1:207-216.

BELLA, J., BEIRAS, R., MARINO–BALSA, J. C., AND FERNANDEZ, N. (2005a). Toxicity of organotin compounds to marine invertebrate embryos and larvae a comparison between the sea urchin embryogenesis bioassay and alternative test species.*Ecotoxicology.* 14:337-353.

DUBEY, S.K AND ROY, U. (2003). Biodegradation of tributyltins (organotins) by marine bacteria. *Applied organometallic Chemistry.* 17:1-6.

CAROLINA,M.L., FERNADA, M.F. AND JONES, B.G. (2018). Organotin compounds Toxicity: Focus on Kidney. *Front. Endocrinol*, 9: 256 - 263.

EBAH, E. E. AND OKPOKWASILI, G. C. (2016). Isolation and Biological Characterization of Tributyltin Degrading Bacteria in Onne port Sediment. *Open Journal of Marine Science*, 6:193-199.

FENT, K. (1996). Ecotoxicology of organotin compounds. *Critical Review Toxicology.* 26:1-17.

FENT, K. AND MULLER, M. D. (1991). Occurrence of organotin in municipal waste water and sewage sludge and behaviour in a treatment plant. *Environment Science Technology.* 25:489-493.

FUKAGAWA, T. S., KONNE, S. C., TAKAMA, K. AND SUZUK, S. (1994). Occurrence of tributyltin (TBT) and methylmecury tolerant bacteria in natural seawater to which TBTwas added. *Journal of marine Biotechnology.* 1:211-214.

FENT, K.AND HUN, J. (1995). Organotin in fresh water harbours and rivers temporal distribution annual trends and fate. *Environmental Toxicology Chemistry*. 14:1123-1132.

GIBS, P. E. AND BRYAN , G. W. (1996). *Reproductive failure in the gastropod Nucella lapillus associated with imposex caused by tributyltin pollution*: a review. In champ M A, sedigman PF (ed) organotin environmental fate and effects champman and effects. Champman and Hall London, 25-28.

GIL, R., AVITAL, G., STEWART, L. AND YEHUDA, B. (2000). Un-regulated use of TBT-based antifouling paints in Isreal: high

contamination and imposex levels in two species of marine gastropods. *Marine Ecology.* 192:229-239.

HAGGERA, J. A., DEPLEDGE, M. H. AND GALLOWAY, T. S. (2005). Toxicity of tributyltin in the marine mollusk *Mytilus edulis. Marine Pollution Bulletin* 51:811-816.

HELOISE, F., EMILIEN, P., MICHEL, L. AND MICHEAL, F. (2010). Toxicology of organotins in marine organism: A Review in Organometallic compound: (ed) Ohin, H.F.Nova Science Pub.Inc. pp.1 – 47.

HORIGUCHI, T., IMA, T., CHO, H.S., SHIRAISHI, H., SUBATA, Y. AND MORTITA, M. (1999). Acute toxicity of organotin compounds to the larvae of the rock shell the Thais clavigere, the disk abalone, haliotis madaka. *Marine Environmental Resource.* 46:469-473.

MEADOR, J. P. AND RICE, C. A. (2001). Impaired growth in the polychaete *Armandia brevis* exposed to TBT in sediments. *Marine Environmental Resource.* 51:23-29.

PETTIBONED, G. W. AND COONEY, J. J. (1986). Effect of organotins in fecal pollution indicator microorganism. *Applied Environmental Microbiology.* 52:562-556.

RUIZ, J. M., BRYAN, G. W. AND GIBBS, P.E. (1995). Effects of TBT exposure on the veliger larvae development of the bivalve *Scrobicularia plana* (da costa). *Journal of Experimental Marine Biology Ecology.* 9186 : 53-63.

SENA, G.C., FREITAS-LIMA, L.C., MERLO., E.,PODRATZ., P.L., DE ARAUJO., J.F.P., BRANDAO., P.A.A. (2017). Environmental obesogen tributyltin chloride leads to abnormal hypothalamic pituitary-gonadal ascis function by distruption in kisspeptin signaling in female rates. *Toxicol Appl Phamacol*. 319:22 -38.

SINGH, K. (1987). *Frontier in Applied Micobiology* (eds Makherjee, S.G., Singh, V.P and Garg, K.L) Publication Print House, New Delhi, 11:297-316.

SUZUKI, S., FUGAKAWA, T. AND TAKMA, K. (1992). Occurrence of tributyltin tolerant bacteria in tributyltin or cadmium contaminating sea water. *Applied Environmental Microbiology* 52:3410-3412.

WHITE, J. S., TOBIN, J. M. AND COONEY, J. J. (1999). Organotincompounds and thire interactions with microoganisms.*Canadian Journal of Microbiology.* 45:541-554.