The Pharma Innovation Journal 2022; 11(5): 1548-1553

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(5): 1548-1553

www.thepharmajournal.com
Received: 05-03-2022
Accepted: 15-04-2022

Mhase PP
Department of Veterinary Microbiology, Krantisinh Nana Patil College of Veterinary Science, Shirwal, Satara, Maharashtra, India

Ramteke MD
Department of Veterinary Microbiology, Krantisinh Nana Patil College of Veterinary Science, Shirwal, Satara, Maharashtra, India

Budhe MS
Department of Veterinary Microbiology, Krantisinh Nana Patil College of Veterinary Science, Shirwal, Satara, Maharashtra, India

Tumlam UM
Department of Veterinary Microbiology, Krantisinh Nana Patil College of Veterinary Science, Shirwal, Satara, Maharashtra, India

Muglikar DM
Department of Veterinary Microbiology, Krantisinh Nana Patil College of Veterinary Science, Shirwal, Satara, Maharashtra, India

Urkude PP
Department of Veterinary Microbiology, Krantisinh Nana Patil College of Veterinary Science, Shirwal, Satara, Maharashtra, India

Pawar PD
Department of Veterinary Microbiology, Krantisinh Nana Patil College of Veterinary Science, Shirwal, Satara, Maharashtra, India

Corresponding Author:
Mhase PP
Department of Veterinary Microbiology, Krantisinh Nana Patil College of Veterinary Science, Shirwal, Satara, Maharashtra, India

Predatory *Bdellovibrio* Bacteria: Novel biological approach against antimicrobial resistance

Mhase PP, Ramteke MD, Budhe MS, Tumlam UM, Muglikar DM, Urkude PP and Pawar PD

Abstract
Antimicrobial resistance (AMR) is an emerging global threat leading to human, animal and environmental health crisis. The development of new antibiotics is at very slow pace and an ongoing process resulted in wide gap between need and its fulfilment in near future. To combat with current scenario of AMR and bridge the gap between critical need and affordable effective solution developing the conventional and nonconventional alternative strategies is gaining the topmost priority. One of the promising novel strategies being advancement in the area of formulation of newer antimicrobial modes like use of "Predatory Bacteria" against pathogenic bacteria especially those expressing AMR or MDR. Predatory bacteria are the naturally occurring bacteria in the animal environment that are found to prey and sustain on other bacteria, especially the Gram negative bacteria. The discoveries suggesting predatory bacteria have gained attention worldwide as they are found useful against many MDR species. Therefore, in this article we have discussed about some of these antibacterial bacteria, their predatory mechanisms, application, scope and recent advancements with future perspective in the field of Veterinary medicine.

Keywords: Predatory bacteria, BaLOs, AMR, Mechanism, application

Introduction
The silent pandemic of antimicrobial resistance (AMR) lurks in the shadow of COVID-19 in present scenario. However, if the staggering figures regarding AMR data are to believed as presented in WHO statement, AMR is supposed to be directly responsible for an estimated 1.27 million deaths worldwide, and estimated loss of 4.95 million human lives in year 2019 itself. For mitigating this problem, different alternative strategies like Ayurveda, Homeopathy, bacteriophage therapy, bacteriocins etc., and / or their combinations with Allopathy, are being exploited since few decades. Recently one more novel approach for treatment of multi drug resistant (MDR) infections is being implemented that is use of predatory bacteria, commonly known as the "*Bdellovibrio* and Like Organisms (BaLO)" likely to be a promising alternative.

Predatory bacteria
The bacterial species known to be predating on other bacteria are *Bdellovibrio bacteriovorus*, *Micavibrio aeruginosavorus*, *Myxococcus xanthus*, *Vampirovibrio cholleravorus*. Collectively known as BaLOs, these are small (0.5-1.5µm), mostly gram-negative bacteria, found ubiquitously in soil, aquatic environments, and some of them even belong to normal healthy gut microbiota of the intestine of animals and human being. BaLOs possess ability to naturally invade, grow in or upon and thus, inhibit and kill other unwanted bacteria specifically and non-specifically. These are highly motile with the aid of single flagellum at the end of cell, are vibrioid in shape and aerobic and microaerophilic demand for growth (Rotem *et al.*, 2014, Cavallo *et al.*, 2021) [1, 2].

Origin and development
The developmental milestones are cited by Cavallo *et al.*, (2021) [2] in a review highliting predatory capability of one bacterium on another prey bacterium for its sole existence as actually an accidental discovery of Stolp and Petzold during year 1962, while isolating bacteriophages from soil sample. It was noticed by them for the first time that it was an an isolate of bacteria which possessed predation mechanism upon other host bacteria just like bacteriophages do. This isolate was later identified and studied and got its identity as *Bdellovibrio bacteriovorus*. As per this review, Cotter and Tomashow in year1992 had

explained host independent mode of life of *Bdellovibrio.* This name was allotted to an isolate because of its characteristic lifestyle. Also as per the cited literature later on Gromov and Mamkaeva in year 1966 had mentioned one isolate they had discovered, which was exhibiting the different mechanism of predation similar to a mythical blood sucking 'vampire', hence, was given the name *Vampirovibrio*. Esteve in year 1983 had isolated and characterized *Vampirococcus*. While, as a recognition of most recent developmental milestone, Schuster in 2004, was referred to have studied molecular fingerprinting and the Genome sequence for the type strain *Bdellovibrio bacteriovorus HD 100*.

Table 1: Classification of major BaLOs

Kingdom	**Bacteria**	**Bacteria**	**Bacteria**	**Bacteria**
Sub- kingdom	Negibacteria			
Phylum	Proteobacteria	Proteobacteria	Cyanobacteria	Proteobacteria
Class	Deltaproteobacteria	Alphaproteobacteria	Melainbacteria	Deltaproteobacteria
Order	Bdellovibrionales		Vampirovibrionales	Myxococcales
Family	Bdellovibrionaceae		Vampirovibrionaceae	Myxococcaceae
Genus	*Bdellovibrio*	*Micavibrio*	*Vampirovibrio*	*Myxococcusthaxter*
Species	*B. bacteriovorus*	*M. aeruginosavorus*	*Vampirovibrio chlorellavorus*	*M. xanthus*

(Source; Michiel Vos *et al*., 2021) [3]

Predatory mechanisms of BaLO's

The predatory lifestyle of BaLOs in general is explained having two distinct phases; first being attack Phase and second is growth and division phase ultimately leading to the death of prey bacteria and release of progenies of predator. There are however, different phases being exhibited by predator bacteria as per their different predatory mechanisms, which are; Endobiotic, Epibiotic and Wolf pack mechanism which are explained by founders.

1] Endobiotic Mechanism of Predation

Bdellovibrio bacteriovorus exhibit the endobiotic mechanism of predation. Here Endo meaning –Inside and biotic meaning – the living organism. Basic concept behind this mechanism of predation is that the *Bdellovibrio* enters inside the periplasm of prey bacterial cell, then absorbs the readymade nutrients present in prey protoplasm and multiplies rapidly by binary fission leading to formation of multiple progenies which mature and escape prey cell by its lysis. The steps of endobiotic mechanism as follows:

A] Attack Phase

1. Motility and Prey Recognition: Attack phase predator cells are highly motile, but are non replicative. The shape of these cells is maintained by MreB2 gene. This is a cytoskeleton protein required for formation of membrane associated filaments in bacteria. It is responsible for regulation of cell wall synthesis and cell elongation necessary for the maintenance of cell shape. The predator bacteria with its single polar flagellum gets propelled to a very high velocity of around 160 µm/sec (Iida *et al*. 2009; Lambert *et al*. 2006) [4, 5]. During this motility, which is essential for encountering the prey, it keeps on moving till it reaches or finds suitable prey host. In higher concentrations of available prey, it becomes easier to find it for predator. Though the exact mechanism of attraction of predator on prey cell is not yet understood, it may be a result of chemotactic forces. It may be explained as a result of availability of Multiple methyl accepting chemotaxis proteins (MCP) on surface of predators which may be having the ability to sense various ligands on host surface just like providing cues towards various proteins, organic / inorganic compounds and oxygen for adhesion in nature (Lambert *et al*., 2003; Rotem *et al*., 2014) [6, 1]. In one of the controlled experiments of Rotem *et al*., 2014 [1] when the MCPs were deleted the ability of predatory bacteria to locate the prey was significantly reduced.

2. Attachment To The Prey: The attachment phase of *Bdellovibrio* to its prey is likely to be dependent upon various external factors such as temperature, pH of medium, oxygen concentration and quantity of prey available. Once the prey is recognised attachment over the surface will occur immediately. This attachment is irreversible in case of specific prey but, in case of non-specific pray it gets separated immediately within few minutes (Rotem *et al*., 2014) [1]. Receptors in the form of Type IVa pili in case of *Bdellovibrio* at the non-flagellated pole are found to be playing essential role for prey attachment and penetration into its periplasm.

3. Prey Invasion and Formation of Bdelloplast- Once the predator gets irreversibly attached over its ligands on prey cell surface it leads to the formation of pore in prey cell wall, through which *Bdellovibrio* enters inside protoplasm of prey bacteria. The membrane pore formation may be explained by virtue of solubilization of N-acetylglucosamine of prey cell wall with the help of enzyme glycanase released by predator. The glycanase producing ability of predator is with sole purpose of penetration and is a controlled activity which later on is specifically controlled by counteraction of N-deacetylase production upon accomplishment of entry of predator. Thus it prevents the premature lysis of prey bacteria. Once Bdellovibrio has penetrated the prey cell wall and entered periplasm it grows into a filamentous form known as 'Bdelloplast' (Sánchez-Amat *et al*, 1990) [7].

B. Growth and Division phase

Once the predator Bdellovibrio becomes intracellular into periplasmic stage, it grows into the filamentous structure known as bdelloplasts. Belloplast utilizes periplasmic nutrients and can grow upto the extent of complete periplasmic content available in prey cell bacteria. Once, the contents are depleted, multicellular bdelloplast mature and starts fragmenting to divide itself into number of unicellular progenies whose number will be directly proportional to the size of the prey cell. Concluding lysis of prey cell to release progenies of predator occurs which are fresh actively motile attack phase predator cells that are ready to encounter new prey cells.

C. Bdellocysts formation phase

Sometimes the conditions for further growth of predators may not remain favaourable and in such a scenario few Bdellovibrio strains are also known to adopt to a different resting phase. In this condition when less number of prey or in low nutrient environment prevails, predator cells start forming cyst like structure known as 'Bdellocysts'. Bdellocysts occur inside the prey cells which are morphologically kidney shaped cysts that can again germinate when favoured by the increased prey cells as well as nutrition and L-glutamate, K+ and NH4 becomes readily available for them (Tudor and Conti, 1978) [8].

2. Epibiotic mechanism of predation

The examples of species of predator bacteria which follow this type of mechanism of predation are *Vampirococcus micavibrio, Vampirococcus aeruginosavorus, Bdellovibrio exovorus* and *Vampirovibrio chlorellavorus.* This mechanism has got its name from mythical vampire like behavior of the organism for their propogation. These highly motile organisms move towards their prey and attach to the cell surfaces of host bacteria. However, unlike *Bdellovibrio*, these do not get internalized, instead remain firmly irreversibly attached over the surface of prey bacterium just like in exo-parasitism. They start sucking out the nutrients and multiply by binary fission rapidly on the surface of the host. Finally, upon exhaustion of all cellular contents the prey cell death occurs (Pérez *et al*., 2020) [9]. Hydrolytic enzymes are transferred into the prey cells via the T4SS (transports protein and DNA across cell membrane) mechanism along with the plasmid. The predator metabolites injected into prey organisms degrade their cellular contents to the extent that it kills bacteria. Upon the death of prey the newly formed highly motile progenies are released to further prey on fresh cells completing the cycle. One more predator *Micavibrio aeruginosavorus* is also known to produce six haemolysin proteins belonging to the RTX (Repeats in the Toxins) toxin family. These toxins bind to the prey cell membrane and known to play an important role in attachment and lysis of the prey bacteria (Rotem *et al*., 2014) [1].

3. Wolf pack mechanism of predation

The best example of a predator bacteria to explain this mechanism of predation is *Myxococcus xanthus*. This predator is found to be non-specific and so regardless of the prey species used, it just needs to reach in the close proximity to any prey bacteria which can fall prey to it. As the name for this mechanism suggests one more essential criteria for these predator bacteria is they attack collectively the prey cell in large number at a time. Further attachment on the surface of prey results in induction of its lysis. Because of lysis of prey cell these predators get benefitted from the biomass of prey bacterium in terms of nutrients required. When *M. xanthus* encounters prey cells in large number, by gliding slowly over its surface it covers the prey bacterial surface. Gliding motility is powered by two mechanisms, the S- and A-motility. The polar type IV pili of predator are extended and retracted to move by gliding resulting in efficient predation (Abram *et al*. 1974; Lambert *et al*. 2006) [10, 5]. *M. xanthus* posses ability to produce several known and unknown metabolites. These can be pigments, siderophores, bacteriocins, and antibiotics which can target bacteria or fungi and kill them. Potent antibiotic substances detected to be produced by *M. xanthus* are Myxovirescin A and Myxoprincomide which exhibit the potent antibacterial properties against prey (Pérez *et al*., 2015) [9]. The role myxoprincomide is proved against *B. subtilis* and Myxovirescin A is observed to form lethal cross-links between the cell wall and the inner membrane of prey cells (Pérez *et al*., 2015) [9]. The survival mechanism in absence of prey has been explained in this mechanism and in unfavourable circumstances these bacteria form fruiting bodies (spores) which can later germinate back under favourable conditions.

Table 2: Known predator bacteria and their prey

Predator bacteria	*Micavibrio aeruginosavorus*	*Vampirovibrio chlorellavorus*	*Bdellovibrio bacteriovorus*	*Myxococcus xanthus*
Pray Bacteria	*Burkholderia Escherichia Klebsiella Pseudomonas Shigella*	*Chlorella species*	*Acinetobacter Aeromonas Bordetella Burkholderia Citrobacter Enterobacter Escherichia Klebsiella Listonella Morganella Proteus Pseudomonas Salmonella Serratia Shigella Vibrio Yersinia*	*Escherichia coli Klebsiella pneumoniae Staphylococcus aureus Staphylococcus epidemidis Staphylococcus saprophyticus Proteus mirabilis Pseudomonas aeruginnosa Enterobacter faecalis Bacillus subtilis Candida albicans*

(Juana Pérez *et al*., 2015; Rotem *et al*., 2011; Bratanis *et al*., 2020) [9, 1, 11]

Isolation of BaLOs

Two methods have been discussed by various researchers, the old conventional method was host dependent method in which the prey bacteria were used as trap, while the recent method is host independent method where alternative cells are employed instead of prey bacterium to grow BaLOs in laboratory.

Host Dependent Method

The cultural isolation of BaLOs is explained to be pretty much similar to the isolation of bacteriophages with molten agar overlay method (Rotem *et al*., 2014) [1]. In this method sample (soil or water) is mixed with potential prey bacteria in molten soft agar. This mixture is then poured onto the nutrient agar plate. BaLO's will form transparent lytic plaques upon incubation which are to be differentiated from those formed by bacteriophages (Oyedara *et al*., 2016) [12]. Limitation of this method is since BALO's have specific prey range, only one type of prey bacteria species cannot be used to isolate all the different strains of BALO's.

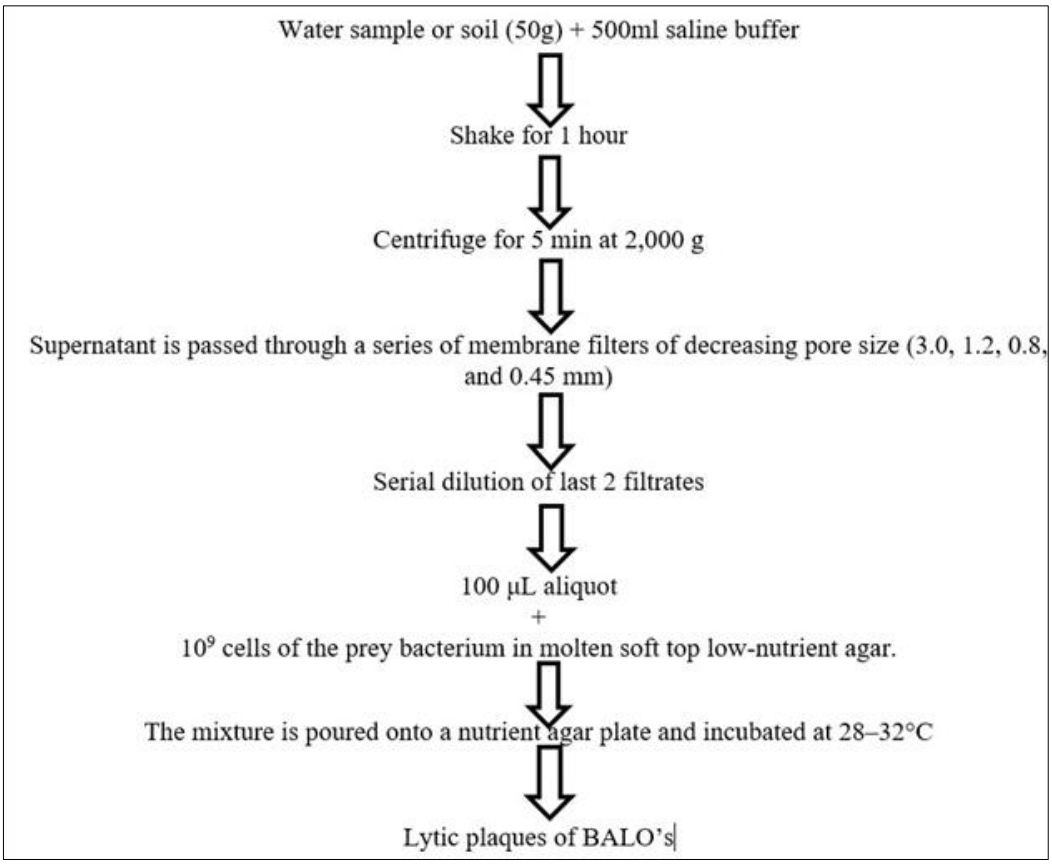

Fig 1: Steps involved in traditional method for isolation from environmental samples.

Marine BALOs require salts to grow. Therefore, the medium used for isolating marine strains should contain at least 25% sea water or appropriate salts. (Rotem *et al*., 2014) [1].

Host Independent Isolation of BaLOs

In host independent method BaLOs are isolated by introducing cell lines like Murine colorectal carcinoma cell line or wild type (WT) cells along with attack phase cells in peptone yeast extract without using prey bacterium. Buffered heat killed prey bacterial biomass can be used for growing the predator bacteria in certain cases. Further double agar overlay technique is used to grow these BaLOs which are detected by plaque formation. A drawback of this approach is the spurious growth of residual prey cells in the medium. To overcome this shortcoming, lytic suspensions are filtered through a 0.45 mm membrane, efficiently separating BaLO cells from prey bacteria but resulting in low recovery rates. The host independent mutants cultivated on heat-killed prey have to be laid over a semisolid medium using the double-layer agar plating (Rosenberg *et al*. 2014) [13].

Applications and scope of BaLOs:

Though the exact knowledge of predator bacteria is in its infancy stage it has been proven that BaLOs possess the potential to fight against pathogens and superbugs with AMR and they can also be exploited in agriculture as well as commercial industry and biotechnology. Scientists have shifted their focus towards applied studies for therapeutic applications of the predator. Based on the in vitro studies proving the effectiveness of BaLOs towards MDR bacteria and superbugs, a variety of in vivo models have been used to estimate efficacy and limitations of BaLOs when used as therapeutic agent.

Safety and Efficacy of BaLOs

Any efficacy studies on antibiotic before it application in animals and human need to be pre-evaluated for their safety and should be proven for being non-hazardous. Accordingly, the predator organisms were evaluated for their cytotoxicity and safety studies so that they can be used as an alternative therapeutic antibacterial agents. In the studies carried out by Gupta *et al*. (2016) [14]
number of cell lines such as corneal-limbal epithelial cells, blood monocytes, macrophages, kidney epithelial cells, liver epithelial cells and spleen monocytes were exposed to BaLOs at a range of
m.o.i. (multiplicity of infection) and were observed at different time intervals. The levels of pro- and anti-inflammatory cytokines produced in response og infection were measured. Though the pro- and anti-inflammatory cytokines IL1B, TNFa, IL6, IL8 and IL10 were stimulated in response to bacterial outer membrane lipopolysaccharide (LPS) which were negligible or low. The morphological changes, cell viability imaging and measurement of cytotoxin levels remained in normal range, reassuring their safety. The first ever *in vivo* study was performed by Atterbury *et al.*, (2011) [15] in day old HY line brown male chicks. The main objective was to estimate the safety levels of *Bdellovibrio* when given as oral inoculum as well as effectiveness against induced *Salmonella* infection. *Bdellovibrio HD 100* strain was used against *Salmonella enteritidis P125109.* The results indicated that after three days of treatment the concentration of *Salmonella* in caeca of chicks was significantly reduced. This study proved the safety of BaLOs when used orally as well as their potential in elimination of *Salmonella* infection without compromising standard growth and performance parameters of birds. Mouse models were also been used by

Shatzkes *et al*., (2015) [16] and BaLOs were found to be safe for intranasal and intravenous inoculation (Kenneth *et al*., 2015) [26]. A low level of predator in absence of prey with no morbidity or adverse histopathology of different organs reconfirmed their safety. Intranasal inoculation of *B. bacteriovorus* against induced infection by *Enterobacteriaceae* evaluated in rat lungs resulted in a decrease of 3.4 log10 CFU/ml in later studies (Shatzkes *et al*., 2015 [16]; Cavello *et al*., 2021 [6]). A systemic injection of *B. bacteriovorus* was attempted against induced *K. pneumoniae* infection in a rat model by these workers and an initial increase in pro-inflammatory cytokines was reported by them which later on returned to baseline levels within 18 h. As well as an efficient clearance of
B. bacteriovorus was observed within 20 days. However, in this study predator organisms were not found effective for treatment of acute blood stream infections. Findlay *et al*., (2019) [17] proved *bacteriovorus* conferring protection against a lethal systemic infection caused by *Yersinia pestis* in SKH-1 mice. Zebrafish larval model was used for in vivo evalution by Willis *et al*., (2016) [18] to demonstrate the safety of *Bdellovibrio* as well as their effect against induced *Shigella* infection, which was reduced significantly and even immunocompromised patients survived the *Shigella* infection. This repeated non-toxic nature of BaLOs can be attributed to their natural co-existance in human and animal environment since initial phase of evolution leading to built up of immune tolerance for them. It might also be attributed to the cell wall of BALO's which have modified Lipid A protein along with Alpha D-mannose instead of negatively charged phosphate group which is present in pathogenic gram negative bacteria (Evans *et al*., 2007). Immune cells have receptors to recognize these phosphate groups, leading to immune reaction. Besides, the TTSS (Type Three Secretory System) that provides gram negative bacteria with unique virulence mechanism enabling them to cause infections is not found in predatory bacteria therefore, are not toxic to human as well as animal cells (Bratanis *et al*.,2020) [11]. One of the major reason for emergence of AMR is horizontal gene transfer by transduction and transformation in superbugs. The novel approach to fight AMR is by using bacteriophages however, presence of ARG's (Antimicrobial Resistance Gene) in them cannot be ruled out (Lood *et al*., 2015) [20]. The importance of elimination of recombinant DNA from the environment by using predatory bacteria like *B. bacteriovorus HD100* for effective removal of recombinant bacterial strains in aqueous and soil slurry environments were demonstrated earlier and such experiments are warranted in future for reducing the environmental hazards due to rDNA technology. Production of bioplastic is one of the major aspects to control and protect the environmental pollution. The extended scope of BaLOs being implemented to the rescue of mankind as cheapest lytic agents for the recovery of intracellular bio-products produced by *Psudomonas* as well as *E. coli* known as PHA (Polyhydroxyalkalonates) has been reviewed by Bratanis *et al*., (2020) [11] in his work.

Limitations of BaLOs

Although predatory bacterial therapy and applications are found to be very safe and effective, their large scale practical applications are still in infancy stage. The isolation of BaLOs is difficult as their presence is highly dependent of the concentration of prey bacteria. Maintaining the culture of BALOs is difficult as the viability and survival reduces with reduction in prey organisms. BaLOs are sensitive to the environmental factors such as temperature, pH, availability of oxygen, environmental pollutants which affect their survivability and predatory activity adversely. Common waste water toxicants such as phenol and urea have been shown to affect the life cycle of *B. bacteriovorus* (Markoleva *et al*., 2002) [21]. Cho *et al*., (2019) [22] found another wastewater pollutant SDS (Sodium Dodecyl Sulphate) adversely affecting *Bdellovibrio*. Herbicides in agricultural application can affect adversely the recovery and efficacy of BaLOs.
Their efficacy can get affected in mixed microbial communities as predation of the favored prey is a biggest limitation factors. Efficacy of BALOs in mixed or multiple infections may not result in complete elimination of prey (Rogosky *et al*., 2006) [23]. Although it is not toxic to human and animal cells the possibility of developing hypersensitivity or autoimmunity cannot be overlooked. Host dependent strains are more effective than independent strains and later may contribute in formation of unnecessary biofilms (Bratanis *et al*., 2020) [11]

Conclusion

BaLOs have natural ability to predate gram negative bacteria due to which they have potential to be used as therapeutic agent to fight prevailing AMR pandemic. The possibility of transfer ARG's via HGT mechanism in BALOs is less likely. With the use of recombinant technology bacterial genome of both prey and predators can be modified so that large scale application through developing broad spectrum of activity is possible. Simpler techniques for isolation, maintenance of culture can be standardized. The possibilities of induction of adverse or hazardous reactions are likely, and so area needs to be explored more. Calculation of doses, route and mode of administration are yet to be scaled up. The literature indicated growing interest of researchers in noval applications of BaLOs as alternative promising antibacterial agents. Lot of areas are being explored and needed to be explored for application of BaLOs for the welfare of human, animal and environmental health.

References

1. Rotem O, Pasternak Z, Jurkevitch E. *Bdellovibrio* and Like Organisms. In: Rosenberg E, DeLong EF, Lory S, Stackebrandt E., Thompson F. (eds) The Prokaryotes. Springer, Berlin, Heidelberg. 2014. https://doi.org/10.1007/978 -3-642-39044- 9_379
2. Cavallo Francis M, Lorea Jordana, Alexander W. Friedrich, Corinna Glasner, Jan Maarten van Dijl. *Bdellovibrio bacteriovorus*: a potential 'living antibiotic' to control bacterial pathogens, Critical Reviews in Microbiology. 2021;47(5):630-646,
3. Michiel Vos *et al*., Integrated Taxonomic Information System – Report. 2021.
4. Iida Y, Hobley L, Lambert C, Fenton AK, Sockett RE, Aizawa S. Roles of multiple flagellins in flagellar formation and flagellar growth post bdelloplast lysis in Bdellovibrio bacteriovorus. J Mol Biol. 2009;394:1011–1021.
5. Lambert C, Evans KJ, Till R, Hobley L, Capeness M, Rendulic S. Characterizing the flagellar filament and the role of motility in bacterial prey-penetration by Bdellovibrio bacteriovorus. Mol Microbiol. 2006;60:274-

286.
6. Lambert C, Smith MC, Sockett RE. A novel assay to monitor predator–prey interactions for Bdellovibrio bacteriovorus 109J reveals a role for methyl-accepting chemotaxis proteins in predation. Environ Microbiol. 2003;5:127-132.
7. Sánchez-Amat A, Torrella F. Formation of stable bdelloplasts as a starvation-survival strategy of marine bdellovibrios. *Appl Environ Microbiol*. 1990;56(9):2717-2725. doi:10.1128/aem.56.9.2717-2725.1990.
8. Tudor JJ, Conti SF. Characterization of germination and activation of Bdellovibrio bdellocysts. *J Bacteriol*. 1978;133(1):130-138. doi:10.1128/jb.133.1.130-138.1978
9. Pérez J, Contreras-Moreno FJ, Marcos-Torres FJ, Moraleda-Muñoz A, Muñoz-Dorado J. The antibiotic crisis: How bacterial predators can help. *Comput Struct Biotechnol J*. 2020;18:2547-2555. Published 2020 Sep 15. doi:10.1016/j.csbj.2020.09.010
10. Abram D, Melo CJ, Chou D. Penetration of *Bdellovibrio bacteriovorus* into host cells. J Bacteriol. 1974;118:663–680.
11. Bratanis, Eleni Andersson, Tilde Lood, Rolf Bukowska-Faniband, Ewa Review Biotechnological Potential of Bdellovibrio and Like Organisms and Their Secreted Enzymes Frontiers in Microbiology. 2020.
12. Oyedara OO, De Luna-Santillana EJ, Olguin-Rodriguez O, Guo X, Mendoza-Villa MA, Menchaca-Arredondo JL. Isolation of Bdellovibrio sp. from soil samples in Mexico and their potential applications in control of pathogens. Microbiologyopen. 2016 Dec;5(6):992-1002. doi: 10.1002/mbo3.382. Epub 2016 Jun 13. PMID: 27297185; PMCID: PMC5221441.
13. Rosenberg Z, *et al.* (eds.) The Prokaryotes – Deltaproteobacteria and Epsilonproteobacteria, 2014. DOI 10.1007/978-3-642-39044-9_379, # Springer-Verlag Berlin Heidelberg
14. Gupta S, Tang C, Tran M, Kadouri DE. Effect of Predatory Bacteria on Human Cell Lines. PLoS One. 2016 Aug 31;11(8):e0161242. doi: 10.1371/journal.pone.0161242. PMID: 27579919; PMCID: PMC5006992.
15. Atterbury RJ, Hobley L, Till R, Lambert C, Capeness MJ, Lerner TR. Effects of orally administered Bdellovibrio bacteriovorus on the well-being and Salmonella colonization of young chicks. Appl Environ Microbiol. 2011 Aug 15;77(16):5794-803. doi: 10.1128/AEM.00426-11. Epub 2011 Jun 24. PMID: 21705523; PMCID: PMC3165243.
16. Shatzkes K, Chae R, Tang C, Ramirez GC, Mukherjee S, Tsenova L. Examining the safety of respiratory and intravenous inoculation of Bdellovibrio bacteriovorus and Micavibrio aeruginosavorus in a mouse model. Sci Rep. 2015 Aug 7;5:12899. doi: 10.1038/srep12899. PMID: 26250699; PMCID: PMC4528228.
17. Findlay JS, Flick-Smith HC, Keyser E, Cooper IA, Williamson ED, Oyston PCF. Predatory bacteria can protect SKH-1 mice from a lethal plague challenge. *Sci Rep*. 2019;9(1):7225. Published 2019 May 10. doi:10.1038/s41598-019-43467-1
18. Willis AR, Moore C, Mazon-Moya M, Krokowski S, Lambert C, Till R. Injections of Predatory Bacteria Work Alongside Host Immune Cells to Treat Shigella Infection in Zebrafish Larvae. Curr Biol. 2016 Dec 19;26(24):3343-3351. doi: 10.1016/j.cub.2016.09.067. Epub 2016 Nov 23. PMID: 27889262; PMCID: PMC5196024.
19. Evans KJ, Lambert C, Sockett RE. Predation by *Bdellovibrio bacteriovorus*. HD100 requires type IV pili [published correction appears in J Bacteriol. 2007 Sep;189(17):6507]. J Bacteriol. 2007;189(13):4850-4859. doi:10.1128/JB.01942-06
20. Lood R, Winer BY, Pelzek AJ, Diez-Martinez R, Thandar M, Euler CW. Novel phage lysine capable of killing the multidrug-resistant gram- negative bacterium *Acinetobacter baumannii* in a mouse bacteremia model. *Antimicrob Agents Chemother*. 2015;59:1983–1991. doi: 10.1128/AAC.04641-14.
21. Markelova NY. Effect of toxic pollutants on *Bdellovibrio*. Process Biochem. 2002;37:1177-1181. 10.1016/S0032-9592(01)00331-4
22. Cho G, Kwon J, Soh SM, Jang H, Mitchell RJ. Sensitivity of predatory bacteria to different surfactants and their application to check bacterial predation. Appl. Microbiol. Biotechnol. 2019;103:8169-8178. 10.1007/s00253-019-10069-w
23. Rogosky AM, Moak PL, Emmert EAB. Differential predation by *Bdellovibrio bacteriovorus* 109J. Curr. Microbiol. 2006;52:81–85. https://www.who.int/publications-detail-redirect/9789240027336
24. Dwidar M, Monnappa AK, Mitchell RJ. The dual probiotic and antibiotic nature of Bdellovibrio bacteriovorus. BMB Rep. 2012 Feb;45(2):71-8. doi: 10.5483/BMBRep.2012.45.2.71. PMID: 22360883.
25. Kenneth, *et al*. "Examining the safety of respiratory and intravenous inoculation of Bdellovibrio bacteriovorus and Micavibrio aeruginosavorus in a mouse model." Scientific reports 12899. 2015 Aug7, 5. doi:10.1038/srep12899