Quest Journals

Journal of Research in Agriculture and Animal Science

Volume 9 ~ Issue 9 (2022) pp: 51-56

ISSN(Online): 2321-9459 www.questjournals.org



Research Paper

Effect of Probiotic Bacteria on the Xenobiotics Degradation Using Freshwater Fish Catla Catla

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Abstract

The analysis of physiochemical parameters were determined by their organisms and the environment. Physicochemical properties analysed from four different fish farm water samples of Thittai, Ammapet, Thiruvaiyaru and Orathanadu, Thanjavur District, Tamil Nadu, India. The maximum physicochemical properties analyzes by Orathanadu fish farm water sample. Bacteria associated from the fish farm water samples such as Bacillus sp., B. cereus, B. coagulans, B. substilis, Flavobacteriumsp., Lactobacillus acidophilus, Lactobacillus sp., Lactococcuslactis, Micrococcus sp., Pseudomonas aeruginosa, Staphylococcus aureusand S.saprophyticuswere isolate and identified. The Lactococcuslactisbacteria were screened by the environ parameters such as ammonia, pH, DO, OC, TDS and temperature. The commercial xenobiotics were degraded by the potential Lactococcuslactisbacteria with different concentration for 12 days of soil conditioners xenobiotics like gypsum and lime.

Keywords: aquaculture, Catlacatla, probiotic, environ parameters, xenobiotic, gypsum and lime

Received 04 Sep, 2022; Revised 17 Sep., 2022; Accepted 19 Sep., 2022 © The author(s) 2022. Published with open access at www.questjurnals.org

I. Introduction

Catlacatla(Hamilton), a fast-growing Indian major carp, is a surface feeder, feeding mainly on zooplankton. It accepts artificial diets and therefore, is a popular species for polyculture in India. Barash and Schroeder (1984) observed that formulated feeds could be partially replaced by organic manures. A reduction in fish meal, the major protein source of fish diets, is desirable due to high cost and scarcity. According to Paulyet al., (2000), fish meal production is not expected to increase further. Current research is directed at the use of plant ingredients in fish feeds (Mbahinzirekiet al., 2001). Food and feeding habits of carps have been a field of interest to fisheries researchers since very long. Natarajan and Jhingran (1961) studied that the food habits of Catlacatla and reported a zooplankton dominated food preference for Catlacatla. Hora and Pillay (1962) reported that the feeding habits of Catlacatla. The food and feeding habits of an Indian major carp Labeorohita(Ham). Rajgopal (1978) described that the foods and feeding habits of some commercial fishes from the Tungabhadra reservoir. Aquaculture is one of the fast growing systems in the world, which has emerged an industry possible to supply protein rich food throughout the world(Prasad, 1996). Fish is an important dietary animal protein source in human nutrition. Production of aquatic species through freshwater fisheries and aquaculture for protein supply is being encouraged throughout the world. In particular, summarized the current knowledge on the direct mechanisms by which probiotics can influence xenobiotics detoxification.

II. Materials and Methods

Sample collection

The aqua water and fish samples *Catlacatla* were collected from different places Thittai, Ammapet, Thiruvaiyaru and Orathanadu, Thanjavur District, Tamil Nadu, India pack in polythene bags. The samples were

carry out to the laboratory in aseptically condition and maintained in the laboratory in a glass aquarium tank and acclimated in aerated tap water with continuous aeration for two weeks prior to experimentation. During this period, fishes were fed with a known amount of fish food.

Physico- Chemical Parameters (Ogbonna and Chinomso, 2010)

Some physical test should be performed for testing of its physical appearance such as colour, pH, Total alkalinity, DO, BOD, COD, Ammonia, Turbidity, TDS, Total alkalinity and Electrical conductivity were analysed. Following different physico-chemical parameters are tested regularly for monitoring quality of water. These physical-chemical properties were determined electrometrically with a multiparameter data logger (Hanna model HI991300).

Isolation of of probiotics from Catlacatla aquaculture water samples (Wankaet al., 2018)

In the laboratory, the water sample was aseptically carried out and serially diluted upto 10^{-6} dilution. From each dilution, 0.1 ml of sample was taken and spread plated on sterile nutrient agar medium. The plates were then incubated at 37^{0} C for 24 to 48 h. The total viable count (TVC) of the colonies was finally noted. For Total Viable Count were counted by using Quebec colony counter. Population density is expressed in terms of colony forming unit (CFU) per gram of soil with dilution factor

Purification of probiotics from Catlacatla quaculture water samples (Surkattiet al., 2021)

Then the bacterial cultures were identified by performing biochemical tests. Morphologically different colonies were isolated, re-streaked to ensure purity and maintained on Nutrient Agar vials for further characterization. The slant was kept in refrigerator at 4°C for short time storage before further studies.

Identification and biochemical characterization of gut microflora (Sirishaet al., 2017)

The cultures were then identified as various genera as per the Bergey's manual of determinative bacteriology.

Screening of potential probiotics with different environ parameters(Jacobsenet al., 1999)

The screening of probiotics with different environment parameters like ammonia, pH, DO, OC, TDS and temperature for *Bacillus* sp., *B. cereus*, *B. coagulans*, *B. substilus*, *Flavobacterium* sp., *Lactobacillus acidophilus*, *Lactobacillus* sp., *Lactococcuslactis*, *Micrococcus* sp., *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *S. saprophyticus*.

Biodegradation of xenobiotics by Lactococcuslactis (Daxini and Mistry, 2018)

The *Lactococcuslactis* was screened for degradation of soil conditioners xenobiotics like gypsum and lime. The isolate was grown on NA broth supplemented with 125ppm, 250ppm, 375ppm and 500ppm gypsum and lime soil conditioners xenobiotics separately and incubated at room temperature for 9 days. At a regular interval of 3 days to assess residual concentrations of each xenobiotics, Culture was inspected visually at intervals for turbidity, color change and oil dispersion. Growth has observed by monitoring optical density at 600nm at regular time interval.

III. Results

The physiochemical parameters were analyzed for fish cultivation. According to the cultivation of fish farming, water has major role for the growth and development of *Catlacatla* fishes like, pH, Total alkalinity, DO, BOD, COD, Ammonia, Turbidity, TDS, Total alkalinity and Electrical conductivity was6.58±0.10, 47.6±18.40 mg/l, 5.5±0.50 mg/l, 2.9±0.60 mg/l, 19.6±5.32 mg/l, 4.3±1.12 mg/l, 29.1±4.7 mg/l, 55.2±19.02 ppm, 19.7±4.10 mg/l and 137.6±75.37 µmhos/cm in Thittai area, 6.52±0.90, 43.1±18.0 mg/l, 5.1±0.08 mg/l, 3.0±0.50 mg/l , 22.5±6.24 mg/l , 3.9±0.98 mg/l , 45.1±15.07 mg/l , 49.8±15.21 ppm, 25.7±3.90 mg/l and 128.5±75. 30 µmhos/cm in Ammapet, 6.24±0.02, 77.6±32.63 mg/l, 2.8±0.20 mg/l, 4.52±0.90 mg/l, 18.6±5.54 mg/l, 3.2±1.02 mg/l,56.2±30.50ppm, 95.2±69.40mg/l, 24.3±3.80mg/l and 144.3±78.26 µmhos/cm in Thiruvaiyaruand 6.28±0.20, 93.70±46.53mg/l, 6.6±0.18mg/l, 4.40±0.90mg/l, 17.9±4.61mg/l, 2.9±0.95mg/l, 34.7±6.80ppm, 145.40±91.01mg/l, 44.3±15.07mg/l and 378.4±130.20 µmhos/cm in Orathanadu area respectively were recorded (Table1).

Isolation of probiotics from water samples

The maximum number of probiotics colonies recorded in Ammapet area water sample than compared to Thittai, Orathanadu and Thiruvaiyaru area water samples (Table 2, 3 and Plate I).

Identification of bacteria from parts of the fish sample

Totally twelve probiotics were recorded from aqua water sample. The population of bacteria was identified with gram staining and biochemical studied followed. The name of the bacteria such as *Bacillus* sp., *B. cereus*, *B. coagulans*, *B. substilis*, *Flavobacterium*sp., *Lactobacillus acidophilus*, *Lactobacillus* sp., *Lactococcuslactis*, *Micrococcus* sp., *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *S.saprophyticus* were identified respectively (Table-4).

Screening of potential probiotics by environmental parameters

In Bacillus sp. was showed following results such as ammonia (7.21mg/L), pH(6.5), DO (69.3mg/L), OC (3.6mg/L), TDS (10.3mg/L) and temperature (32°C), B. cereuswas ammonia (1.35mg/L), pH (6.9), DO (59.4mg/L), OC (2.0mg/L), TDS (10.2mg/L) and temperature (37°C), B. coagulans showed as ammonia (2.06mg/L), pH (7.1), DO (52.5mg/L), OC (2.7mg/L), TDS (10.7mg/L) and temperature (32°C), B. substilushas showed ammonia (6.50mg/L), pH (7.5), DO (50.1mg/L), OC (3.2mg/L), TDS (14.3mg/L) and temperature (33°C), Flavobacteriumsp. ammonia (2.49mg/L), pH (6.7), DO (41.3mg/L), OC (2.1mg/L), TDS (13.4mg/L) and temperature (37°C), Lactobacillus acidophilus expressed ammonia (5.93mg/L), pH (6.6), DO (47.8mg/L), OC (2.8mg/L), TDS (16.7mg/L) and temperature (38°C), Lactobacillus sp. was expressed following ammonia (6.74mg/L), pH (7.1), DO (51.3mg/L), OC (3.4mg/L), TDS (13.4mg/L) and temperature (35°C), Lactococcuslactishas showed expected results such as ammonia (8.25mg/L), pH (7.0), DO (43.7mg/L), OC (1.4mg/L), TDS (17.4mg/L) and temperature (35°C), Micrococcus sp. was found following ammonia (3.25mg/L), pH (7.3), DO (61.4mg/L), OC (2.0mg/L), TDS (12.1mg/L) and temperature (33°C), Pseudomonas aeruginosaas showed ammonia (2.41mg/L), pH (7.0), DO (65.7mg/L), OC (2.7mg/L), TDS (15.2mg/L) and temperature (37°C), Staphylococcus aureus was ammonia (5.62mg/L), pH (6.8), DO (68.9mg/L), OC (3.7mg/L), TDS (14.2mg/L) and temperature (35°C) and S. saprophyticus was showed ammonia (5.42mg/L), pH (6.54), DO (70.8mg/L), OC (4.0mg/L), TDS (13.8mg/L) and temperature (37°C). The maximum ammonia production and Total dissolved solids were identified from *Lactobacillus lactis* recorded (Table 5).

Effect of Lactococcuslactison the degradation of soil conditioners

In the gypsum soil conditioners xenobiotics were degraded by Lactococcuslactisin 125ppm, 250ppm, 375ppm and 500ppm was expressed following degradation such as $0.25\pm0.08IU/ml$, $0.28\pm0.09IU/ml$, $0.36\pm0.12IU/ml$ and $0.41\pm0.13IU/ml$ in 3rd day. In 6th day of Lactococcuslactiswas expressed for 125ppm, 250ppm, 375ppm and 500ppm following quantity of xenobiotic degradation such as $0.22\pm0.07IU/ml$, $0.27\pm0.09IU/ml$, $0.33\pm0.11IU/ml$ and $0.38\pm0.12IU/ml$.Similarly, in 9th day has showed $0.46\pm0.14IU/ml$ m $0.63\pm0.21IU/ml$, $0.72\pm0.24IU/ml$ and $0.76\pm0.25IU/ml$ quantity for 125ppm, 250ppm, 375ppm and 500ppm.

In the lime soil conditioners xenobiotics were degraded by Lactococcuslactisin 125ppm, 250ppm, 375ppm and 500ppm was expressed following degradation such as $0.21\pm0.07IU/ml$, $0.22\pm0.07IU/ml$, $0.25\pm0.08IU/ml$ and $0.32\pm0.10IU/ml$ in 3rd day. In 6th day of Lactococcuslactis was expressed for 125ppm, 250ppm, 375ppm and 500ppm following quantity of xenobiotic degradation such as $0.55\pm0.18IU/ml$, $0.33\pm0.11IU/ml$, $0.55\pm0.18IU/ml$ and $0.46\pm0.15IU/ml$.Similarly, in 9th day has showed $0.53\pm0.17IU/ml$ m $0.63\pm0.21IU/ml$, $0.62\pm0.20IU/ml$ and $0.82\pm0.27IU/ml$ quantity for 125ppm, 250ppm, 375ppm and 500ppm were recorded respectively.

Table 1:Physico-chemical parameters of *Catlacatla* aquaculture water samples

Physico-chemical parameters	Different Sampling places					
r nysico-chemicai parameters	Thittai	Ammapet	Thiruvaiyaru	Orathanadu		
Colour	Greenish white					
pH	6.58±0.10	6.52±0.90	6.24±0.02	6.28±0.20		
Total alkalinity (mg/l)	47.6±18.40	43.1±18.0	77.6±32.63	93.70±46.53		
DO (mg/l)	5.5±0.50	5.1±0.08	2.8±0.20	6.6±0.18		
BOD (mg/l)	2.9±0.60	3.0±0.50	4.52±0.90	4.40±0.90		
COD (mg/l)	19.6±5.32	22.5±6.24	18.6±5.54	17.9±4.61		
Ammonia (mg/l)	4.3±1.12	3.9±0.98	3.2±1.02	2.9±0.95		
Turbidity (ppm)	29.1±4.7	45.1±15.07	56.2±30.50	34.7±6.80		
Total dissolved solid (mg/l)	55.2±19.02	49.8±15.21	95.2±69.40	145.40±91.01		
Total hardness (mg/l)	19.7±4.10	25.7±3.90	24.3±3.80	44.3±15.07		
Electrical conductivity (µmhos/cm)	137.6±75.37	128.5±75.30	144.3±78.26	378.4±130.20		

Table 5: Screening of probiotics by using different environ parameters

8	Different parameters					
Name of the bacteria	Ammonia (mg/l)	pН	DO (mg/l)	OC (mg/l)	TDS (mg/l)	Temperature (°C)
Bacillus sp.	7.21	6.5	69.3	3.6	10.3	32
B. cereus	1.35	6.9	59.4	2.0	12.2	37
B. coagulans	2.06	7.1	52.5	2.7	10.7	32
B. substilis	6.50	7.5	50.1	3.2	14.3	33
Flavobacteriumsp.	2.49	6.7	41.3	2.1	13.4	37
Lactobacillus acidophilus	5.93	6.6	47.8	2.8	16.7	38
Lactobacillus sp.	6.74	7.1	51.3	3.4	13.4	35
Lactococcuslactis	8.25	7.0	43.7	1.4	17.4	35
Micrococcus sp.	3.25	7.3	61.4	2.0	12.1	33
Pseudomonas aeruginosa	2.41	7.0	65.7	2.7	15.2	37
Staphylococcus aureus	5.62	6.8	68.9	3.7	14.2	35
S.saprophyticus	5.42	6.4	70.8	4.0	13.8	37

pH - Hydrogen iron Concentration, DO -Dissolved oxygen, OC - Organic carbon, TDS - Total dissolved solid

Table 6: Effect of *Lactococcuslactis* on the degradation of different commercial xenobiotics by invitromethod

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Different				Different xenobiotics (IU/ml)						
concentrati on (ppm)	Soil cond	litioners	Therapeutants			Anesthetics		Feed additives		
	Gypsum	Lime	Hydrogen peroxide	streptomycin	erythromycin	Benzocain	Formalin	Vitamin - C		
	After 3 days treatments									
Control	0.19±0.12	0.15±0.05	0.10±0.07	0.02±0.03	0.10±0.04	0.11±0.05	0.07±0.03	0.03±0.06		
125	0.25±0.08	0.21±0.07	0.12±0.04	0.03±0.01	0.15±0.05	0.13±0.04	0.06±0.02	0.05±0.01		
250	0.28±0.09	0.22±0.07	0.15±0.05	0.06±0.02	0.12±0.04	0.15±0.05	0.06±0.02	0.11±0.03		
375	0.36±0.12	0.25±0.08	0.17±0.05	0.06±0.02	0.16±0.05	0.16±0.05	0.05±0.01	0.15±0.05		
500	0.41±0.13	0.32±0.10	0.21±0.71	0.14±0.04	0.23±0.07	0.24±0.08	0.11±0.03	0.14±0.04		
	After 6 days treatments									
Control	0.12±0.05	0.11±0.12	0.10±0.05	0.12±0.11	0.13±0.10	0.12±0.11	0.10±0.04	0.12±0.05		
125	0.22±0.07	0.55±0.18	0.14±0.04	0.06±0.02	0.35±0.11	0.54±0.18	0.11±0.03	0.10±0.03		
250	0.27±0.09	0.33±0.11	0.25±0.08	0.14±0.04	0.38±0.12	0.55±0.18	0.12±0.04	0.12±0.04		
375	0.33±0.11	0.55±0.18	0.36±0.12	0.20±0.06	0.44±0.14	0.51±0.17	0.12±0.04	0.13±0.04		
500	0.38±0.12	0.46±0.15	0.36±0.12	0.26 ± 0.08	0.45±0.15	0.63±0.21	0.13±0.04	0.13±0.04		
After 9 days treatments										
Control	0.09±0.12	0.03±0.17	0.06±0.03	0.08±0.07	0.04±0.12	0.12±0.10	0.11±0.04	0.10±0.03		
125	0.46±0.14	0.53±0.17	0.11±0.03	0.23±0.07	0.34±0.11	0.34±0.11	0.19±0.06	0.15±0.05		
250	0.63±0.21	0.63±0.21	0.13±0.04	0.44±0.14	0.43±0.14	0.52±0.17	0.13±0.04	0.16±0.05		
375	0.72±0.24	0.62±0.20	0.58±0.19	0.41±0.11	0.64±0.21	0.53±0.17	0.16±0.05	0.16±0.05		
500	0.76±0.25	0.82±0.27	0.59±0.19	0.42±0.14	0.62±0.20	0.61±0.20	0.14±0.04	0.13±0.04		

Standard deviation ±error

IV. Discussion

Delince (1992) stated that the abundance of phytoplankton and zooplankton is responsible for the determination of the color of an aquatic body and Green, bluish green/ brown greenish color of water indicates good plankton population hence, good for fish health [National Agricultural Extension and Research Water Quality Management in Fish Culture,, 1996]. In the present study, the pond water color is light green so the pond water is good for fish productivity.

The pH between 6 and 9 was appropriate for increased fish production. Electrical conductivity (EC) is a useful tool to evaluate the purity of water [ICMR, 1975] Turbidity and the appearance of water are important considerations in pond aquaculture. This is a measure of the ability of water to transmit the light that restricts light penetration and limit photosynthesis. Bio-chemical Oxygen demand is a parameter to assess the organic load in a water body. It is the measurement of total dissolved oxygen consumed by microorganism for biodegradation of organic matter. Dissolved oxygen is an important parameter in water quality assessment and reflects the physical and biological processes of aquatic life.

COD is an important parameter for establishing the quality of water. It determines the amount of oxygen required for chemical oxidation of organic and inorganic matter. Organic matter and anthropogenic activities are the main factors responsible for higher COD. Total hardness of water is the parameters used to describe the effect of dissolved minerals (mainly Ca and Mg), determining suitability for domestic and industrial purposes which is attributed to the presence of bicarbonates, sulfates, chlorides and nitrates [Solomon *et al.*, 2013]. Calcium and Magnesium are essential for bone and scale formation.

In the present investigation that the physicochemical parameters were analyzed in four different places of Thittai, Ammapet, Thiruvaiyaru and Orathanadu, Tamilnadu, India. The maximum physiochemical parameters was determined in Orathanadu fish farm water especially pH, Dissolved Oxygen, Ammonia and turbidity than compared to other area water samples, the parameters are main recognized factors for better

growth of fish cultivation. Probiotics have the potential to improve immune status as well as performance of farmed fish but the use of non-native bacteria derived from endothermic terrestrial species may undermine a successful colonization of the GIT of farmed fish. probiotic candidates should exhibit high growth rates at the respective rearing temperatures, be easily stored in cryo-cultures, oxygen tolerant and effectively supplemented to the diet assuring sufficient viability (Martínez Cruz *et al.*, 2012).

Probiotic were identified addressing antagonism towards a major pathogen of turbot, *Tenacibaculummaritimum*, synthesis of essential FA and metabolization of plant-specific anti-nutrients, using saponin as a model substance. Among the autochthonous microbial community closely associated with the tissue surface of the fish intestine, afew pathogens were identified here. This confirms that pathogens are part of the teleost microbiom, even in fish that do not exhibit any symptoms of disease (Xing *et al.*, 2013).

In the present study, the twelve probiotics were isolated and identified by the fish farming water samples. More number colonies isolated by *Lactococcuslactis*. Based on the morphological characteristics four (4) isolates were identified as *Lactobacillus* spp. After gram staining the isolated bacteria were rod shaped, convex, rough, smooth, shiny, irregular, circular, gram positive, facultative anaerobic, nonspore forming which indicate them to be the member of *Lactobacillus* spp. (Bauer *et al.*, 1966).

In the present investigation to suggests that they include the bacterial from the fish farm water samples some of the bacteria associated such as *Bacillus* sp, *B. cereus*, *B. coagulans*, *B. substilis*, *Flavobacterium*sp., *Lactobacillus acidophilus*, *Lactobacillus* sp., *Lactococcuslactis*, *Micrococcus* sp., *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *S.saprophyticus* were isolate and identified.

Cahill, (1990)Studied that the microbiological diversity of fresh fish muscle depends on the fishing grounds and environmental factors around it It has been suggested that the type of micro-organisms that are found associated with particular fish depends on its habitat. The bacterial pathogens associated with fish have been classified as indigenous and non-indigenous. The non-indigenous contaminate the fish or the habitat one way or the other and examples include *Escherichia coli, Clostridium botulinum,Shigelladysenteriae, Staphylococcus aureus, Listeria monocytogens* and *Salmonella*. The indigenous bacterial pathogens are found naturally living in the fish s habitat for example *Vibrio* species and *Aeromonas* species (Rodricks, 1991).

The influence of probiotics on xenobiotics metabolism has been the subject of many studies. Considering the possibility of the formation of derivatives with various properties, the investigation of the impact of the probiotics on changes of biological activity of xenobiotics currently forms an important direction of research. Xenobiotics penetrate the organism primarily via the oral route (Claus *et al.*, 2016). The biotransformation of xenobiotics via probiotics with gut microflora cooperation can alter xenobiotic half-life, toxicity, and bioavailability as well as the endocrine-disrupting potential (Abdelsalam*et al.*, 2020; Clarke *et al.*, 2019; Złoch*et al.*, 2020). The most commonly used probiotics are lactic acid bacteria (LABs), *Bifidobacterium* sp., and the yeast *Saccharomyces* (*S. cerevisiae* var. boulardii). These strains have various beneficial properties that can be important in xenobiotic detoxification, such as strong ability to bind, tolerate or detoxify, high tolerance to acid and bile, strong adhesion to the gut mucosa, and strong antioxidant or immunoregulatory capacities enabling them to adapt to xenobiotic-induced changes in the gut environment.

In this investigation of screening of potential probiotic bacteria from using different environmental parameters like ammonia, pH, DO, OC, TDS and temperature. They were screened potential bacteria as *Lactococcuslactis* was based upon the ammonia and organic carbon presence. And *Lactococcuslactis* was taken for the gypsum and lime soil conditioners xenobiotics degradation for 9 days at 3 days after interval with different concentration of culture such as 125ppm, 250ppm, 375ppm and 500ppm. They are showed maximum degradation in 3rd day was 500rpm in both gypsum and lime xenobiotics. In 6th day has revealded 500ppm in gypsum is high and 125 and 250ppm was high in lime. Simalarly, observed in both gypsum and lime soil conditioners xenobiotics of 500ppm was showed maximum of the degradation.

V. Conclusion

Microbial communities have great potential to mediate the successful biodegradation process of xenobiotic-contaminated soil/water environments. However, the greater part of mainstream microorganisms involved in bioremediation are still undefined because not all organisms in nature could be cultured under *in vitro* environments, but reside in viable-but-non-culturable (VBNC) environments. Xenobiotics are released into the environment by human activities, and they often cause problems such as environmental pollution, since most such compounds cannot be readily degraded, and have harmful effects on human beings and the natural ecosystem. However, some microorganisms that degrade man-made xenobiotics have been isolated. Most of these aerobic xenobiotics-degrading bacterial strains use xenobiotics as their sole source of carbon and energy, and thus they are excellent models for studying the adaptation and evolution of bacteria in the environment.

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