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Development Of Cactus Oil Dispersant Suitable For Oil-Degrading Marine Bacteria And Various Seawater Temperatures

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***Abstract*— Following an oil spill, dispersants are sprayed in large volumes onto the ocean surface and work by emulsifying oil particles. However, most chemical dispersants contain toxic properties and inhibit natural oil-degradation by bacteria. This study investigates the effects of a natural dispersant from *Opuntia ficus-indica* cactus mucilage on oil-degrading *Marinobacter* growth and oil emulsification by testing marine broth absorbance and qualitatively rating dispersion respectively. The *Opuntia ficus-indica* was selected due to its unique surface-active properties, allowing it to naturally enhance dispersion. The results indicate that the mucilage enhances oil-degrading *Marinobacter* sp, *M. aestuarii, M. antarcticus,* and *M. maritimus* populations. Microscopic inspection suggests that mucilage is an effective dispersant both alone and in combination with the *Marinobacter*. The mucilage was also equally effective at various temperatures and without certain standard pre-treatment from previous studies. Lastly, a cell viability test indicated that both acidic and neutralized pH cactus mucilage did not damage cell growth, and is thus safe to use in oceanic waters. In short, cactus mucilage is a natural nontoxic dispersant that promotes oil-degrading *Marinobacter* and works well at various oceanic conditions.**

I. Introduction

When oil spreads across the ocean surface following an oil spill, less sunlight is able to penetrate the water, reducing phytoplankton population and harming marine wildlife [1]. To prevent oil from clumping on the surface, dispersants are applied to emulsify oil through amphipathic properties [2]. However, there has been much controversy surrounding the use of dispersants due to the toxicity of early generation of chemical dispersants. For instance, chemical dispersants may suppress natural oil degradation by marine bacteria [3].

This study tests the oil-dispersing abilities of the cactus *Opuntia ficus-indica*, a natural nontoxic alternative to chemical dispersants, at various ocean temperatures and conditions. In this study, a slimy fluid visible in the cross section of the pad was extracted and studied [4]. In the past, powder cactus mucilage was found to be an effective and eco-friendly dispersant at various salinities [5]. Although insoluble in water, the mucilage swells in water, allowing it to potentially precipitate ions, bacteria, and particles from aqueous solutions. The mucilage also holds unique surface-active properties, allowing it to enhance dispersion and reduce high polarity liquid surface tension [5]. Since there are currently no studies on the effect of cactus mucilage on oil-degrading *Marinobacter* growth, this study investigates whether cactus mucilage can coexist with *Marinobacter* oil-degradation and the conditions under which this would be a possibility.

II. Methods

Cactus mucilage was extracted from the pads of *Opuntia ficus-indica* by blending, heating, and neutralization [4].

The effect of cactus mucilage on oil-degrading *Marinobacter* sp., *Marinobacter aestuarii, Marinobacter antarcticus,* and *Marinobacter maritimus* population growth was first tested by incubating each species in marine broth with 10% diluted mucilage, measuring absorbance at 600nm after 48 hours, then calculating growth rate.

The efficacy of oil dispersion by the mucilage on diesel oil was then observed via visual microscopic inspection of a drop of oil-water-dispersant solution once, mixed with methylene blue and second, under fluorescent light. Images were rated 1-5 based on number and size of oil particles and oil-water drop diameter ratio was calculated. Emulsification was not only analyzed for the mucilage alone, but also in combination with the various *Marinobacter* species.

The same two tests were repeated for mucilage after various pre-treatment including autoclave heating, alcohol-drying, etc. to find optimal conditions. The effect of seawater temperatures 4°C and 25°C were also tested.

Lastly, a cell cytotoxicity test on SK-N-MC cells was conducted to ensure that the cactus mucilage is non-toxic to marine wildlife. SK-N-MC, neuroblastoma cancer cells from the brain, were chosen to simply test if the mucilage had any adverse effects on general cell growth. The cells were seeded on a 12-well plate with DMEM, fetal bovine serum and penicillin streptomycin. After 48 hours, the cells were treated with diluted mucilage. Cell viability was calculated using a CCK-8 kit and measuring absorbance at 450nm.

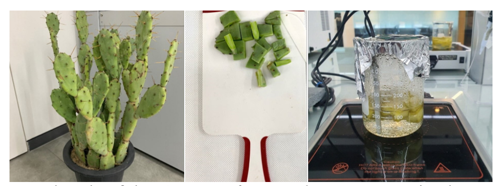

Figure 1. Nopal pads of the *Opuntia ficus-indica* were cut, its thorns removed, heated, and neutralized with 0.1M NaOH.

Table 1. *Marinobacter* sp. characteristics

	Marinobacter sp.	*Marinobacter aestuarii*	*Marinobacter antarcticus*	*Maribacter maritimus*
Catalogue number	KCTC 22332	KCTC 52913	KCTC 23684	KCTC 62399
Culture media	Marine agar	Marine agar	Marine agar	Marine agar

III. Results

A comparison of average growth rate for each *Marinobacter* species showed that *M.* sp., *M. aestuarii,* and *M. antarcticus* respectively all increased after the addition of cactus mucilage in marine broth. *M. maritimus* did not show significant growth, however. Since *M. aestuarii* showed the highest growth rate among the *Marinobacter* species, this bacteria was used to test oil emulsification in the next step.

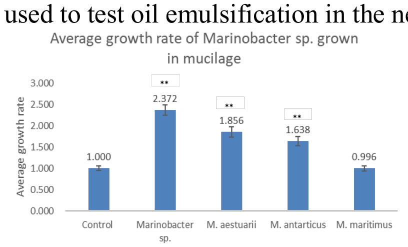

Figure 2. Average growth rate of *Marinobacter* sp. grown in mucilage. The data are shown as mean ± S.E.M. (*n*=3). $^{**}P<0.01$, vs control.

To measure oil emulsification efficacy, oil layer ratio was calculated as seen below. A lower ratio means more effective oil emulsification. For all diesel concentrations, cactus mucilage alone reduced oil layer ratio. Combined mucilage and *M. aestuarii* further reduced the ratio at 10% and 20% diesel. Overall, the greatest emulsification was at 20% diesel for the mucilage and *M. aestuarii* combined.

Comparison of the various pre-treated mucilage from the cacti indicated no significant differences in *Marinobacter* growth and oil emulsification at various conditions. Although neutralized mucilage at pH 7.8 showed higher marine broth absorbance than at pH4.5, both mucilage pH groups still showed higher absorbance than the control (no mucilage). The only outlier was *M. aestuarii.* This meant that regardless of whether the cactus mucilage was left acidic or neutralized, the mere presence of mucilage increased *Marinobacter* growth compared to the control. Thus, mucilage acidity and other pre-treatment factors had little effect on the *Marinobacter* and oil emulsification.

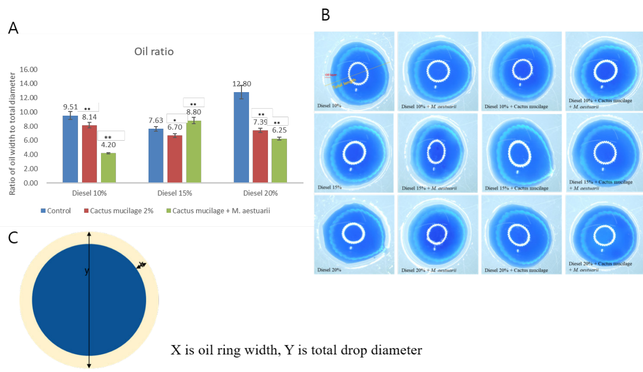

Figure 3. Oil dispersion efficacy of mucilage with *M.* aestuarii, A. Ratio of width to total diameter for methylene blue drop test, B. Microscopic image of methylene blue oil-water drops, C. Calculation method of ratio. The data are shown as mean ± S.E.M. (n=3). **P<0.01, *P<0.05, vs control.

Additionally, differences in seawater temperature did not show any adverse effects on *Marinobacter* and oil dispersion. Although all four species exhibited slightly higher absorbance at 25°C than 4°C, given the nature of these bacteria, such an effect is reasonable. Moreover, the correlation between emulsification score and temperature was insignificant as seen by overlapping error bars. The only outliers were Mucilage + *M. aestuarii* and with *M. maritimus*. This indicates that the cactus mucilage can maintain *Marinobacter* populations while enhancing emulsification at both arctic and tropical oceanic temperatures.

Lastly, in the cell cytotoxicity test, both pH4.5 and pH7.8 mucilage showed similar absorbance to those of both controls as seen in the overlapping error bars. Thus, the results indicate that this acidity does not pose a significant threat to cell viability, meaning that the mucilage will likely be non-toxic to marine wildlife. As such, cacti neutralization may not be necessary as seen in previous studies [5].

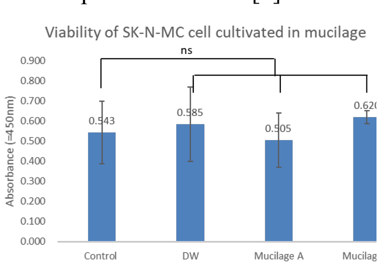

Figure 4. Viability of cells as calculated by absorbance. Control= no mucilage; DW= distilled water; Mucilage A= pH4.5; Mucilage B= pH7.8. The data are shown as mean ± S.E.M. (n=3). ns: Not significant vs control.

IV. Discussion & Conclusion

Overall, cactus mucilage did not harm but instead promoted the growth of *Marinobacter*, and oil emulsification was effective both alone and in combination with *Marinobacter* species at low and high oceanic temperatures. Furthermore, pH, heating temperature, and alcohol drying did not have a significant effect on *Marinobacter* growth and oil dispersion, meaning that certain standard procedures may be eliminated from previous studies [5]. Lastly, mucilage at both acidic and neutral pH did not harm cell viability, ensuring that the cactus will be safe to use in oceanic waters.

Still, further research needs to be done on the interaction between oil-degrading bacteria and natural dispersants by observing the effect of dispersants on not only the bacteria's population, but it's oil-degradation rate. Other methods of preparation and application other than using mucilage in powdered and liquid form may also be studied such as long-term freezing, mixing with other dispersants, etc. For instance, an alternative oat-derived dispersant can be prepared through a variety of drying techniques such as fluid bed drying, subjecting the powder to a vacuum with or without the addition of heat, or simply air drying [6]. In short, this study provides an eco-friendly alternative to chemical dispersants that works well with oil-degrading marine bacteria at various oceanic conditions.

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