Fighting wildfires with bubble nests, a clean alternative to fluorosurfactants.

Monterrey is the city that witnessed the birth and growth of our iGEM team; therefore, we, as a team, have a responsibility towards its community. Monterrey is located in northeastern Mexico, in a region named "Sierra Madre Oriental" (S.M.O.). The S.M.O. is a mountain chain with an approximate length of 1,350 kilometers, stretching along northern Mexico. It extends from the south of the Rio Grande River, crossing eight states in the north of the country to join the Neovolcanic Axis. Its area represents 11% of Mexico's continental territory (Valdez et al., 2004).

In the state of Nuevo Leon, part of the S.M.O. is protected in the National Park "Cumbres Monterrey", holding more than 189 species of flora and fauna, 73 of which are endangered, threatened, endemic or under special protection (Estrada-Castillón, 2007). It is also part of the migratory route of the monarch butterfly (Danaus plexippus), being the first mountain range with forest habitat on their southern migration. This national park is of great socio-economic importance to the region since it provides most of the drinking water to our metropolitan area and is an important hotspot for tourism in Northern Mexico.
Its topography creates a wide range of microclimates to which many ecosystems have adapted, resulting in a wide range of species. Because of this, it is one of the most biodiverse orographic regions in Mexico. The predominant vegetation types in the S.M.O. are oak forest, pine forest, sub-montane and desert shrubbery, among others (Estrada et al., 2007), and it houses more than 7% of Mexico's endemic plants, making it an area of great ecological significance (Salinas, 2018).

Its dry and arid climate makes this region optimal for fire spreading. However, its ecosystems have adapted to resist and regenerate after these events, to the point that a regular fire regime promotes adequate functioning of ecosystems in the zone (Pérez & Rodríguez, 2011). Nonetheless, these ecosystems have not adapted to the abnormal fire regimes caused by human activities and climate change. Natural weather patterns have been modified due to global warming, changing the levels of environmental humidity, affecting the incidence of natural fires, and causing higher intensity, but with lower frequency (Yocom et al., 2010). If we also consider the fires caused by human activities in areas in the process of recovery, the future of these ecosystems seems dire.

This scenario already exists in other parts of Mexico, where wildfires are becoming more prevalent. States like Jalisco, Oaxaca, and Durango lost 205,562 acres of land due to wildfires last year (CONAFOR, 2020). Similar events are happening in other parts of the world, like the recent wildfires in Australia and California.

With this in mind, foam is one of the essential tools used by emergency response personnel to mitigate fire. Fluorosurfactants (PFAS) are standard components in aqueous film-forming foams for fire suppression, and they have been used since the 1960s (Adams & Simmons, 1999). The fluorine content helps create a low surface tension film that spreads rapidly across the surface of burning material. However, in recent years, many people have switched to fluorine-free foams in response to environmental concerns. Since the fluorinated chemicals are not biodegradable, they accumulate in the food chains and show toxicity over time, mainly towards aquatic ecosystems and amphibian species (Ross, Miles &

Storch, 2019; Kalabokidis, 2000). PFAS can enter the food chain through environmental contamination. One example of the trophic transfer of PFAS in an ecosystem as shown in a study of the Baret's sea food web, where the examined species included sea ice amphipod (Gammarus wilkitzkii), polar cod (Boreogadus saida), black guillemot (Cepphus grylle) and glaucous gull (Larus hyperboreus). They observed that PFAS acts similarly to lipid soluble contaminants, despite accumulation through different pathways (Haukås, Berger, Hop, Gulliksen & Gabrielsen, 2007).

We face a significant problem with social, economic, and environmental implications. In addition to the increased severity of fires, the currently available foam's demand is high, and due to its high cost, Mexican firefighter departments cannot afford it. Besides, as previously mentioned, the additives for improving the effectiveness of existing foams are environmentally harmful. On top of that, the Monterrey metropolitan area continuously expands towards the mountains, increasing the risk of people being exposed to wildfires.
Our project tackles this problem directly. We plan to produce our own Class-A fluorine-free firefighting foam because we want to solve this fire hazard problem by making an effective, environmentally friendly alternative. This proposal has the potential to be upscaled to mass production in bioreactors to reduce its manufacturing costs. Synthetic biology offers an excellent opportunity to achieve this goal.

First, we had to choose a surfactant agent, a necessary component for foam-making liquids, that does not harm the environment. Considering this, our foam is composed of surfactant proteins as an alternative to fluorosurfactants. We got the idea from the long-lasting bubble nests naturally built by the frogs from the genus Leptodactylidae, which contains over 180 species, 3 of which are vulnerable, and 6 are endangered (IUCN, 2020). One of these species, Leptodactylus fragilis, inhabits the S.M.O. and is currently classified as "least concern" (Smithsonian Tropical Research Institute, 2020). It builds nests that can last more than a week exposed to the environment. This has been attributed to a mixture of surfactant proteins that maintain the nest's integrity, which are named Ranaspumins 1-6 (Cooper, Vance, Smith & Kennedy, 2017) (Mackenzie et al., 2009). For that reason, we chose ranaspumin-2, alongside with surfactin, to produce our firefighting foam.

With this proposal, we are offering a tool that could be useful to fight fires that endanger ecosystems, while avoiding the use of chemicals that can threaten wildlife. We are increasing the chances to protect endemic plant species like Abies vejari (Farjon, 2013), Picea martinezii (Thomas & Farjon, 2013), Agave nickelsiae (González-Elizondo, 2019), or endangered animals such as Rhadinaea montana (Mendoza-Quijano, 2007), Rhynchopsitta terrisi (BirdLife International. 2017), and Tampichthys mandibularis (Mejia, 2019), among many others (IUCN, 2020).

By using proteins produced by a frog species from our locality in our project, we show the importance of wildlife conservation in its ecosystems, since it provides us useful resources that could go undiscovered. We believe that the same species that are threatened by the loss of their habitat can be the ones that can give us the tools to protect it. We also hope that projects like ours make people reflect on the economic, social, and ecological importance of local ecosystems, and with your help, we will reach our goal.

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