Promoter regulation in *Pseudomonas putida* - Working towards bio-sustainability

***Pseudomonas putida* is a bacterium that is naturally found in soils and that is capable of growing on various compounds composing lignin, one of the major polymers found in wood. However, for this bacterium to convert these compounds into useful chemicals, it needs to be genetically engineered so that relevant proteins are added or removed. For this purpose, it is important to be able to tune protein production using appropriate signals that are found on the DNA sequence of the corresponding genes and called promoters. In the present study, a set of three promoters are characterized and their response to the presence of different lignin compounds is investigated.**

Over 50 million tons of lignin is being produced yearly as a side product of the pulp and paper industry. This lignin stream is made up of many molecules with different sizes and impurities, which makes it difficult to use in conventional chemical processes. Fortunately, the bacterium *Pseudomonas putida* naturally uses many of those compounds for growth and further genetic manipulation has enabled the bacterium to convert two aromatics commonly found in the lignin stream – guaiacol & vanillin - into muconic acid, a precursor chemical to nylon and PET production.

However, making a bacterium able to utilize a substrate and produce a compound of interest is only a part of the struggle. To make it into a commercially viable process, the bacterium must be an efficient workhorse, meaning that it must be able to produce a lot of product in as little time as possible and at sufficient concentration. Enzymes are the machines responsible for converting substrates into desired products. In many cases, increasing the number of enzymes leads to faster conversion of substrates and a higher productivity. A promoter is a genetic element which promotes gene expression by binding of the RNA polymerase. By using a stronger promoter, higher protein expression can be achieved.

I evaluated the strength of three strong promoters, named p14c, lacIq-Ptrc and p14g, on different substrates by placing a gene coding for a green fluorescent protein (GFP) behind these promoters and measuring the corresponding fluorescence. It was found that p14g promoter led to the highest GFP expression. This does not make the weaker promoters useless because producing too much protein can burden the cell and lead to lower growth. The lacIq-Ptrc promoter was also special because it needed to be activated by adding a lactose mimicking molecule, giving the opportunity to control when we want the protein to be produced.

Vanillin, a nice smelling chemical, was found to be easily consumed by *P. putida* for growth without affecting GFP production too much. Guaiacol, although not pleasantly smelling, caused more than 20% higher GFP production. However, it also decreased cellular growth, presumably due to the molecule toxicity. Whether the increase was promoter or growth related is unknown and should be further examined.

Altogether, it can be concluded that p14g is the strongest promoter tested in this study and that it worked equally well when guaiacol and vanillin were present. Hopefully, this work contributes to further developments in this amazing bacterium and a bio-sustainable future!