THE BOOLEAN
SNAPSHOTS OF DOCTORAL RESEARCH AT UCC

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Brewing the Future

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Abstract

Nowadays the environmental concerns and the limited availability of fossil resources have resulted in the development of bioeconomy and biorefineries. It is crucial to adapt production processes within this context. In Ireland cheese whey and derivatives are some of the main wastes generated by the industry. This article aims at the possibility of using this dairy waste for production of interesting biochemicals and compounds using a microbe known as *Kluyveromyces marxianus*, which is a yeast adapted to live in milk.

Keywords: cheese whey, yeast, biorefinery.

Life, uh, finds a way.

— *Ian Malcom*

The Environmental Problem

Nowadays we are all aware of the environmental issues the planet and society are facing: global warming, resource scarcity or plastic pollution are some of the main ones. All these important problems share similar origin and are connected with an oil-based economy. Concerns have been raised at the ways we generate energy, make food, or manufacture diverse products. These production chains need to become sustainable and circular in order to balance the impact humanity has over the planet.

One interesting solution that was soon considered and is rocketing nowadays is the concept of biorefinery. Traditional crude refining and biorefining both are based on the idea of creating a wide variety of products (fuels, plastics, chemicals or aromas) from a raw material. However, while in a normal refinery crude oil is the only raw material distilled and separated in its different fractions for later manufacturing, biorefineries use different substrates to create this vast array of compounds. Simplifying this idea, a refinery scheme or flowchart would look like a pyramid while a biorefinery scheme resembles an hourglass (Fig. 1).

In a biorefinery plant the starting material is usually some kind of waste or a by-product of biological origin, like wood chips, cheese whey, fish scales or spent coffee grounds. These

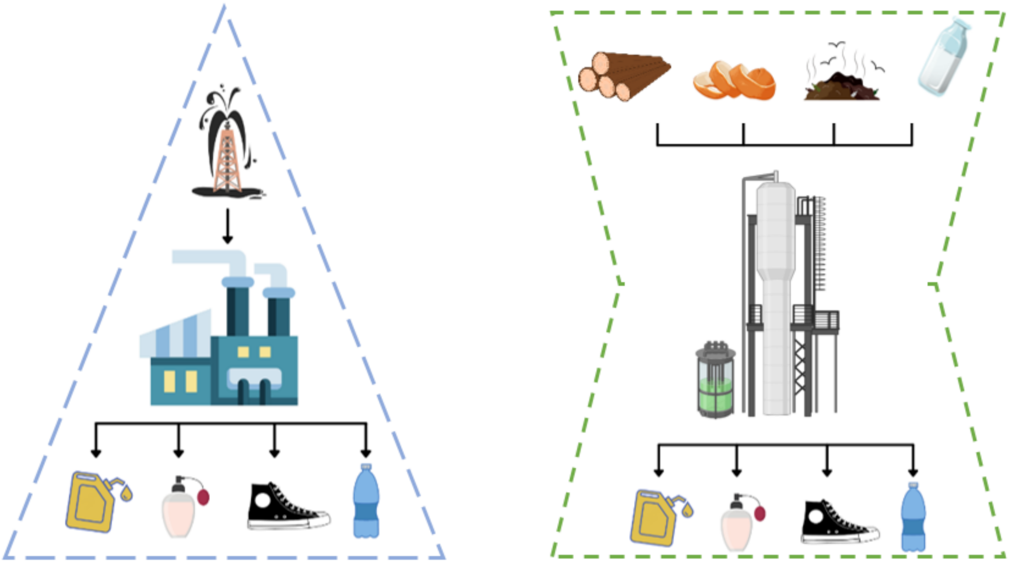

Figure 1: Simple schemes of a traditional refinery process (left) and a biorefinery flowchart (right).

substrates need to be broken down into the main molecules that form life, namely sugars, amino acids and lipids. Then, these main building-blocks of life are fed to a wide spectrum of organisms, generally microscopic, that will use them as food and will produce all the valuable goods (Fig. 1). Finally, but most important, for a bioprocess to become a success it is crucial that it generates benefit, as if there is no reward for companies to invest in sustainability the waste will just be dumped.

It is worth to mention that, while a refinery is an actual plant with big tanks and distilling columns, and we can have a fixed image of how it looks like, biorefinery is more a concept or a collection of different bioprocesses and it will be shaped depending on the raw material, the microorganisms used and the product generated. And because sustainability and circularity are the main goals, specific bioprocesses need to be designed for the wastes generated in each region.

Sustainable Dairy Industry

In Ireland one of the main raw materials one can count with is cheese whey, which is a by-product from the cheese production process. In this process, the solids contained in the milk are intentionally clotted and separated from the liquid in which they are dissolved in a step called curdling. Once the curds have formed, they are removed to make the cheese. The resulting liquid, rich in lactose (the main sugar present in milk), nitrogen and minerals, is known as cheese whey. To produce 1kg of cheese, approximately 10L of milk are needed and 9L of cheese whey are produced. Traditionally cheese whey was dumped in rivers or other water streams but, nowadays, this is illegal. Cheese whey is a big problem for the cheese industry for two reasons:

- First, is the main product generated by the dairy industry, adding up to 90% of what a cheese plant manufactures. Despite of that, it is considered a waste and it has to be disposed of.
- Second, is a nutrient-rich waste, meaning that is a potential pollutant in water bodies because it promotes spoilage microorganisms to grow and eutrophication, potentially causing big problems in rivers or lakes.

A first approach to get some profit out of cheese whey is to concentrate the few nutrients still left in this liquid. These approach yields a secondary product that is usually known as whey powder and is a food ingredient very valuable for the dairy industry. Whey powder can be produced by different filtration and drying steps. However, it does not solve the issue of cheese whey since there is still another by-product generated known as whey permeate, which is basically a poorer and less nutritious cheese whey.

Hence, a different solution needs to be applied to solve this problem. By applying circular economy, a wide variety of sustainable products can be obtained from cheese whey: bioplastics, aromas or probiotics among others. However, because economic feasibility is the most important aspect of a bioprocess, opting for producing high-added value molecules is always preferred.

An interesting product with potentially a very high-added value are proteins. Proteins have multiple uses in industry (therapeutics, supplements, meat substitutes, chemical catalysers...) and they are produced by different means. When the function of a protein is not very important, bacteria is usually the preferred microorganism since they are able to make very high quantities of proteins with low and cheap means. When the proteins are for therapeutical use, like antibodies or cytokines, cultivating animal cells is preferred because these cells provide better protein quality and activity. Yeast cells, however, are somewhere in between: They produce proteins with higher quality than bacteria but, likewise, they use basic resources.

1 The Milkman's Yeast

Traditionally, the preferred yeast in both industry and research is the well-known baker's yeast, *Saccharomyces cerevisiae*. This microorganism is the common yeast we use to bake loaves or brew beer among other uses. Moreover, *S. cerevisiae* is also a well-studied microorganism widely used in laboratories and, for this same reason, in the biotechnological industry. However, *S. cerevisiae* presents several drawbacks when used in industry. It is not able to use all the resources present in raw materials, for example lactose, which reduces the overall yield of the bioprocesses designed. Moreover, it is a yeast very well-suited for fermentation but, for protein production, oxygenated processes are preferred due to higher efficiency. Besides, it finds difficulties to grow when temperatures are higher than 37°C.

Among yeasts, one that is particularly interesting for use under these conditions is *Kluyveromyces marxianus* (Lane & Morrissey, 2010). *K. marxianus* is a yeast capable of thriving with lactose, is thermotolerant and one of the fastest growing eukaryotic microorganisms. For these reasons it is an ideal candidate for a biorefinery process within the dairy industry. *K. marxianus* is closely related to the baker's yeast *S. cerevisiae*, which means that part of the rich knowledge available for *S. cerevisiae* is adaptable and can be applied almost directly. However, there are still gaps to be filled in order to equate *K. marxianus* to *S. cerevisiae*; my research focuses on filling some of these gaps.

Designing a Yeast

We are often explained in a simple way a species' genome is like a book with the instructions for life where all the characteristics of that life being are written in the form of genes. There are different books, each of them explaining the instructions of a particular species. Each gene would be a word that, if read, a protein is produced. But a book is not only made of words, these words also need to have a sense. When we read a sentence, it is important to recognise when words start and end, if it is a question or when a sentence ends and a new one starts. In the instructions of life, these features are also very important. There are genetic elements that separate genes between each other and increase or decrease the strength with which genes are expressed (Fig. 2). These features are known as genetic regulators and they are usually not mentioned or highlighted but play a role as important as the gene *per se*:

- Promoter: Is a genetic sequence, found before the gene, that indicates the start of a gene and promotes its expression. Promotes can be strong or weak and this strength represents the level at which the gene is read.
- Secretion tag: Is a label for the yeast's protein machinery that indicates the tagged protein is to be secreted out of the cell. It is a useful feature in protein production because it facilitates the protein separation from the yeast's culture.
- Terminator: Is a genetic sequence right after the gene that indicates the end of the gene.

When we want to use a microorganism, like *K. marxianus*, to produce a protein, we need to modify genetically the microorganism by including the gene of the protein within the microorganism's genome. Following the analogy of the book, what we want to do is to include a particular word from a different book in the *K. marxianus* instructions. But, as explained before, we cannot just copy the gene, we need to surround it with the right regulatory parts, so the yeast is able to read it good and loud.

A wide variety of genetic regulators in yeasts have been isolated and characterised from *S. cerevisiae* but only few have been discovered in *K. marxianus*, and they can have an effect completely different even if the yeasts are similar. One of the aims of my PhD is finding out more of these parts so the protein production can be better controlled using this dairy yeast.

To do this, I cultured *K. marxianus* in cheese whey at high temperature, 42°C, in laboratory bioreactors (high volume vessels for microbe culture) to take samples at different points (Fig.

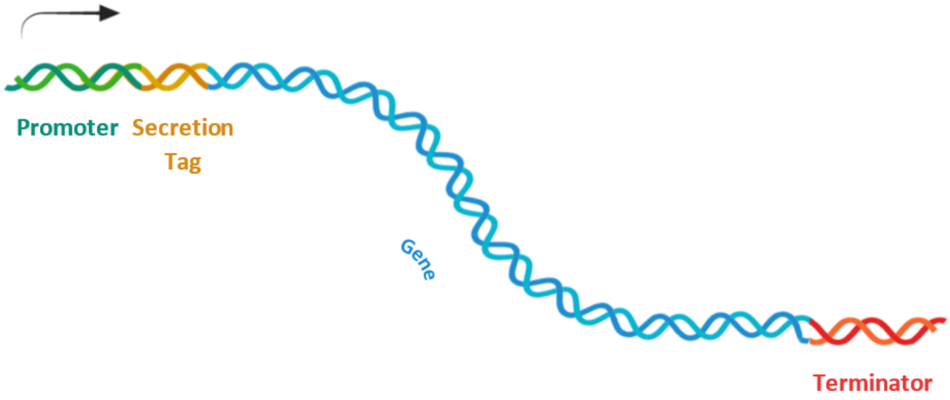

Figure 2: Representation of a gene with its promoter (green), secretion tag (yellow) and terminator (red).

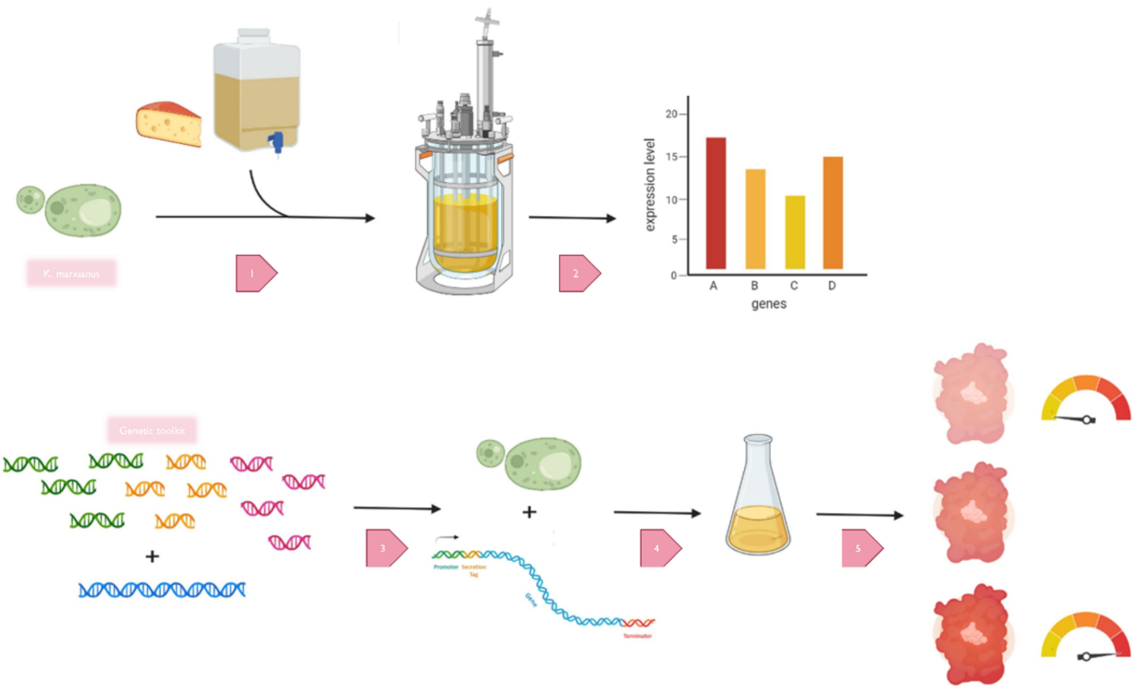

Figure 3: Flowchart of my project. 1. Culture of *K. marxianus* on whey at 42° C in bioreactor 2. Identification of overexpressed genes and creation of a genetic toolkit 3. Combinatory assembly of different parts in *K. marxianus* 4. Growth of the modified strains on cheese whey at 42° C in flask 5. Measurement of the fluorescence level of each constructed strain (red fluorescent protein representation).

3). With this samples, I will be able to identify which are the genes that are highly expressed by sequencing them and isolating the different genetic components that govern their high expression: promoters, secretion tags and terminators. These regulatory parts will be specific for the conditions I am growing the yeast, higher temperature and lactose utilisation. Once identified, I will isolate and combine the different promoters, secretion tags and terminators to see their effect in the expression of a reporter gene expressing a fluorescent protein. This way, the different genetic regulators can be characterised depending on how strong the fluorescence is.

Conclusion

With this work I hope I will add a valuable bit of knowledge to the yet unknown world of non-conventional yeasts. Enhancing the production of proteins is crucial to provide nutritive food, produce diverse medicines and enhance sustainability and circularity all over the world, and yeasts can help to get there!

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