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Group Contribution as Selection Criteria for Nutrients Fermentation Media in the Production of Tacrolimus

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Group contribution methods are suitable tools for estimating many physicochemical properties of pure compounds and mixtures. The classical group contribution method decomposes each chemical compound into first, second, and third-order functional groups based on its molecular structure. The most significant advantage of these methods is that they need only the compounds chemical structure without any other input information. From these approaches, structural fragments and subsequent group contribution methods can be established. The knowledge of the molecular structure and its decomposing into molecular fragments can optimize the production of biosynthesis compounds for chemical and pharmaceutical industries. The current research proposes an innovative group contribution application as selection criteria to culture media carbon and nitrogen sources. The concept is based on group contribution to identifying molecular fragments present in tacrolimus structure. Tacrolimus is a macrolide lactone originally obtained from fermentation of *Streptomyces tsukubaensis* broth. This drug decreases the occurrence and severity of refractory rejection episodes. In addition, tacrolimus is recommended for the treatment of autoimmune diseases. In order to investigate the application of the group contribution, tacrolimus molecular structure and fermentation results of this macrolide were analyzed. This approach can enhance productivity of this important immunosuppressant.

1. Introduction

The coronavirus pandemic changed our lives, particularly that population on waiting list for a transplanted organ, whose perspective is full of hope. When a kidney disease is detected, the patient hopes for a quick cure, but suffers when knows his life will be governed by dialysis. During dialysis, the patient hopes to find a compatible organ. When finds a compatible organ and performs the surgery, he has the hope that his organism will accept the new organ, and starts administering immunosuppressant that, when adapting the organism to the graft, reduces his immunity and exposes him to the most diverse types of infection, such as Covid-19. In addition, the patient has the hope of having the immunosuppressant at his disposal, so that he begins to sow the hope of living (Bertan and Cremasco, 2020). This reflection clearly points to important social issue to be resolved, which necessarily involves investments in research and development and innovation in the production of tacrolimus. This compound is recommended as immunosuppressive drug for therapy of kidney and liver transplantation treatment. In addition, tacrolimus, as pointed in Figure 1, is recommended for the treatment of autoimmune diseases, rheumatoid arthritis, and lichen planus (Kovarik et al., 2003; Sanchez et al., 2004), as well as in bronchial asthma treatments, dermatological disorders as vitiligo, psoriasis, atopic dermatitis (Chaudhari et al., 2012; Dähnhardt et al., 2019), and eye diseases like uveitis (Erdinest et al., 2019).

Tacrolimus, known as FK-506 and fujimycin, is a macrolide lactone with molar mass 804.018 g/mol and empirical formula C44H69NO12, can be obtained via fermentation by several species of Streptomyces genus, usually Streptomyces tsukubaensis. The improving FK-506 production can be done by mutant of S. tsukubaensis (Du et al., 2014; Sing et al., 2017) or from different nutrient media for ordinary S. tsukubaensis bacteria (Kino et al., 1987; Chen et al., 2012; Wang et al., 2017; Silva et al., 2019; Moreira et al., 2020). In the second case, these authors use distinct carbon and nitrogen sources, without specify clearly the criteria for their choose. However, from analysis of metabolic pathways of tacrolimus production it is possible to observe some precursor, such as methylmalonyl-CoA, malonyl-CoA, methoxymalonyl-CoA, pipecolate among others

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(Huang et al., 2013). Some authors apply precursor in the media nutrients to improve the tacrolimus production (Mishra and Verma, 2012; Turlo et al., 2012). It is important to point that the structures of these precursors are present in tacrolimus structure or, in other words, the tacrolimus structure presents fragments of this precursors or classical structures, such as amino acids (L-lysine, L-proline for example).
The tacrolimus structure presents other fragments as fatty acids, that are present in classical carbon sources for its productions. As can be seen, it is possible to identify classical structures in the FK-506 structures. In this case, the group contribution methods from thermodynamics, suitable tools for estimation of many physicochemical properties of pure compounds and mixtures. The crucial advantage of these methods is they need knowledge only of the chemical structure of the compounds without any other input information (Kolská et al., 2005). Then, these methods could be extended for identification of fragments of complex molecule, whose objective is to improve its production.

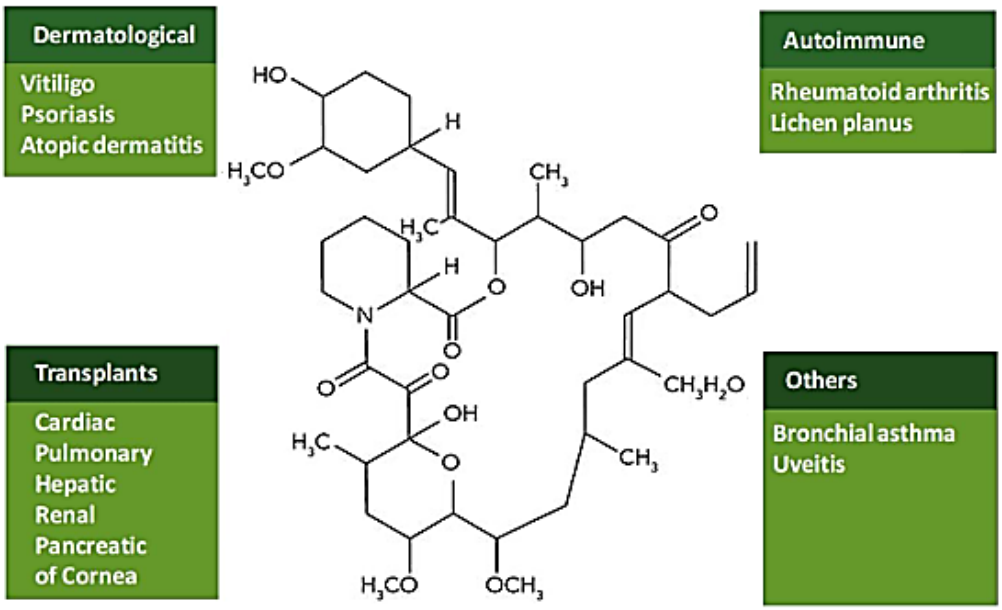


Figure 1: Molecular structure of tacrolimus and applications (Adapted from Bertan and Cremasco, 2020).

2. Methodology

The group contribution approach considers that the properties of molecules are established from the contributions of their functional groups. The classical group contribution method decomposes each chemical compound into first, second, and third-order functional groups based on its molecular structure. The application of these methods is usually broad and simple because only the structural formula of a compound is needed (Poling el al., 2001). In the domain of property prediction models, group contribution method is applied to predict thermodynamic properties of pure compounds, however the approach of this method is extended to lethal concentration, LC50 (Kashinath et al., 2020; Hukkerikar et al., 2012), thermophysical properties of complex components such as ionic liquids (Soares et al., 2019), and other applications as cost analyzes of recovery in solvent extraction (Silva et al., 2018).
The determination of optimum initial sources of carbon and nitrogen in the medium is an essential step in optimizing the fermentation process and, consequently, the desired product, as tacrolimus. In general, the metabolic route suggests precursors that are used as strategies to assess productivity (Mishra and Verma, 2012; Turlo et al., 2012, Huang et al., 2013; Ferrari, 2017; Silva, 2018). In this context, the optimization of fermentation media using response surface methodology is common (Mishra and Verma, 2012; Antolinez; 2016). Then, the present work proposes an innovative group contribution application as selection criteria to culture media carbon and nitrogen sources. The concept is based on the group contribution to identify molecular fragments to increase the tacrolimus productivity. It is an analogy to the classical group contribution, in which the fragmentation scheme is relevant to the calculation property. The tacrolimus molecule was fragmented, and the groups are the precursors in the fermentation media. In order to investigate this application, studies were evaluated to obtain tacrolimus from fermentation of *Streptomyces tsukubaensis* (Table 1) and *Streptomyces sp.* (Table 2). The selected studies considered similar operational conditions

about temperature, pH, time, and rotation, the values of these variables were, respectively, 27-30 °C, 6.7-7.0, 5-7 days, and 110-240 rpm. From these previous studies it is possible to relate the structure-dependence in media culture sources to tacrolimus productivity.

Table 1: Strain of Streptomyces tsukubaensis.

Authors	Strain	Carbon source (including amino acids and proteins)	Nitrogen source	Production TAC (mg/L)
Kino et al. (1987)	*Streptomyces tsukubaensis*	Glycerol, corn starch, glucose, corn steep liquor, soluble starch, yeast extract	Seed meal, corn steep liquor	13.6
Okuhara et al. (1990)	*Streptomyces tsukubaensis*	Sucrose, glucose, cottonseed meal, corn steep liquor, glycerine, soluble starch	Dryed yeast	14-23
Turlo et al. (2012)	*Streptomyces tsukubaensis*	Glucose, maltose, malt extract, yeast extract, corn steep liquor, soy peptone, picolinic acid	Corn steep liquor, soy peptone	32.5
Ferrari (2018)	*Streptomyces tsukubaensis*	Malt extract corn steep liquor, soy peptone, picolinic acid, Brazil nut oil	Corn steep liquor, soy peptone	47.4
Silva (2019)	*Streptomyces tsukubaensis*	Glucose, maltose, malt extract, yeast extract, corn steep liquor, soy peptone, Brazil nut oil	-	41.7

Table 2: Genetic variant of Streptomyces tsukubaensis.

Authors	Strain	Carbon source (including amino acids and proteins)	Nitrogen source	Production TAC (mg/L)
Mishra and Verma (2012)	*Streptomyces sp.*	Soy oil, soybean meal, L-lysine	$(NH_4)_2SO_4$	135.6
Dumont et al. (1992)	*Streptomyces sp. (MA 6858) ATCC n. 55098*	Glucose, dextrose, asparagine, soluble starch	Dried yeast, corn steep liquor, asparagine	10 - 37.8
Vaid (2007)	*Streptomyces sp. (Strains PSCS) FERM B027; MA 6858, ATCC n. 55098; Mutant P5C*	East extract, malt extract, glucose, glycerin, cotton seed oil, ground oil, soy oil, sunflower oil	Cotton seed meal, corn steep liquor, dried yeast, wheat germ, feather meal, peanut powder, $(NH_4)_2SO_4$	150 - 250
Chen et al. (2012)	*Streptomyces tsukubaensis* ZJU01	Glycerol, soybean meal, soluble starch, glucose, soybean oil, L-lysine	$(NH_4)_2SO_4$	46.9
Huang et al. (2013)	*Streptomyces tsukubaensis* D852	Yeast extract, soybean meal, soy peptone	Soy peptone	177.8

3. Results

The structure of tacrolimus was analyzed and decomposed into molecular groups, that were identified and associated with sources of carbon and nitrogen (Figure 2). Figure 3 presents some analogs compounds from which the fragments were identified.

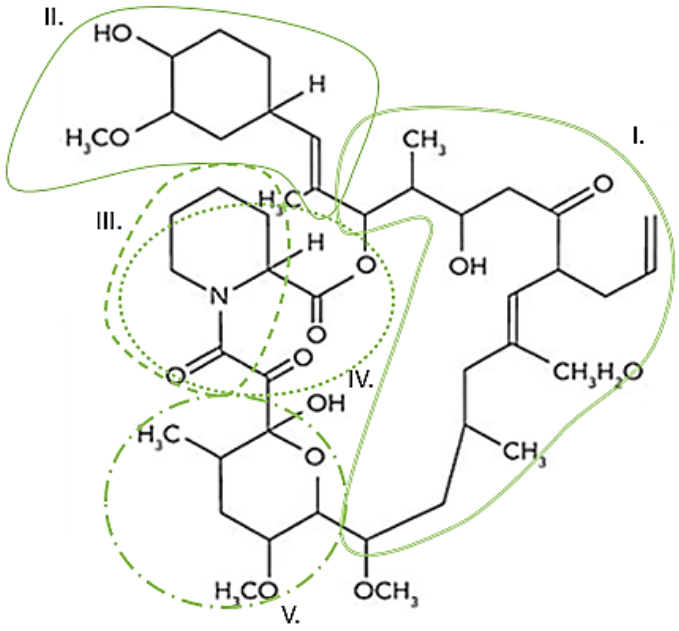
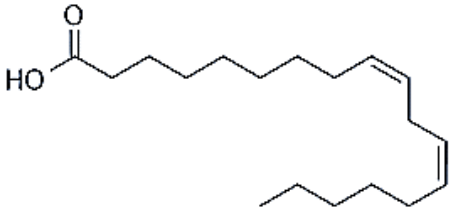
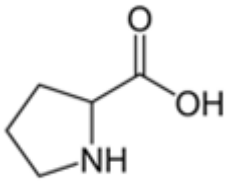


Figure 2: The molecular structure of tacrolimus decomposed into fragments.



Fragment I analogous to linolenic acid



Fragment IV analogous to picolinic acid

Figure 3: Carbon and nitrogen sources.

Similarly to the first level of contribution founds in the thermodynamic approach, the fragments were identified as follows:

Fragment I (Figure 2) corresponds to linoleic acid, the major fatty acid present in soybean oil, sunflower oil, cotton seed oil, and Brazil nut oil added in the composition of the media studied respectively, by Mishra and Verma (2012); Chen et al. (2012); Wang et al. (2017); Vaid (2017); Ferrari (2018), and Silva (2019).

Fragment II (Figure 2) corresponds to the structure of antioxidants and vitamins, although not mentioned directly in the composition of the medium, that may have contributed to the increase in productivity when vegetable oils were added.

Fragment III (Figure 2) corresponds both to the structure of amino acids such as L-lysine and to the soy peptone and is also present in the composition of the most productive media (Turlo et al., 2012; Ferrari, 2018; Silva, 2019; Mishra and Verma, 2012; Chen et al., 2012; Huang et al., 2013, and Wang et al., 2017). Okuhara et al. (1990) also presents the soy peptone, however, unlike the other studies it does not insert vegetable oil in the composition of the medium.

Fragment IV (Figure 2) corresponds to the picolinic acid present in the media studied by Turlo et al. (2012) and Ferrari (2018).

Fragment V (Figure 2) corresponds to starch added directly as glucose, fructose, maltose, sucrose or other carbohydrates and cereal alcohols.

Analyzing Table 1, it is verified that with more complex medium compositions, such as the addition of picolinic acid or Brazil nut oil, productivity becomes more expressive. In this respect, the medium studied by Ferrari (2018) and Turlo et al. (2012) are similar. However, the addition of linoleic acid through Brazil nut oil may explain the higher productivity in Ferrari (2018) and Silva (2019) studies, 47.4 mg/L and 41.7 mg/L, respectively.
In Table 2, mutant strain of *Streptomyces tsukubaensis* were used. Higher yields are associated with compositions in which vegetable oils rich in linoleic acid, L-lysine and soy peptone have been added. The study by Huang et al. (2013) showed higher productivity, 177.8 mg/L. It´s important to signalize that the interaction between the compounds L-lysine from the soybean meal combined with soy peptone can be associate to a second level of contribution. Based on these results, it is possible to affirm that the knowledge of the compound molecular structure and the decomposition into fragments can support the selection of more efficient carbon and nitrogen sources.

3. Conclusions

As pointed by Bertan and Cremasco (2020), the year 2020 enters to history of humanity, exposing its fragility due to a virus, affecting people that remain invisible to public policies. In this case, it is essential to seek scientific and technological solutions to overcome the difficulty of those who depend on medicines, such as tacrolimus. Once this immunosuppressant is obtained by fermentation, it is essential to define the nutrient medium for the action of the microorganism, which, in the present study, refers to Streptomyces sp. The medium basic is defined by presence of carbon and nitrogen source. The strategy proposal in this paper is to analyze the tacrolimus molecular architecture and to find central fragments that contains carbon and nitrogen source, considering the analogy with thermodynamics group contribution approach. The knowledge of molecular architecture can be the auxiliar key to optimizing productivity by fragmentation of tacrolimus structure. This approach can enhance productivity of this important immunosuppressant and can be extended to other compounds produced from fermentative processes.

Acknowledgments

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