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Artificial Intelligence applied to the production of high added-value Dinoflagellates Toxins.

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Abstract . Trade in high value-added toxins for therapeutic and biological use is expanding. These toxins are generally derived from microalgae belonging to the dinoflagellate family. Due to the difficulties to grow these sensitive planktonic species and to the complexity of methods used to synthesize these molecules, which are generally complex chemical structures, biotoxin manufacturers called on *artificial intelligence technologies*. Through the development of *specific learning neural networks* applied to each phases of biotoxin production: photo-bioreactors operating at optimal yield; - new chemical synthesis research processes; - toxin biosynthetic research pathways offering short-cut possibilities, manufacturing processes have been greatly improved.

Key words : Artificial Intelligence, Biotoxins, Dinoflagellates, Drug Discovery, Neural networks.

Introduction

Hundreds of thousands of microalgae species are distributed on the surface of the globe in marine, freshwater or brackish. The long-lasting interest in bioactive molecules (toxins) produced by microalga has risen in recent years (**Sun 2016, Khan 2018**). Microalgae Market is valued at USD 54.64 Million in 2018 and expected to reach USD 76.37 million by 2025 with a compound annual growth rate of 4.9% over the forecast period. (**Brandessence Market Research 2019**). Microalgae could be seen as “Cell mills producing recombinant commercial molecules”, which have encouraged the creation of numerous start-ups, specialized in the production of microalgae species (Green algae, Dinoflagellates, Red algae, Euglenoids) mainly in the western countries (**Cadoret 2008**). The reason for the growing interest of microalgae species is their ability to synthesize a wide variety of complex molecules

recognized for their biological features with great potential for use as pharmaceuticals therapies and/or biological probes. Among myriads of microalgae species, the family of dinoflagellates is of particular interest since they exhibit wide varieties of natural compounds. The main known toxins extracted from dinoflagellate microalgae species with strong biological credit profiles are given in **table 1** :

Table1-Biotoxins of potential therapeutic use produced by dinoflagellates species (for a complete list of dinoflagellates biotoxins see references Cadoret 2018 and Assunção 2017)

Biotoxin	Dinoflagellate species biotoxin producers	Therapeutic use
Saxitoxin	Alexandrium (minutum, tamarense, catenella) Gymnodinium (catenatum) Pyrodinium (bahamense)	Treatment of pain
Karlotoxin	Amphidinium genus.	Haemolytic activity
Palytoxin	Ostreopsis (siamensis, mascarenensis, lenticularis, ovata, fattorussoi)	Wide spectrum of biological effects
Tetrodotoxin	Alexandrium (tamarense)	Nerves and muscles action potential blocker
Okadaic acid	Prorocentrum genus (lima, concavum, belizeanum, maculosum) Dinophysis genus (acuta, acuminate, fortii)	Potent tumor promoter
Brevetoxin	Karenia brevis (formerly known as Gymnodinium breve or Ptychodiscus brevis)	Powerful Neurotoxic
Yessotoxin	Protoceratium reticulatum, Lingulodinium polyedra Gonyaulax spinifera	Biological and pharmacological probes
Gambierol	Gambierdiscus toxicus	Modulatory action upon voltage gated K ⁺ channels

Most of these dinoflagellate drugs have complex chemical structures, which development requires efficient chemical synthesis processes through the use of new designed retrosynthetic schemes. The case of palytoxin is a significant example of high market value since its price is around 700 US \$ 100 μ g. This molecule has 64 stereogenic centers, its synthesis requires more than 140 steps (**Hoffmann 2012, Newhouse 2009**). Start-ups, which economic business model is based on the production and sales of dinoflagellate bioactive toxins with high added value have to face three major concerns:

-Dinoflagellate in general grow slowly and are shear-sensitive species. It is the reason why dinoflagellate are difficult to culture in photo-bioreactors compared to other classes of microalgae. Consequently chemical-physical conditions (temperature, pH, media composition, light, stirring conditions) to find economical and viable options regarding bioreactor design and culture strategies have to be optimized. Moreover, taking into account those drastic dinoflagellate culture conditions, scale-up biotoxin production requests the development of high capacity bioreactors with a view to overcoming dinoflagellate biomass weight limitations (**Assunção 2017**)

-Due to the complex chemical structure of biotoxins of interest, their direct chemical synthesis through multi-steps synthesis requires design of efficient retrosynthetic schemes with focus on the most performing. Besides the possibility of their direct chemical synthesis, hemisynthetic routes can be also envisaged. Numerous examples of hemisynthesis of natural complex compounds are described in literature (**Shabir 2015, Ten Lohuis 1995**). To process biotoxin production through hemisynthetic technology, the most suitable metabolite involved in the metabolic pathway should be first selected, before to be included in the retrosynthetic scheme envisaged for the targeted biotoxin synthesis. Such pathway selection implies the total knowledge of the metabolic pathways involved in the metabolism of this biotoxin, before finalized a possible retrosynthetic scheme.

-To circumvent quantities limitations of the targeted toxin, and in order to enhance the yields, genetic and metabolic engineering (**Segler 2018**) could be ways to overcome those constraints. For this purpose, the knowledge of the different metabolism pathways specific to each dinoflagellate species, is required. Assuming that such pathways are not currently available, data mining have to be first undertaken before handling genomic experiments.

After having analyzed difficulties relative to the production of these high-added value biotoxin production from dinoflagellate microalgae, one can ask the question:

“ How Artificial Intelligence (AI) helps biotoxins manufacturers to overcome the whole problems raised in the previous paragraphs?”

AI is a valuable tool that can contribute to the achievement of each important phases (chemical synthetic processes, photo bio -reactor engineering, biological pathway identification) involved in the biotoxin cycle production.

1-AI in dinoflagellate culture production

A deep-learning neural network that considered the whole physical parameters (temperature, pH, irradiance, turbulence airflow, bioreactor capacity, light energy sources), as well as biological parameters (composition media, nutritional requirements) involved in the production of dinoflagellates, helps to define the optimized conditions which allow to get the best dinoflagellate biomass yield for a given dinoflagellate specie;

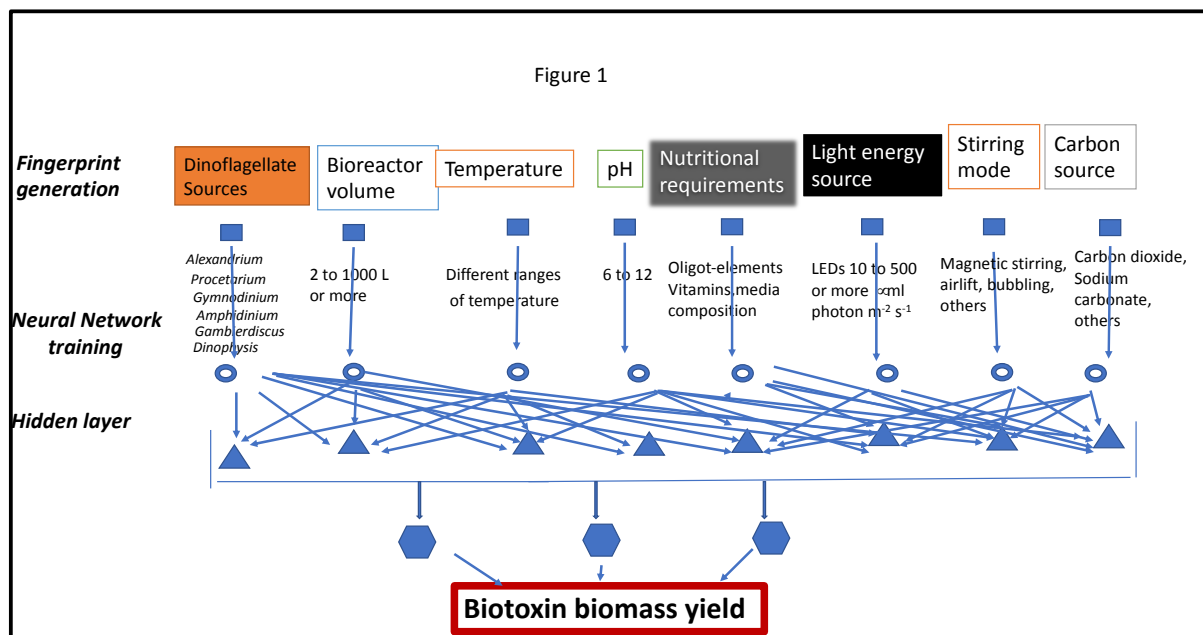


Figure 1: Learning Neural Network applied to biotoxin Dinoflagellate production

The learning neural network presents in Figure 1 is an important step towards the goal of developing an algorithm which can help the tuning of a photo-bioreactor regarding

optimized conditions for the preparation of a targeted biotoxin. This algorithm which predicts the best conditions to produce a given dinoflagellate specie, is part of a learning machine, adapted to a photo-bioreactors devoted to the automatic dinoflagellate production.

Optimization of production can be achieved by using a wide range of AI techniques from classical "one-factor-at-a-time" to modern statistical and mathematical techniques, viz. artificial neural network (ANN), genetic algorithm (GA) etc. Every technique comes with its own advantages and disadvantages, and despite drawbacks some techniques are applied to obtain best results. (Singh 2016)

Moreover knowledge-based or Artificial Intelligence techniques are used increasingly as alternatives to more classical techniques to model environmental systems, but we believe that AI techniques could be also applied to biological systems production. These techniques can be artificial neural networks, fuzzy models, genetic algorithms, cellular automata, multi-agent systems, swarm intelligence, reinforcement learning and hybrid systems. (S.Hamilton, 2008)

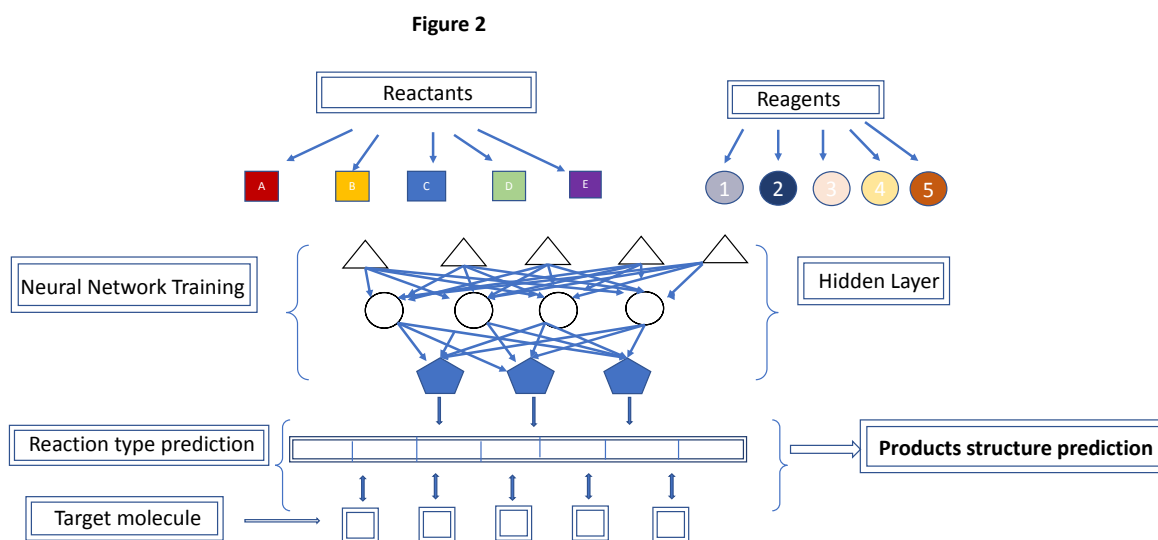
2- AI and biotoxins chemical synthesis and hemisynthesis.

Synthesis of molecules remains one of the most important challenges in organic chemistry. Standard approaches based on the development of specific retrosynthetic schemes are repetitive and time consuming task. Through the use of machine learning, substantial progresses in organic chemical synthesis have been made over the past decades (Coley 2016 ,Ahneman 2018, Klucznik 2018). For some some dinoflgellates biotoxin molecules, de novo synthesis could be considered as feasible (saxitoxin, tetrodotoxin, akadaic acid) but for other biotoxins such as palytoxin, yessotoxin, brevetoxin, gamberiol, de novo synthesis is considered exceedingly complex and economically unfeasible.

-AI and de novo dinoflagellate biotoxin chemical synthesis

Using finger prints derived from a neural algorithm, Wei et al. (Wei 2016), have identified chemical reaction types involved in compounds chemical synthesis with more than 80% accuracy. Since the development of this algorithm, new deep learning neural networks, which involved around 12 millions of known single-step reactions, and allowed the prediction of reactions which can be used in any single step of multi-step synthesis, has been developed (Coley 2016). More recently was developed by Klucznik et al.(Klucznik 2018), an highly effective *Chematica Computer Program* capable of designing novel efficient

synthesis of medicinally relevant drugs, making *Chematica algorithm* able to propose optimized synthetic pathways for the design of any molecules of interest. For example, a standard de novo synthesis of saxitoxin, a toxin used in the treatment of chronic pain, has been achieved in 10 steps with a final yield < 5% , (**Fleming 2006**) in 2006. As represented on Figure 2, using deep learning neural networks, medicinal organic chemists can use valuable retrosynthetic schemes allowing total drug synthesis in better yields, reducing the number of steps, and predicting compound stereochemistry, structure identification (NMR, UV, IR), some physical-chemistry properties .



F.Peiretti and J-M Brunel. ACS Omega 2018, 3, 10, 13263-13266

Figure 2: Learning Neural Network predicting probability of reaction types applied to biotoxin synthesis

-AI and saxitoxin hemisynthesis.

Knowing the metabolic pathways involved in the production of natural compounds, which chemical structure is quite complex, it is sometimes possible to isolate a particular intermediate metabolite, which is part of the metabolic pathway of the targeted natural drug. Starting from this metabolite, are carried specific chemical reactions in order to get the desired final drug in less steps and better yields (**Shabir 2015**). In this perspective, AI technologies have been used at different levels

-saxitoxin biosynthesis pathway determination (**Tsuchiya 2016 , Hunter 1993**).

-hemi-biosynthesis of saxitoxin analogues starting from a specific intermediate named “*shunt product (C’)*” derived from biosynthetic precursors involved in saxitoxin pathway (**Cho 2017**)

-detection and relative toxicity of saxitoxin analogues nonsulfated, monosulfated and disulfated (**Lukovski 2019**)

3- AI and saxitoxin genomic studies.

Artificial Intelligence has made faster, cheaper and more accurate DNA sequencing, and has allowed gain perspective on the particular genetic blueprint that orchestrates the whole activities of a given organism. In this feature, it has been reported (**Kellmann 2008**) that saxitoxin biosynthetic pathway is encoded by more than 35 kb and comparative sequence analysis assigns 30 catalytic functions to 6 proteins. Using in silico functional inferences based on saxitoxin *open reading frames* combined with liquid chromatography–tandem mass spectrometry analysis, the description of the saxitoxin biosynthetic pathway has been revised, and a saxitoxine biosynthetic gene cluster (stx) has been identified and shown to be involved in saxitoxin production. Knowing the right saxitoxin metabolic pathway, it could be possible to construct an algorithm which satisfies not only each constraint but also allows several alternative pathways that by-pass key enzymes. Recently, new methods in artificial intelligence which offer the possibility to integrate chemistry and biology into complex reaction schemes to provide access to complex molecules not achievable with either alone, have been reported (**Lin 2019**). These authors concluded that in the next future to build a xenobiotic molecule of a scale of a natural product, will require integrated computer-aided design packages that combine retrosynthesis, metabolic flux analysis, protein engineering and genetic circuit design automation. Such AI technologies have been already applied for systems biology with a high research impact (**Cevora 2019**).

Conclusion

Dinoflagellates is one of the rich biotechnological source of biotoxins, with interesting biological activities not only in pharmacological and medical fields, but also as promising tools for chemical biology. Despite such recognized value, scarcity of such biotoxins remains a major issue, that new marine natural products start-ups have to face economically and in terms of viability. The problem becomes even more complex since these high value biotoxins

are mainly found in dinoflagellates species which are extremely fragile microalgae and which culture required high tech bioreactors. To circumvent those problems, available AI technologies based on learning neural networks, could be applied at each phase of biotoxin production: chemical synthesis and hemisynthesis, biotoxin structural identification, bioreactor engineering systems, biological pathways identification through marker-passing algorithm. AI clearly appears as a promising tool to help new start-uppers to jump in the restricted biotoxin market, in proposing not only biotoxins at reasonable prices but also allowing the discovery of new drugs, considering that dinoflagellates marine organisms, are the sources of several thousand drugs of interest, which remain to be discovered. **(Kobayashi 2016)**. Of course to generalize AI technique to other dinoflagellate species to produce other high value toxins , we will need to generate enough data to train a deep neural network.

Nevertheless, our perspectives are to use the knowledge we acquired with the use of artificial intelligence networks for the optimization of the production of new high added value toxins from new species of dinoflagellates, known to be difficult to grow.

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