ECRONICON OPEN ACCESS

EC MICROBIOLOGY
Research Article

Infectious Pathogens as a Novel Source for Secondary Metabolites

Fernandez-Bunster Guillermo*

Campus San Felipe, Universidad de Valparaíso, Chile

***Corresponding Author:** Fernandez-Bunster Guillermo, Universidad de Valparaíso, Camino la Troya esq. El Convento, San Felipe, Chile.

Received: May 27, 2019; **Published:** July 12, 2019

Abstract

Infectious diseases are caused by a number of pathogenic microorganisms, such as bacteria, fungi, viruses, and parasites. These organisms can be spread from one person to another, as well as after exposure to an infected animal that contains pathogenic organisms for humans. According to WHO, in 2016, three of the top ten causes of death worldwide were produced by infectious diseases, such as lower respiratory infections, diarrheal diseases, and tuberculosis. But not all is evil with pathogens, they can be also used for good: They have encoded (sometimes silently) in their genome the ability to produce secondary metabolites. Microorganisms use them as a defense system, competitiveness, and communication, but also these compounds may be of pharmaceutical interest for us *in-silico* analysis, i.e. the use of current computational software can predict the presence/absence of secondary metabolite genes and/or gene clusters by solely inputting the nucleotidic sequence of the query organism, such as bacteria and fungi. With a correct prediction and the use of heterologous expression systems, these genes can be inserted in model organisms or better-understood organisms, giving us the ability to express cryptic gene clusters from unusual sources to obtain compounds of interest.

Keywords: *Infectious Diseases; Bioinformatics; Gene Mining; Secondary Metabolites*

Abbreviations

WHO: World Health Organization; GC/MS: Gas Chromatography/Mass Spectrometry

Introduction

Historically, infectious diseases have been affecting negatively the numbers of human population: According to Dye (2014), in 1990, an estimated 16 million people died from infections, while in 2010, the number had fallen slightly to 15 million deaths. On this topic, WHO has predicted 13 million deaths caused by this type of diseases in 2050. The majority of deaths produced by infectious diseases are (and will be) caused by a small number of pathogens, as showed by the statistics proving that in 2010, two-thirds of deaths produced by infections were caused only by twenty species, comprising viruses and bacteria [1].

According to WHO, several infectious diseases have been cataloged as an epidemic, or worst, pandemic diseases, produced mainly by viruses and bacteria. A few examples are cholera, produced by the bacterial strain *Vibrio cholerae*, serovar O1 and O139, producing large amounts of watery diarrhea that lasts a few days, but could lead to severe dehydration [2], plague, an infectious disease produced by the zoonotic bacteria *Yersinia pestis*, has a case-fatality ratio up to 100% if left untreated. Others may be epidemic, as well as endemic of certain regions, such as meningitis, produced by the human pathogen *Neisseria meningitidis*, a bacterial infection that causes development impairment and it is recognized as one of the major causes of bacterial meningitis, as well as other invasive bacterial infections across the world [3].

A great number of infectious diseases are usually treated with antibiotics and antiviral agents, but the apparition of antibiotic-resistant strains as well as mutant microorganisms have led to more persistent and harder-to-treat infections. Moreover, some bacterial infections produce biofilms as defense mechanisms, difficulting their treatment with common antibiotics [4]. One way to combat this problem is by using novel compounds, such as natural-derived ones from plants, fungi, and bacteria. These molecules can be obtained, as an example, from the secondary metabolism of living organisms. Secondary metabolites are, as definition, low molecular mass molecules whose production is not essential for the life of the producer organism [5], as the main difference with primary metabolites, which are essential for development and growth. As an example, citric acid from *Aspergillus niger* and gluconic acid, produced by *A. terreus*, are produced during active growth [6]. A wide variety of organisms, particularly bacteria, fungi, and plants, can produce an extremely diverse "range" of secondary metabolites, including penicillin, cephalosporins, and statins, to name but a few [7].

One way to explain the existence of non-vital metabolites is associated with the use of these type of products as defense, by producing compounds that can inhibit competitively the growth of other organisms, as communication signals, such as a quorum-sensing communication-like system by using homoseryl lactones and specialized peptides, or related to sporulation in bacteria [8] and fungi [9] among other functions.

Metabolite screening

The search for new drugs, aside from attempting a complete chemical synthesis, is by screening natural products, considering that several diseases have been treated, for example, with herbal medicinal products.

The most common way to discover novel natural compounds mainly involves the screening of crude extracts of the organism grown on specific growth media, followed by the analysis of the crude extract by different analytical techniques, such as GC/MS or LC/MS for detection, and NMR/IR for structure elucidation. As an example, the approach of testing different growth media and extracting the secondary metabolites produced under different growth conditions led to the successful isolation of aspoquinolones A-D from *Aspergillus nidulans* [10]. Such non-specific screening of this nature has certainly yielded novel compounds but has often also resulted in the rediscovery of previously described compounds [11].

Another method to identify novel molecules of pharmaceutical interest from microorganisms against pathogenic ones is by phenotypic screening: Of a total of nearly 6 million compounds screened to date against *Plasmodium falciparum*, the pathogen organism responsible of malaria, an infectious diseases produced by parasitic protozoans of the genus *Plasmodium* [12], more than 25000 showed promising results, having an IC_{50} of 1 uM or below against the pathogen. The search for new drugs, aside from attempting a complete chemical synthesis, is by screening natural products, considering that several diseases have been treated, for example, with herbal medicinal products.

Gene mining and gene cluster prediction

Microorganisms are known to be a rich source of bioactive compounds, also partial and full genome sequencing has revealed the presence of more secondary metabolite gene clusters than compounds recorded, indicating the potential to source vastly more bioactive substances than previously imagined possible. Genome mining has already proved to be an excellent tool for cloning of known genes related to secondary metabolites, as well as the neighboring genes that could compose the whole gene cluster [13]. Current discovery techniques have now moved on to whole-genome sequencing projects, generating information that can be used to discover novel compounds and/or fill some missing key steps in biosynthetic pathways of compounds already known [14]. In some cases, the gene (or gene clusters) identified can be correlated with compounds known to be produced by the organisms, but most clusters discovered this way are cryptic or silent.

Given the number of partial and/or full genome sequens of several pathogens of interest determine to date and their possible content of cryptic secondary metabolism gene clusters, there must be a wide range of novel secondary metabolites to discover, many with potential

applications [15]. The basis of this approach is the initial identification of the main synthase gene (for example, polyketides, peptides, terpenes, bacteriocins, etc…) and further prediction of the function of neighboring genes to obtain precursors of the compound, as well as the fully-synthesized product. Software packages, such as antiSMASH (antibiotics & Secondary Metabolite Analysis Shell - 16), NaPDos (Natural Product Domain Seeker) and PRISM (Prediction Informatics for Secondary Metabolomes – Nathan Magarvey Lab at MacMaster University) may predict potential genes related to secondary metabolism, nucleotide, and amino acid sequences, and depending on the identity percentage obtained from the query sequence against known gene clusters, the search of common domains/features with other known secondary metabolite genes/gene clusters.

Materials and Methods

To show the potential given by these type of prediction packages, full genome sequences were retrieved randomly from NCBI, using as a search term "*Bordetella*" and "*Neisseria*", at the Genome category, and two obtained results were selected, *i.e. Bordetella pertussis B1917* (Accession number: NZ_CP009751, sequenced by [17] and *Neisseria gonorrhoeae FA 1090* (Accession number: NC_002946, sequenced by Lewis., *et al* 2003. Then, the analysis was performed through the antiSMASH software [16], a specialized software to search for novel and cryptic gene clusters that encode for secondary metabolites.

Results and Discussion

In-silico genome analysis

The results obtained from the software antiSMASH are shown in table 1 and 2 for *Bordetella pertussis B1917* and *Neisseria gonorrhoeae* FA 1090, respectively.

For *Bordetella pertussis B1917*, circular DNA of 4102 kbp				
Region	**Type**	**Gene size**	**Region size (nt)**	**Other known genes in the region**
1	Bacteriocin	870	10870	TonB-dependent receptor MFS transporter
2	Siderophore Alcaligin siderophore biosynthesis protein AlcC 50% similarity to Desferrioxamine B Bisucaberin B	1857	11857	Alcaligin siderophore biosynthesis transcriptional regulator AlcR Alcaligin siderophore export MFS transporter Bcr Alcaligin siderophore biosynthesis protein AlcE Alcaligin siderophore biosynthesis protein AlcD Alcaligin siderophore biosynthesis protein AlcB Alcaligin siderophore biosynthesis protein AlcA
3	Terpene squalene synthase HpnC	1695	21696	HdeD family acid-resistance protein

Table 1: *AntiSMASH results for Bordetella genome, accession number NZ_CP009751.*

For *Neisseria gonorrhoea*e FA 1090, circular DNA of 2153 kbp				
Region	**Type**	**Gene size**	**Region size (nt)**	**Other known genes in the region**
1	Terpene squalene/ phytoene synthase	873	19017	Acetyl-CoA carboxylase carboxyl transferase subunit alpha chaperone protein HscA
2	Terpene squalene/ phytoene synthase	828	20,828	ADP-heptose--LPS heptosyl transferase II thiol: disulfide interchange protein
3	Bacteriocin	843	10,843	Threonyl carbamoyl-AMP synthase RNA polymerase sigma factor

Table 2: *AntiSMASH results for Neisseria genome, accession number NC_002946.*

Showing the potential of pathogens organisms to produce secondary metabolites of interest, such as terpenes and bacteriocins: Three secondary metabolite gene/gene clusters were found for each bacterial strain, and this number could be higher if other complementary prediction softwares are used.

Bacteriocins, as the name suggests, are peptidic toxins produced by bacterial strains to inhibiting competitively other similar or related bacterial strains. These bacteriocins are ecologically, functionally and structurally diverse, even studied to use them as antibiotics of narrow specter [18]. The second result shows several genes involved in the metabolic pathway of alcaligin, which is defined as a siderophore, iron chelators (siderophore) of low-molecular-mass from a bacterial origin that works as a receptor and transport compound, responding to iron starvation, with wide uses in biotechnology [19]. Terpenes, formed by isoprenoid building blocks, are known for their particular smell and are used as repellants, perfume preparation and even possess medicinal features, among others [20]. One member of the terpene family are squalenes, described as a natural compound that can be found in the human body as a biochemical precursor of steroids via oxidation by squalene monooxygenase to obtain, in the end, lanosterol into cholesterol and other steroids [21].

The software, as shown in the table, additionally provides the nucleotidic and amino acid sequence of the described gene, as well as propose the function proposal of the surrounding open reading frames, facilitating the task of the researcher to decide if one gene is enough to obtain the product, or if more genes are necessary to retrieve the final product.

Heterologous expression

Genome mining helps to find known and/or cryptic gene clusters, i.e. ones that have a low expression or no known expression at all, as well as global/specific gene regulators [22]. One approach is to manipulate global secondary metabolite regulators that could affect the target gene cluster, potentially activating orphan gene clusters [23], but the overexpression of global and/or specific regulators could lead to affect other biological processes, aside of the overproduction of the desired compound. Once a gene cluster has been selected, its function can be studied by using different strategies, including gene knock-out, which leads to a total loss of production of the expected compound if the core synthase gene is inactivated, or an accumulation of compound precursor and/or intermediates if tailoring enzymes are removed. Another approach is to analyze the function of each gene (separately and/or in combination with other genes) by expression in heterologous hosts, such as *Saccharomyces cerevisiae*, *Escherichia coli* and *Aspergillus oryzae,* as demonstrated by Davison., *et al.* [24], who used gene knockout and heterologous expression to study spititatic acid, a tropolone from *Talaromyces stipitatus* [25].

Several microorganisms are not always easy to grow on a large scale and/or may take a long time to achieve detectable production of the desired compound(s). Also, some novel compounds can come from human pathogens that require special requirements for its handling and growth, so in less-studied organisms, or when genetic manipulation has not been possible, a different methodology to study secondary metabolites is using the heterologous expression of partial or complete biosynthetic gene/gene clusters. For example, the expression in *E. coli* of a terpene synthase gene from *Streptomyces avermitilis* [26] resulted in the production of the sesquiterpene avermitilol when growth was in the presence of farnesyl diphosphate. By expressing heterologous genes fused to its native promoters jointly with the synthase in *E. coli*, the same group obtained avermitilol, avermitilone, as well as germacrenes A and B [27]. In bacteria, the expression of the gene cluster was successful only because of the compatibility of prokaryotic promoters. Fungal sources, on the other hand, requires specific promoter change to be compatible for the system host and intron removal prior to gene insertion, because of the prokaryotic system would not be able to remove (even recognize) effectively intronic sequences within the gene, among other factors.

The genes can be inserted in plasmids by using enzyme restriction sites added to the target sequence by PCR and addition of the open reading frames by a ligase into the plasmid, as well as it may be added by using the property of homologous recombination in the yeast *Saccharomyces cerevisiae* (yeast recombination [28]), a methodology used to join DNA fragments to a linearized plasmid vector if there is an overlap of homologous sequence between them and generating a circular recombinant plasmid.

In-silico compound analysis

Once one or more compounds are obtained by heterologous expression, several online and off-line informatic tools can be used to analyze if the metabolite can, in theory, have the desired effect. The first required step is to structurally characterize the compound(s) to learn about its physicochemical properties and the association with target enzymes and proteins. As described by Boruah., *et al.* [29], there are six main bioinformatics areas related to compound analysis: Target identification and validation, lead identification and validation, prediction of drug-like properties and preclinical pharmacology and toxicology.

Target identification requires a good knowledge about the pathology and its related metabolic pathways, to be able to direct the efforts to certain(s) key enzyme(s), whose disruption can interfere in vital processes, resulting in the death of the pathogen. Genome sequencing and gene identification have led to the detection of novel targets, and 10 - 35% of the novel compounds used in the pharmaceutical industry have been based on genome analysis [30]. Once the target is identified, it must be validated in animal models. Lead identification, on the other hand, requires the study of the biological activities in a target. Firstly, a lead can be defined as any compound that shows a biological activity of interest on a specific target. These type of compounds can be obtained for a broad range of natural sources, such as animals, plants, fungal and bacterial strains, but also, they may be produced through semi-synthetic (chemical, followed by biological modifications, such as enzymatic reactions, or biological synthesis, followed by chemical modifications) or synthetic reactions [29]. Several databases can be used to search for the "ideal" compound that fits correctly and competitively against the corresponding substrate in the selected active site of the target, avoiding the protein to function properly by the fitting of the "incorrect" compound, or to by fitting into a key position at the protein to produce conformational changes at the active site by broaden or narrow it, avoiding the perfect "fit" between enzyme and substrate (according to the induced fit model, the fitting between substrate and protein will produce a small structural change, crucial for the protein to work).

In all cases, this identification phase may be divided on virtual screening (Figure 1), bioinformatics, pharmacophore mapping (database searching, modeling), molecular docking, among others: The mapping characterization requires a correct structure identification of the lead to predicting the tri-dimensional arrangement of its functional groups, as well as fingerprinting, a key feature used to compare structural similarities between novel and known compounds. Afterward, molecular docking, an *in-silico* tool only, allows to position the compound of interest into the (predicted) active site within the target protein. For this, a 3-D structure of the protein is required. Fortunately, several databases, such as RCSB Protein Data Bank (https://www.rcsb.org/) and World Wide Data Bank (https://www.wwpdb.org/) have a large number of protein structures ready to download and free-to-use for any researchers interested to perform bioinformatics into their proteins.

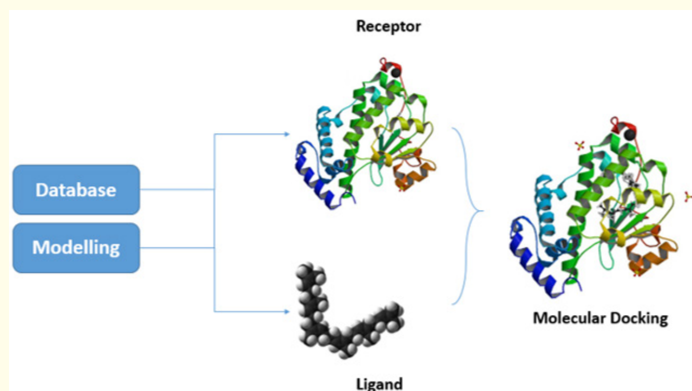

Figure 1: *Stages of Structural-Based Virtual Screening. The target protein (or receptor) can be obtained from a database or by modeling. Molecular docking completes the structure-based virtual screening. Adapted from Giulatti, 2012 [31].*

Continuing with the demonstration, a squalene synthase was detected in both bacterial strains (Bordetella and Neisseria), and if the prediction and heterologous expression of the gene cluster was successful, a novel compound would be obtained. Using "Squalene" as a term in PDB Data Bank [32]. Several results are retrieved: Most of them related to the structure of the squalene synthase (important to understand how the enzyme works), but also, protein structures that use squalene as a ligand. One of them is the protein labeled "4OMK", corresponding to the crystal structure of the SEC14-like domain of the Supernatant Protein Factor (SPF), part of the cholesterol endosynthetic pathway. Squalene and 2,3-oxidosqualene are located at the active site of the protein [33]. The research shows, as described before, the importance of the analysis of the active sites of the proteins, describing in this particular case, that ligand affinity is dominated mainly by hydrophobic interactions, instead of the epoxide group expected to bind. This is only a small example of bioinformatics can do. Several other analysis and experiments that can be performed after the processes described before, but the main aim of this review is simply to demonstrate the ability of bioinformatics to obtain results from databases and mining.

Maithri., *et al.* [34] performed a very exhaustive list of software packages with different functions, even some of them free to use for academics. The following table shows a few of the available software packages for protein and compound analysis (Table 3).

Program	Description	License	Function
DockingServer	Integrates a number of computational chemistry software	Commercial	Docking Studies
AutoDock (Vina)	Automated docking of ligand to a macromolecule	Freeware	Docking Studies
Q-Dock	Low-resolution flexible ligand docking with pocket-specific threading restraints	Freeware	Docking Studies
MultiCASE (MC4PC)	Computational toxicity predictions	Commercial	Developmental and reproductive toxicity
VMD	Visual Molecular Dynamics (Molecular visualization program for displaying, animating, and analyzing large biomolecular systems using 3-D graphics and built-in scripting)	--	Molecular dynamic studies
CAESAR	Specifically dedicated for validation of QSAR models: bio-concentration factor, skin sensitization, carcinogenicity, mutagenicity, Developmental toxicity	Free	Quantitative Structure-Activity Relationship (QSAR) Studies
ChEMBL	Database of small molecules. Includes interactions and functional effects of small molecules binding to their macromolecular targets, and series of drug discovery databases	Free	Databases used in virtual high throughput screening
PubChem	Database of chemical compounds maintained by the National Center for Biotechnology Information (NCBI), along with bioassays results. Allows similar compounds as a search term (2D and 3D).	Free	Databases used in virtual high throughput screening

Table 3: *List of some software packages for in-silico analysis. Adapted from [34].*

Conclusion

Pathogens related to infectious diseases can be used positively as a producers of secondary metabolites of interest for us. The common way to obtain these compounds (Growth at large scale and compound extraction/characterization) has been replaced successfully by using *in-silico* analysis, allowing us to initially identify the potential genes involved in the production of the desired compound, and then express them in a well-studied host, such as *E. coli* and *S. cerevisiae,* in a faster and more reliable way, as well as allowing the insertion of

other tailoring enzymes that can modify the properties of the compound features. Further structural analysis of the compound and/or the target can be done by several *in-silico* tools, allowing us to select and properly modify the compounds to enhance their properties, such as affinity binding to a receptor or interference in key metabolic pathways. In simple words, we can turn our enemies into allies.

Conflict of Interest

There is no financial interest or any conflict of interest involved in this paper.

Bibliography

1. Dye C. "After 2015: infectious diseases in a new era of health and development". *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 369.1645 (2013): 20130426.
2. Harris JB., *et al*. "Cholera". *Lancet* 379.9835 (2012): 2466-2476.
3. Harrison LH. "Epidemiological profile of meningococcal disease in the United States". *Clinical Infectious Diseases* 50.2 (2010): S37-S44.
4. Kim KJ., *et al*. "Natural Products for Infectious Diseases". *Evidence-Based Complementary and Alternative Medicine* (2016): 9459047.
5. Berdy J. "Bioactive microbial metabolites". *Journal of Antibiotics (Tokyo)* 58.1 (2005): 1-26.
6. Calvo AM., *et al*. "Relationship between secondary metabolism and fungal development". *Microbiology and Molecular Biology Reviews* 66.3 (2002): 447-459.
7. Brakhage AA., *et al*. "Aspects on evolution of fungal beta-lactam biosynthesis gene clusters and recruitment of trans-acting factors". *Phytochemistry* 70.15-16 (2009): 1801-1811.
8. Hopwood DA. "The Leeuwenhoek lecture, 1987. Towards an understanding of gene switching in Streptomyces, the basis of sporulation and antibiotic production". *Proceedings of the Royal Society of London. Series B, Biological Sciences* 235.1279 (1988): 121-138.
9. Bu'Lock JD. "Intermediary metabolism and antibiotic synthesis". *Advances in Applied Microbiology* 3 (1961): 293-342.
10. Scherlach K., *et al*. "Discovery of aspoquinolones A-D, prenylated quinoline-2-one alkaloids from Aspergillus nidulans, motivated by genome mining". *Organic and Biomolecular Chemistry* 4.18 (2006): 3517-3520.
11. Winter JM., *et al*. "Genomics-inspired discovery of natural products". *Current Opinion in Chemical Biology* 15.1 (2011): 22-31.
12. Caraballo H., *et al*. "Emergency department management of mosquito-borne illness: malaria, dengue, and West Nile virus". *Emergency Medicine Practice* 16.5 (2014): 1-23.
13. Kroken S., *et al*. "Phylogenomic analysis of type I polyketide synthase genes in pathogenic and saprobic ascomycetes". *Proceedings of the National Academy of Sciences USA* 100.26 (2003): 15670-15675.
14. Crawford JM., *et al*. "Microbial genome mining answers longstanding biosynthetic questions". *Proceedings of the National Academy of Sciences USA* 109.20 (2012): 7589-7590.
15. Bergmann S., *et al*. "Activation of a silent fungal polyketide biosynthesis pathway through regulatory cross talk with a cryptic nonribosomal peptide synthetase gene cluster". *Applied and Environmental Microbiology* 76.24 (2010): 8143-8149.
16. Medema MH., *et al*. "antiSMASH: rapid identification, annotation, and analysis of secondary metabolite biosynthesis gene clusters in bacterial and fungal genome sequences". *Nucleic Acids Research* 39 (2001): W339-W346.

17. Bart MJ., *et al*. "Complete Genome Sequences of Bordetella pertussis Isolates B1917 and B1920, Representing Two Predominant Global Lineages". *Genome Announcements* 2.6 (2014): e01301-e01314.
18. Cotter PD., *et al*. "Bacteriocins — a viable alternative to antibiotics?" *Nature Reviews Microbiology* 11.2 (2012): 95-105.
19. Brickman T., *et al*. "Alcaligin siderophore production by Bordetella bronchiseptica strain RB50 is not repressed by the BvgAS virulence control system". *Journal of Bacteriology* 184.24 (2002): 7055-7057.
20. Kandi S., *et al*. "Biomedical Significance of Terpenes: An Insight". *Biomedicine and Biotechnology* 3.1 (2015): 8-10.
21. Bloch KE. "Sterol, Structure and Membrane Function". *Critical Reviews in Biochemistry and Molecular Biology* 14.1 (1983): 47-92.
22. Corre C., *et al*. "Heavy tools for genome mining". *Chemistry and Biology* 14.1 (2007): 7-9.
23. Bok JW., *et al*. "GliZ, a transcriptional regulator of gliotoxin biosynthesis, contributes to Aspergillus fumigatus virulence". *Infection and Immunity Journal* 74.12 (2006): 6761-6768.
24. Davison J., *et al*. "Genetic, molecular, and biochemical basis of fungal tropolone biosynthesis". *Proceedings of the National Academy of Sciences USA* 109.20 (2012): 7642-7647.
25. Birkinshaw JH., *et al*. "Studies in the biochemistry of micro-organisms: Stipitatic acid, C(8)H(6)O(5), a metabolic product of Penicillium stipitatum Thom". *Biochemical Journal* 36.1-2 (1942): 242-251.
26. Chou W., *et al*. "Genome mining in Streptomyces avermitilis: cloning and characterization of SAV_76, the synthase for a new sesquiterpene, avermitilol". *Journal of the American Chemical Society* 132.26 (2010): 8850-8851.
27. Komatsu M., *et al*. "Genome-minimized Streptomyces host for the heterologous expression of secondary metabolism". *Proceedings of the National Academy of Sciences USA* 107.6 (2010): 2646-2651.
28. Barr MM. "Super models". *Physiological Genomics* 13.1 (2003): 15-24.
29. Boruah L., *et al*. "In-Silico Drug Design: A revolutionary approach to change the concept of current Drug Discovery Process". *Indian Journal of Pharmaceutical and Biological Research* 1.2 (2013): 60-73.
30. Caron PR., *et al*. "Chemogenomic approaches to drug discovery". *Current Opinion in Chemical Biology* 5.4 (2001): 464-470.
31. Giuliatti S. "Computer-Based Methods of Inhibitor Prediction, An Integrated View of the Molecular Recognition and Toxinology - From Analytical Procedures to Biomedical Applications". IntechOpen (2013).
32. Berman HM., *et al*. "The Protein Data Bank". *Nucleic Acids Research* 28.1 (2000): 235-242.
33. Christen M., *et al*. "Structural insights on cholesterol endosynthesis: Binding of squalene and 2,3-oxidosqualene to supernatant protein factor". *Journal of Structural Biology* 190.3 (2015): 261-270.
34. Maithri G., *et al*. "Computational Drug Design and Molecular Dynamic Studies-A Review". *International Journal of Biomedical Data Mining* 6 (2016): 123.

Volume 15 Issue 8 August 2019
©All rights reserved by Roberto Ligugnana and Alessandro Ligugnana