

Chemical Composition And Rheological Study Of Polysaccharide From Eggs Of *Aplysia Dactylomela*

Jao Manarivo Ulrich Anito, Fenoradosoa Taratra André, Delattre Cédric, Michaud Philippe, Kall Briant

Faculté des Sciences de l'Université d'Antsiranana,
BP O, 201, Antsiranana, Madagascar
jaomanarivo@yahoo.com

Faculté des Sciences de l'Université d'Antsiranana,
BP O, 201, Antsiranana, Madagascar
radosoataratra@yahoo.fr

Université Clermont Auvergne - Polytech Clermont - 2, avenue Blaise Pascal –
TSA 20206 - 63178 Aubière - France
Blaise Pascal TSA 60206, CS 60026, 63178 Aubière, France
cedric.delattre@uca.fr

Université Clermont Auvergne - Polytech Clermont - 2, avenue Blaise Pascal –
TSA 20206 - 63178 Aubière - France
philippe.michaud@uca.fr

Faculté des Sciences de l'Université d'Antsiranana,
BP O, 201, Antsiranana, Madagascar
briantkall@yahoo.com

Abstract : *Aplysia dactylomela* is a sea hare having eggs with high potential as polysaccharide collected in the coastal sea of Madagascar (Vohemar). To determine the chemical compositions, rheological characteristics, colorimetric assays, HPAEC and viscometer, measurements were carried out. Crude polysaccharides (EPPOAD) were extracted from *Aplysia dactylomela* eggs by the aqueous solvent extraction method under neutral and alkaline conditions. The extraction yield was 20.9 % and 22.89 % under those both conditions respectively. The results showed 31.24 % total sugar, 27.51 % neutral sugar, 17.92 % uronic acid, 3.93 % protein and 38.93 % sulphate. It is composed of fucose, arabinose, glucosamine, galactose, galacturonic acid and glucuronic acid assigned in g/g of sample 0.022, 0.049, 0.048, 0.146, 0.020, 0.023, respectively. The result showed that the EPPOAD extract has a shear-thinning behavior and a recovery of the critical concentration (C^*) at 17.42 g/L.

Keywords : Polysaccharide, *Aplysia*, *dactylomela*, rheology

1. Introduction

The polysaccharides are macromolecules that exist throughout nature. The structures of those polysaccharides are influenced by their origins and the extraction method. They potentially have many useful therapeutic and pharmaceutical applications [1], [2], [3], [4], [5]. Marine organisms are valuable, underexploited sources of polysaccharides with chemical structures newly discovered. Marine natural polysaccharides were previously isolated from algae [6], [7] fishes [8] and marine invertebrates [9]. The natural polysaccharides from marine animals are a hot research topic because of their various biological activities. However, the extraction of polysaccharides from sea hares has thus far received little attention from scientists and researchers who are working in the field of marine natural products. Although some polysaccharides structures obtained from sea hare muscle have been reported in recent years [10], [11] but there was only one investigation of polysaccharides from its eggs, that of *Notarchus leachii* freeri. Sea hare eggs were a new raw material for polysaccharide extraction although their structures are not yet well known. Therefore, it is important to study its structures. The sea hare *Aplysia dactylomela* is a marine opisthobranch gastropod mollusk belonging to the *Aplysiidae* family. It is a circumtropical aplysid and occurs worldwide in tropical to warm temperate waters [12]. The sea hare and its eggs are used as traditional nutritious food source in Asian countries

[13], [14] and its eggs have also been used as traditional medicine in China since Ming Dynasty [15]. The egg mass of an *A. dactylomela* is a resistant gelatinous string that can reach 8 m in length with variable colors, reaching tones of yellow, orange or pink [12]. In the present work, we extract for the first time polysaccharide from *Aplysia dactylomela* egg collected in the coastal waters of Madagascar (in Indian Ocean). We carry out an preliminary study for structure polysaccharide eggs of *Aplysia dactylomela* with the determination global composition of polysaccharide using colorimetric assay and the monosaccharides composition using HPEAC. Finally, the physicochemical characterization is determined using viscometer.

2. Materials and methods

2.1. Material

eggs of *Aplysia dactylomela* were collected in the shore sea at Vohemar district of Madagascar (latitude 13 degrees 21 minutes 33 seconds South, longitude 50 degrees 0 minutes 43 seconds east, 18 m above sea level). The eggs were cleaned manually with sea water and then dried at 40 °C in oven (Drying oven, DHG-9030A) before been stored in bags at a shaded and ventilate site. Before each polysaccharide extraction, the sample was washed abundantly with water and dried during 30 hours at 65 °C.

2.2. Extraction

Then, extractions of the polysaccharide have been done with water (pH 6,0) at solvent ratio of 1/30 (w/v) and conducted under condition of 80 °C for 3 hours. Separation of the residue from the extracted solution was performed by filtration with a very fine mesh fabric. The residue was briefly washed with additional distilled water and the procedure was repeated several times to maximize polysaccharide recovery. Then, the solution was centrifuged at 4500 rpm for 30 minutes and the supernatant was precipitated with ethanol (3 vol.) and collection. The procedure is repeated again with the obtained polysaccharide using acetone. The polysaccharide is collected after wringing out the acetone and dried at 40 °C in the oven (Drying oven, DHG-9030A). Extraction of polysaccharide with 750 mL of 0,3 M NaOH was applied to 25 g of dried *Aplysia dactylomela* egg, at 80 °C during 3 hours. The same extraction procedure with neutral pH is followed to obtain the crude polysaccharide. The percentage yield of crude polysaccharides (EPPOAD) were calculated according to the following formula :

$$\text{Yield (\%)} = \frac{\text{The weight of polysaccharides extracted}}{\text{The weight of dry powder from sea hare eggs strings}} \times 100 \%$$

2.3. Global composition analysis

The total sugar content of EPPOAD was determined by the phenolsulfuric acid method and glucose acted as the standard [16]. Total neutral sugar content was determined by the reaction with resorcinol in presence of sulfuric acid using glucose as a standard [17]. The total uronic acid content was colorimetrically determined by the m-hydroxydiphenyl assay using galacturonic acid as a standard [18]. Sulphur content was determined by the turbidimetric method [19]. The protein content was determined by Folin reaction [20].

2.4. Monosaccharides composition analysis

The monosaccharide composition was determined using highperformance anion exchange chromatography (HPAEC), on a guard CarboPac PA1-column (4 × 50 mm) and analytical CarboPac PA1-column (4 mm × 250 mm). Detection was performed with a pulsed amperometric ED50 detector (Dionex Corp., Sunnyvale, CA). The polysaccharides from *Aplysia dactylomela* eggs (10 mg) dissolved in 4 M TFA (1 mL) were heated at 100° C during 8 hours. The hydrolysates were neutralized with ammonia solution (4 M). 25 microliters of sample (with 10 mg/mL of concentration) were injected. Each carbohydrate concentration was determined after integration of respective areas [Chromleon management system (Dionex)] and comparison with standard curves obtained with all relevant monosaccharide standards. To investigate the neutral monosaccharides, the elution has been achieved isocratically with 16 mM NaOH at a flow rate of 1 mL min⁻¹. On the other hand, to elucidate the acidic sugars composition of all samples, a gradient of 160 mM NaOH (solvent A) and 600 mM ammonium acetate in 160 mM NaOH (solvent B) were applied at a flow rate of 1 mL min⁻¹. The gradient contained four steps (expressed in percent B in A) : 0 % during 10 minutes ; 0-100 % from 10 to 40 minutes ; 100 % from 40 to 45 minutes ; 100-0 % from 45 to 50 minutes.

2.5. Rheological measurements

Flow measurements were performed using a viscometer NDJ-1 on spring torque LV with 4 speeds (rpm) transmissions. All the rheological studies were conducted at 25 °C using cylindrical spindles (#61 LV, #64 LV) and disc spindles (#62 LV, #63 LV). The quantity of the samples was 600 ml for containers with internal diameter of 83 mm. Polysaccharides solutions 0.2 % to 5 % (w/v) were prepared in distilled water stirred for 2 hours at room temperature. The shear flow behavior was assessed over shear rates of 1 to 13 s⁻¹. The critical concentration recovery (C*) indicates the limit between dilute to semi-dilute regime measured in water at 25 °C. It was determined using the log-log plot of the specific viscosity (η_{sp} : specific viscosity measured at zero shear) vs the concentration of polysaccharide ($\log \eta_{sp} = f(\log C)$).

3. Results and discussions

3.1. Extraction of polysaccharide from *Aplysia dactylomela* eggs

The polysaccharide from *Aplysia dactylomela* eggs was extracted with aqueous solvent in neutral and alkaline conditions. The yield of the polysaccharide fraction extracted in neutral condition was 20.9 %, which was higher to 4.9 % and 3 % than that obtained from *Notarchus leachii* freeri egg [15] and from *Bursatella leachii* viscera [11] respectively. Alkaline extraction was tested to increase the yield of polysaccharide from above 22.89 %. However, alterations of monosaccharide composition and substitution were often observed with alkaline extraction [6] and the color of polysaccharide powder was not good enough. The polysaccharide obtained by neutral condition (EPPOAD) was selected for the differents analyzes.

3.2. Composition of polysaccharide

The polysaccharide extracted contain 31.24 % of total sugar, 27.51 % of neutral sugar, 17.92 % of uronic acid and 3.93 % of proteins were found in EPPOAD. Our uronic acid and protein results were higher than 7.97 %, 1.58 % respectively reported for *Notarchus leachii* freeri egg polysaccharide but his total sugar to 85.43 % was seemed to be much higher [15]. We noted the high amount of sulfate at 38,93 %, which was higher than 28 % obtained from *Bursatella leachii* viscera polysaccharide [11]. The retention time of eight standard monosaccharides were determined by HPAEC. The result of HPAEC analysis shows that the native polysaccharide from *Aplysia dactylomela* eggs contains fucose, arabinose, glucosamine, galactose, galacturonic acid and glucuronic acid assigned in g/g of sample 0.022, 0.049, 0.048, 0.146, 0.020, 0.023, respectively. The polysaccharide was composed of galactose (0.146 g/g of sample) as major sugar and the presence of glucosamine confirms that the polysaccharide is appart of glycosaminoglycan family. Comparing with another result, glucose is the dominant monosaccharides in *Notarchus leachii* freeri egg polysaccharide [15]. The principal backbone was constituted of sulfated lactosamine, repeating galactose linkage with glucosamine : keratane sulfate (KS) structure. The literature reports that the keratane sulfate have not an acid residues [21]. The presence of galacturonic acid and glucuronic acid indicates that polysaccharide contain several glycosaminoglycans. The total amount of KS is significantly higher and we suggest a presence of second

glycosaminoglycan probably. Previous work demonstrated that monosaccharide like arabinose, galactose, xylose and mannose was related to the immunomodulatory activity of macrophages [22]. EPPOAD contained those two monosaccharides, which suggested that EPPOAD may have immunological activity.

3.3. Rheological measurements

We have conducted rheological measurements to determine hydrodynamic properties at various concentrations in water and the critical concentration recovery. First, the behavior rheology of the polysaccharide EPPOAD was carried out in solution with polymer concentrations between 3 % and 5 % and shear rate swept from 1 to 13 s⁻¹ (Fig. 1). For all concentrations, the viscosity of the polysaccharide extracted from *Aplysia dactylomela* eggs has rheofluidifying behavior (shear thinning). The rheofluidifying behavior is found in this interval shear rate and is characterized by the independence of the viscosity that decreases under shear strain. The polysaccharide solution had a behavior of shear-thinning fluids attributed to the disorientation and disentanglement of the macromolecular chains with high molecular mass under influence of shear rate. In rheology, shear thinning is the non-newtonian behavior of fluids and it is sometimes considered synonymous for pseudoplastic behaviour [23], [24].

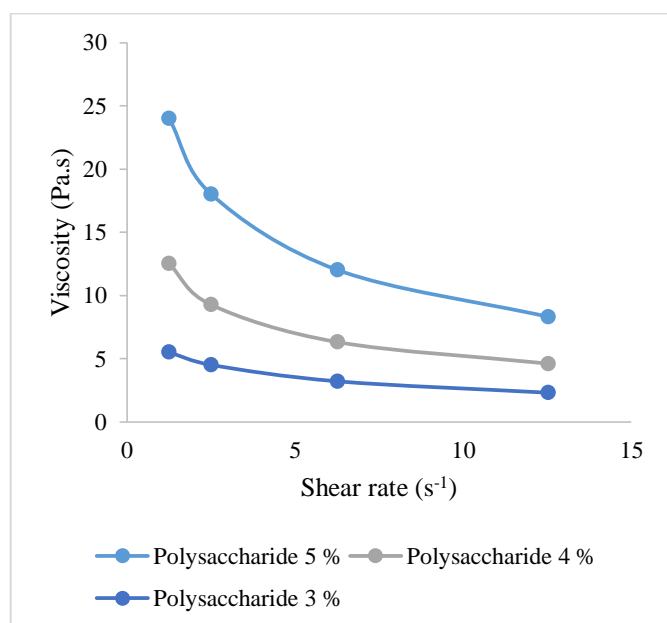


Figure 1 : The steady-shear viscosity of a 3 %, 4 % and 5 % (w/v) EPPOAD solution, at 25 °C.

By using a representation of log η_{sp} versus log C [25], the obtained straight lines present intersections which correspond to the transition from the dilute to semi-dilute regime (C^*). The figure 2 shows the beginning of such chain entanglement. The critical concentration recovery of polysaccharide EPPOAD was 17.42 g/L at 25°C. The investigation in this rheologic parameter is not yet reported in literature for glycosaminoglycan family. However, if we compare our results from themselves, this value C^* is smaller than that of polysaccharide extracted from *Cereus triangularis cladodes* for which C^* was 22 g/L [26]. This result could suggest that the polysaccharide has a high molecular mass, as mentioned in literature, because of the

higher molar mass of the polysaccharides and the lower critical concentration C^* recovery [27].

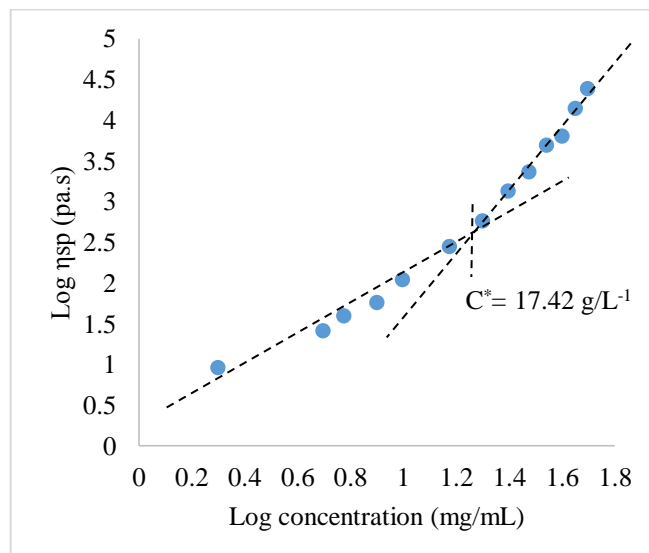


Figure 2: Recovery of the critical concentration C^* of EPPOAD

4. Conclusion

In this study, we extracted the polysaccharides from *Aplysia dactylomela* eggs, collected in the marine coasts of Madagascar (Indian Ocean), for preliminary structural determination and physico-chemical characterization. Several analytical approaches include elemental analysis such as colorimetric assays and HPAEC. Those analyses have shown that the extract (EPPOAD) has a similar chemical structure to that of GAGs. From results, we also know that this polysaccharide has uronic acids in its composition and is highly sulphated. Fucose, arabinose, glucosamine, galactose, glucuronic acid and galacturonic acid were also detected. Galactose was the predominant sugar, suggesting that branched units of glucosamine and galactose were assigned to the main backbone of the polysaccharide structure. The result showed that the EPPOAD extract has a rheofluidifying (shear-thinning) behavior and a recovery of the critical concentration C^* at 17.42 g/L in ambient temperature. Next studies will focus on structural characterization using FTIR and NMR analysis for the determination of characteristic groups and glycosidic linkages. A search for biological properties will act as an antioxidant or anti-coagulation test because the polysaccharides present interesting elements in these compositions.

REFERENCES

- [1]. N. Karaki, C. Sebaaly, N. Chahine, T. Faour, A. Zinchenko, S. Rachid and H. Kanaan, « The antioxidant and anticoagulant activities of polysaccharides isolated from the brown algae *Dictyopteris polypodioides* growing on the Lebanese coast ». Journal of Applied Pharmaceutical Science, vol. 3, no. 02, pp 043-051, 2013.
- [2]. W. Yang, D. Chen, Z. He, L. Zhou, Y. Cai, H. Mao, N. Gao, Z. Zuo, R. Yin, J. Zhao, « NMR characterization and anticoagulant activity of the oligosaccharides from the fucosylated

- glycosaminoglycan isolated from *Holothuria coluber* ». *Carbohydrate Polymers*. Vol. 233, 115844, 2020.
- [3]. Y. Sun, Z. Liu, S. Song, B. Zhu, L. Zhao, J. Jiang, N. Liu, J. Wang, X. Chen, « Anti-inflammatory activity and structural identification of a sulfated polysaccharide CLGP4 from *Caulerpa lentillifera* ». *International Journal Biological Macromoles*. Vol. 146, pp 931-938, 2020.
- [4]. Y. Yu, Q. Song, L. Huang, M. Shen, Q. Yu, Y. Chen, J. Xie, « Immunomodulatory activities of sulfated *Cyclocarya paliurus* polysaccharides with different degrees of substitution on mouse spleen lymphocytes. *Journal Functional Foods* ». Vol. 64, 103706, 2020.
- [5]. R.O. Souza, S.J.C. Madeira, F.D.S. Chagas, L.A. Parreiras, G.R.C. Santos, A.M.S. Assreuy, A.O. Mour and M.G. Pereira., « Purified polysaccharides of *Geoffroe aspinosa* barks have anticoagulant and antithrombotic activities devoid of hemorrhagic risks ». *Carbohydrate Polymers*. Vol. 124, pp 208-21, 2015.
- [6]. T.A. Fenoradosoa, C. Delattre, C. Laroche, A. Wadouachi, V. Dulong, L. Picon, P. Andriamadio, P. Michaud, « Highly sulphated galactan from *Halymenia durvillei* (Halymeniales, Rhodophyta), ared seaweed of Madagascar matine coasts ». *International journal of biological macromolecules*. Vol. 45, pp 140-145, 2009.
- [7]. M.H. Rakotoarisoa, T.A. Fenoradosoa, F. Abdoul, G. Pierre, J.H. Andriamadio, C. Delattre, P. Michaud and L. Clément, « Structural characterization and evaluation of biological activity of polysaccharides extracted from *Haloptilon subulatum* ». *American Journal innovative research & applied sciences*. Vol 13, no 1, pp 304-312, 2021.
- [8]. A.V. Nogueira, G.R. Rossi, M. Iacomini, G.L. Sasaki, E.S. Trindade, T.R. Cipriani, « Viscera of fishes as raw material for extraction of glycosaminoglycans of pharmacological interest ». *International Journal Biological Macromoles*. Vol. 121, pp 239-248, 2019.
- [9]. L. Chahed, R. Balti, S. Elhiss, N. Bouchemal, N. Ajzenberg, V. Ollivier, F. Chaubet, R.M. Maaroufi, M.B. Mansour, « Anticoagulant activity of fucosylated chondroitin sulfate isolated from *Cucumaria syracusana*. *Process Biochemistry* ». Vol. 91, pp 149-157, 2020.
- [10]. J.H. Ryu, J. Sung, C. Xie, M.-K. Shin, C.-W. Kim, N.-G. Kim, Y.J. Choi, B.D. Choi, S.S. Kang, D. Kang, « *Aplysia kurodai*-derived glycosaminoglycans increase the phagocytic ability of macrophages via the activation of AMP-activated, protein kinase and, cytoskeletal reorganization in RAW264.7 cells ». *Journal of Functional Foods*. Vol. 27, pp 122-130, 2016.
- [11]. M. Dhahri, S. Sioud, R. Dridi, M. Hassine, N.A. Boughattas, F. Almulhim, Z. Al Talla, M. Jaremko, A.M. Emwas, « Extraction, characterization, and anticoagulant activity of a sulfated polysaccharide from *Bursatella leachii* Viscera ». *ACS Omega*, Vol. 5, no 24, pp 14786-14795, 2020.
- [12]. H. Matthews-Cascon, C.A. Rocha-Barreira, C.A.O. Meirelles, « Egg masses of some Brazilian mollusk ». *Fortaleza : Expressão Gráfica e Editora*. 119 p, 2011.
- [13]. D.M. Pereira, P. Valentao, N. Teixeira, P.B. Andrade, « Amino acids, fatty acids and sterols profile of some marine organisms from Portuguese waters ». *Food Chemistry*. Vol. 141, no 3, pp 2412-2417, 2013.
- [14]. T. Braga, M.J. Rodrigues, H. Pereira, J. Varela, L. Barreira, M. González-Wangüemert, L. Custódio, « *Bursatella leachi* from Mar Menor as a source of bioactive molecules : preliminary evaluation of the nutritional profile, in vitro biological activities, and fatty acids contents ». *Journal of Aquatic Food Product Technology*. Vol. 26, no 10, pp 1337-1350, 2017.
- [15]. Q. Pan, Y. Sun, X. Li, B. Zeng, D. Chen, « Extraction, structural characterization, and antioxydant and immunomodulatory activities of a polyasaccharide from *Notarchus leachii* freeri eggs ». *Bioorganic Chemistry*. Vol. 116, 105275, 2021.
- [16]. M. Dubois, K.A. Gilles, J.K. Hamilton, P.A. Rebers, F. Smith, « Colorimetric method for determination of sugars and related substances ». *Analytical Chemistry*. Vol. 28, no 3, pp 350-356, 1956.
- [17]. M. Monsigny, C. Petit, and A.-C Roche, « Colorimetric determination of neutral sugars by a resorcinol sulfuric acid micromethod ». *Analytical Biochemistry*, Vol. 175, no 2. pp 525-530, 1988.
- [18]. N. Blumenkrantz and G. Asboe-Hansen, « New method for quantitative determination of uronic acids ». *Analytical Biochemistry*. Vol. 54, no 2, pp 484-489, 1973.
- [19]. K.S. Dodgson and R.G. Price, « A note on the determination of the ester sulphate content of the sulphated polysaccharide ». *Biochemical journal*. Vol. 84, pp 106-110, 1962.
- [20]. O.H. Lowry, N.J. Rosenbrough, A.L. Farr, R.J. Randall, « Protein measurement with the Folin phenol reagent. *Jounal biology chemistry* ». Vol. 193, pp 265-275, 1951.

- [21]. J.L. Funderburgh, « Keratan sulfate : structure, biosynthesis, and function ». *Glycobiology*. Vol. 10, no 10, pp. 951-958, 2000.
- [22]. T.C.T. Lo, Y.H. Jiang, A.L.J. Chao, C.A. Chang, « Use of statistical methods to find the polysaccharide structural characteristics and the relationships between monosaccharide composition ratio and macrophage stimulatory activity of regionally different strains of *Lentinula edodes* ». *Analytica Chimica Acta*. Vol. 584, no 1, pp 50-56, 2007.
- [23]. T.G. Mezger, « The rheology handbook : for users of rotational and oscillatory rheometers (2., rev. ed.) ». Hannover :Vincentz Network, 34 p, 2006.
- [24]. R.P. Heldman and D.R. Singh, « Introduction to food engineering (5thed.) ». Amsterdam : Elsevier, 160 p, 2013
- [25]. L. Utracki, and R. Shima, « Corresponding state relations for the viscosity of moderately concentrated polymer solutions ». *Journal of Polymer Science*. Vol. 1, pp 1089-1098, 1963
- [26]. B. Petera, C. Delattre, G. Pierre, C. Vial, P. Michaud, T. A. Fenoradosoa, « Rheological Study And Prebiotic Potential Of *Cereus Triangularis Cladodes* Extract ». *International Journal of Advanced Research and Publications*. Vol. 4, no 3, pp 12-22, 2020.
- [27]. R.J. Redgwell, C. Schmitt, M. Beaulieu, D. Curti, « Hydrocolloids from coffe : physicochemical and functional properties of an arabinogalactan-protein fraction from green beans ». *Food Hydrocolloids*. Vo. 19, pp 1005-1015, 2005.



FENORADOSOA Taratra Andrée
Doctor HDR - Option leader Biochimie Alimentaire et Valorisation des Ressources Naturelles (BAVRN) at the faculty of sciences Antsirana, Madagascar. Laboratory director of GreenMadag naturally



DELATTRE Cédric
Associate Professor - University Clermont Auvergne & Institut Universitaire de France (IUF), Institut Pascal. International expert in polysaccharide field, biobased and bio-inspired material.



MICHAUD Philippe
Professor, University Clermont Auvergne, Institut Pascal, Clermont Ferrand, France. Research in biochemistry and biological engineering.

AUTHORS PROFIL



JAO Manarivo Ulrich Anito
2nd year doctoral at the faculty of sciences Antsirana, Madagascar.



KALL Briant
Professor, University Antsirana – Madagascar
University President of Antsirana