



JAGIELLONIAN UNIVERSITY  
IN KRAKÓW

Kraków, 25<sup>th</sup> May 2026

FACULTY OF BIOCHEMISTRY, BIOPHYSICS AND BIOTECHNOLOGY

Department of Plant Physiology and Biochemistry

Dariusz Latowski, PhD, DSc

e-mail: [dariusz.latowski@uj.edu.pl](mailto:dariusz.latowski@uj.edu.pl)

Gronostajowa 7, 30-387 Kraków, Poland

### Review of the doctoral dissertation

Author (Candidate): Ms. Marta Saldat, M.Sc.,

Title: *“Biotechnological potential of diacylglycerol acyltransferases from *Nannochloropsis oceanica* for boosting lipid production in diverse hosts”*

Supervisor: dr hab. Krzysztof Zienkiewicz, prof. NCU

Assistant supervisor (co-supervisor): prof. Zhi-Yan (Rock) Du

Reviewer: dr hab. Dariusz Latowski, prof. UJ

Podstawa prawna: Uchwała nr 12 Rady Dyscypliny Nauki Biologiczne Uniwersytetu Mikołaja Kopernika w Toruniu z dnia 20 marca 2026 roku w sprawie wyznaczenia recenzenta w postępowaniu o nadanie stopnia doktora mgr Marcie Saldat

Legal basis: Resolution No. 12 of the Council of the Discipline of Biological Sciences at Nicolaus Copernicus University in Toruń of March 20, 2026, appointing the reviewer in the doctoral proceedings for Ms. Marta Saldat, M.Sc.

**1. Subject of the Dissertation** (*Does the subject of the doctoral dissertation address issues that are significant for the development of the discipline of biological sciences?*)

The subject of the dissertation—functional and biotechnological characterization of DGAT-type enzymes (DGTTs) from *Nannochloropsis oceanica*—is highly justified and scientifically well-founded in the context of current biological research.

From a fundamental perspective, triacylglycerols (TAGs) represent the principal storage lipids in eukaryotic organisms and play essential roles in energy storage, stress adaptation, and cellular homeostasis across plants and microalgae. The final and rate-limiting step of TAG biosynthesis is catalyzed by diacylglycerol acyltransferases (DGATs), which are therefore considered key regulators of lipid accumulation and fatty acid composition.

At the same time, contemporary literature clearly indicates that microalgae are among the most promising biological systems for sustainable lipid production, due to their rapid growth,

high photosynthetic efficiency, and ability to accumulate large quantities of lipids (often exceeding 50–70% of biomass under stress conditions).

Importantly, TAGs synthesized by microalgae constitute major precursors for biofuels and industrial bioproducts, positioning them at the center of modern research in renewable energy and circular bioeconomy.

Within this context, *Nannochloropsis oceanica* has emerged as a model oleaginous microalga of exceptional relevance, due to its high TAG productivity, well-annotated genome, and availability of genetic engineering tools.

Crucially, genomic analyses have revealed an unusually large expansion of DGAT/DGTT gene families in this organism, yet the functional roles of many isoforms remain poorly understood.

This gap has been repeatedly emphasized in the literature: despite the recognized importance of DGAT enzymes, their functional diversity, specialization, and contribution to metabolic regulation in microalgae are still insufficiently resolved, especially at the level of individual isoforms.

Therefore, the decision to focus specifically on:

- DGTT diversification,
- previously unexplored isoforms,
- and their cross-system functionality

addresses a clearly defined and acknowledged research gap of high importance.

From an applied perspective, this topic is equally justified. The development of efficient strategies for enhancing TAG accumulation is currently one of the central challenges in metabolic engineering of microalgae.

Understanding enzyme-level control of carbon flux toward lipid storage—particularly through DGATs—has been identified as a key bottleneck in improving lipid yield and economic feasibility of algal biofuels.

In summary, the selected topic is:

- fully aligned with current global challenges (renewable energy, carbon neutrality),
- strongly grounded in contemporary literature, and
- highly relevant for both basic and applied biological sciences.

Thus, the choice of the dissertation topic must be assessed as fully justified, timely, and scientifically significant, contributing directly to both the advancement of biological knowledge and the development of future biotechnological applications.

**2. Candidate's Knowledge** (*Assessment, with justification, of whether the doctoral dissertation demonstrates the general theoretical knowledge of the candidate applying for the Ph.D. degree in the discipline of biological sciences.*)

The dissertation clearly demonstrates that the candidate possesses broad and well-integrated theoretical knowledge in biological sciences, particularly in:

- lipid metabolism across kingdoms,
- enzymology of acyltransferases,
- algal physiology and stress biology,
- molecular genetics and metabolic engineering.

The introductory chapters provide a comprehensive and up-to-date overview of TAG metabolism in plants and microalgae, including regulatory, physiological, and evolutionary aspects. The candidate critically discusses the expansion and diversification of DGTT gene families and identifies knowledge gaps that justify the undertaken research.

Moreover, the candidate shows strong command of:

- advanced molecular biology techniques,
- bioinformatic analysis of protein structure and localization,
- biochemical characterization of enzymes,
- cross-species experimental design.

The ability to integrate knowledge from multiple biological systems (yeast, algae, plants) reflects a high level of conceptual understanding and interdisciplinary competence. Overall, the thesis convincingly demonstrates doctoral-level expertise in biological sciences.

**3. Independence of the Candidate** (*Assessment, with justification, of whether the doctoral dissertation demonstrates the candidate's ability to conduct independent scientific research.*)

The dissertation provides strong evidence of the candidate's ability to independently conduct scientific research.

This is demonstrated by:

- the formulation of a clearly defined and original research problem,
- development of a coherent multi-system experimental strategy,
- application of diverse and advanced methodologies,
- rigorous data analysis and interpretation.

The study required:

- independent cloning and engineering of multiple gene constructs,

- design and execution of heterologous expression systems,
- integration of in silico, biochemical, and physiological approaches.

Importantly, the candidate addressed a previously unexplored subset of DGTT genes (non-nitrogen-responsive isoforms), which required independent hypothesis formulation and experimental validation.

The cross-kingdom validation (yeast → algae → plant) further reflects a high degree of scientific maturity, planning, and autonomy.

**4. Originality of the Dissertation** (*Assessment, with justification, of whether the doctoral dissertation constitutes an original solution to a scientific problem, or an original contribution regarding the application of the candidate's own research results in the economic or social sphere.*)

The dissertation constitutes an original and significant contribution to biological sciences.

The main elements of originality include:

- First comprehensive functional analysis of non-nitrogen-responsive DGTT isoforms in *N. oceanica*.
- Identification of NoDGTT12 as a highly efficient TAG synthase, surpassing previously studied isoforms.
- Demonstration that NoDGTT12 activity is an intrinsic and conserved enzymatic property, maintained across different biological systems.
- Integration of multi-host validation (yeast, microalga, Arabidopsis), which significantly strengthens conclusions.
- Establishment of a functional hierarchy within an expanded enzyme family, addressing a major knowledge gap.

Furthermore, the work provides applied originality, identifying NoDGTT12 as a promising tool for:

- metabolic engineering,
- sustainable lipid production,
- biofuel and industrial biotechnology applications.

The study bridges fundamental biology and practical application, which significantly enhances its scientific value.

## 5. Points Requiring Clarification

### 5.1. Limited insight into regulatory networks controlling DGTT activity

Although the dissertation convincingly demonstrates functional differences among DGTT isoforms, it only marginally addresses the regulatory mechanisms governing their activity, such as transcriptional control, signaling pathways, and post-translational modifications.

This represents a limitation because contemporary research indicates that TAG biosynthesis in microalgae is a multi-level process controlled not only by enzyme abundance but also by complex regulatory networks, including stress signaling, transcription factors, and metabolic flux adjustments. Thus, enzyme activity alone does not fully explain the physiological contribution of DGTTs *in vivo*.

#### Discussion question:

*How do you envisage the integration of NoDGTT12 activity into the broader regulatory network of TAG biosynthesis in microalgae, and what experimental approaches would you propose to validate its regulation at transcriptional and post-translational levels?*

### 5.2. Dependence on heterologous expression systems

A substantial part of the functional characterization relies on heterologous systems (*Saccharomyces cerevisiae*, *Arabidopsis thaliana*). While methodologically justified, this approach has inherent limitations.

It is well established that heterologous hosts may not faithfully reproduce native metabolic contexts, particularly in terms of:

- acyl-CoA substrate pools,
- membrane architecture,
- protein folding and localization,
- interaction with endogenous partners.

Moreover, DGAT enzymes often exhibit substrate specificity tuned to organism-specific lipid metabolism, meaning that activity observed in yeast may not directly reflect their physiological function in microalgae.

#### Discussion question:

*To what extent do you consider the activity of NoDGTT12 observed in yeast and Arabidopsis to reflect its native function in Nannochloropsis, and how would you experimentally disentangle intrinsic enzymatic properties from host-specific metabolic effects?*

### 5.3. Incomplete resolution of functional redundancy within DGTT family

The thesis identifies a functional hierarchy among selected isoforms; however, the broader issue of genetic redundancy and compensatory interactions within the expanded DGTT family is not fully resolved.

This is important because microalgae typically possess multiple DGAT paralogs that function within distributed metabolic networks, often showing partial redundancy or context-dependent specialization.

Such redundancy can mask phenotypic effects and complicate interpretation of single-gene studies.

**Discussion question:**

*How would you design experiments (e.g., multi-gene knockouts or CRISPR approaches) to determine whether NoDGTT12 functions redundantly or uniquely within the DGTT network in *N. oceanica*?*

**5.4. Limited analysis of carbon flux and metabolic trade-offs**

While increased TAG accumulation is convincingly demonstrated, the work provides relatively limited insight into global carbon partitioning and metabolic trade-offs, such as:

- effects on growth,
- membrane lipid remodeling,
- energy balance.

This is a significant aspect because TAG accumulation in microalgae is tightly linked to redistribution of carbon flux from growth-related pathways to storage lipids, often involving trade-offs that determine overall productivity.

Without such analysis, it is difficult to fully assess the biotechnological efficiency of the proposed engineering approach.

**Discussion question:**

*Did overexpression of NoDGTT12 affect carbon allocation, growth rate, or other metabolic pathways, and how would you evaluate potential trade-offs between lipid accumulation and cellular fitness in an industrial context?*

**5.5. Limited structural/mechanistic explanation of superior enzyme performance**

Although the thesis identifies NoDGTT12 as the most efficient enzyme, the molecular basis of its superior catalytic properties is only partially addressed.

This is a limitation because recent research emphasizes that DGAT/DGTT activity is determined by:

- precise structural features,
- membrane topology,
- substrate-binding domains,
- protein–protein interactions.

A deeper structural analysis (e.g., mutagenesis, modeling) would strengthen mechanistic conclusions.

### **Discussion question:**

*Which specific structural features of NoDGTT12 do you consider most critical for its high activity, and how would you experimentally validate their functional importance (e.g., by site-directed mutagenesis)?*

The identified limitations do not diminish the **high scientific value of the dissertation**; rather, they indicate natural extensions of a complex and ambitious research topic. Importantly, each limitation corresponds to active research frontiers in microalgal lipid metabolism, confirming that the thesis operates at the cutting edge of the discipline.

### **6. Final evaluation of the doctoral dissertation**

In light of the detailed analysis of the dissertation, it can be concluded that the work presented by Marta Saldat fully meets the formal and substantive requirements for a doctoral thesis in the discipline of biological sciences.

The dissertation is logically structured, methodologically coherent, and scientifically rigorous. It demonstrates a clear link between the theoretical background, identified research gap, applied methodology, and obtained results. The candidate has successfully addressed an important and current scientific problem related to lipid metabolism and metabolic engineering of microalgae, contributing both to fundamental biological knowledge and to the development of applied biotechnology.

The study is characterized by:

- a well-justified and timely research topic, aligned with global scientific and technological challenges (renewable energy, bioeconomy),
- advanced methodological design, integrating *in silico*, molecular, biochemical, and physiological approaches,
- a high level of scientific independence,
- and convincing experimental validation across multiple biological systems.

Particularly noteworthy is the conceptual coherence of the work, from hypothesis formulation through experimental realization to interpretation of results.

### **7. Assessment of whether the dissertation is outstanding**

In my opinion, this dissertation should be considered a distinguished (outstanding) doctoral work.

### **Justification**

The thesis stands out above the average doctoral dissertation due to several key features:

### **7.1. Addressing a clearly defined and globally relevant research gap**

The work tackles a significant unresolved issue—functional specialization of DGTT enzymes in microalgae—identified in contemporary literature. The focus on non-nitrogen-responsive isoforms represents a novel and insightful extension beyond the current state of knowledge.

### **7.2. High degree of originality and scientific contribution**

The identification of NoDGTT12 as a highly efficient, cross-kingdom TAG synthase constitutes a substantial and original scientific finding. Moreover, the establishment of a functional hierarchy within a large enzyme family significantly advances our understanding of lipid metabolism in microalgae.

### **7.3. Exceptional experimental design (multi-system validation)**

A particularly strong element is the integration of three biological systems (yeast, microalga, plant), allowing:

- verification of intrinsic enzymatic activity,
- validation across different metabolic contexts,
- demonstration of evolutionary conservation.

Such a comprehensive approach is rare at the doctoral level and reflects advanced scientific maturity.

### **7.4. High biotechnological relevance**

The work goes beyond descriptive biology and provides a clear pathway toward application in:

- metabolic engineering,
- sustainable lipid production,
- biofuel and industrial biotechnology.

This dual (basic + applied) value significantly enhances the impact of the study.

### **7.5. Scientific maturity and methodological competence**

The candidate demonstrates:

- the ability to design complex experimental workflows,
- critical analysis of limitations,
- and awareness of broader biological context.

This level of integration and interpretation is characteristic of researchers beyond the early doctoral stage.

## **8. Final statement**

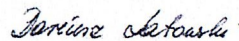
Taking all aspects into consideration, I conclude that:

The doctoral dissertation of Marta Saldat represents a highly valuable, original, and methodologically advanced contribution to biological sciences, and it deserves to be classified as an outstanding doctoral thesis.

Its scientific merit, interdisciplinary scope, and strong applicability place it clearly above the standard level expected for a PhD degree, justifying recognition as a distinguished achievement.

Ja, niżej podpisany stwierdzam, że recenzowana rozprawa doktorska Pani magister **Marty Saldat** spełnia warunki określone w art. 187 Ustawy z dnia 20 lipca 2018 r. Prawo o szkolnictwie wyższym i nauce (Dz. U. z 2018 r. poz. 1668 z późn. zm.) i wnioskuję do Rady Dyscypliny Nauki Biologiczne Uniwersytetu Mikołaja Kopernika w Toruniu o dopuszczenie Pani magister **Marty Saldat** do dalszych etapów postępowania ws. nadania stopnia doktora w dziedzinie nauk ścisłych i przyrodniczych w dyscyplinie nauki biologiczne.

Jak wskazałem powyżej, wnioskuję też o wyróżnienie rozprawy doktorskiej Pani mgr Marty Saldat.



Podpisany elektronicznie przez  
Dariusz Latowski  
25.05.2026  
10:16:39 +02'00'