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Selezione per titoli e colloquio ai sensi dell'art. 8 del "Disciplinare concernente le assunzioni di personale con contratto di lavoro a tempo determinato", per l'assunzione, ai sensi dell'art. 141 del CCNL del Comparto "Istruzione e Ricerca" 2019-2021, sottoscritto in data 18 gennaio 2024, di una unità di personale con profilo professionale di Ricercatore III livello, presso l'Istituto di Biologia e Biotecnologia Agraria del CNR sede di Milano. Programma di ricerca "GoodByO - Multi-commodities microbial-driven BiOrefinery based on food-processing industry wastes, biogenic CO2 and bioprocess wastewaters", finanziato da Unione Europea - Circular Bio-based Europe Joint Undertaking (CBE JU) - codice CUP B53C24005620006

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SERIE DOMANDE N. 1 – BUSTA ESTRATTA

- Il/La candidato/a illustri come il proprio background possa non solo supportare, ma anche potenziare lo sviluppo futuro del progetto GoodByO, indicando eventuali ambiti di innovazione o miglioramento a cui potrebbe contribuire.
- Il/La candidato/a discuta cosa si intende per bioraffineria microalgale e quali sono i principali prodotti e co-prodotti che possono essere ottenuti in un approccio a cascata.

Conoscenza dell'informatica di base

- Cosa si intende per *phishing* e come ci si può difendere da questo tipo di attacco?

Conoscenza della lingua inglese

Improving high carbon dioxide tolerance and carbon dioxide fixation capability of *Chlorella* sp. by adaptive laboratory evolution

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A B S T R A C T

CO_2 capture by microalgae is a promising method to reduce greenhouse gas emissions. It is critical to construct a highly efficient way to obtain a microalgal strain tolerant to high CO_2 concentrations with high CO_2 fixation capability. In this study, two evolved *Chlorella* sp. strains, AE10 and AE20 were obtained after 31 cycles of adaptive laboratory evolution (ALE) under 10% and 20% CO_2, respectively. Both of them grew rapidly in 30% CO_2 and the maximal biomass concentration of AE10 was 3.68 ± 0.08 g/L, which was 1.22 and 2.94 times to those of AE20 and original strain, respectively. The chlorophyll contents of AE10 and AE20 were significantly higher than those of the original one under 1–30% CO_2. The influences of ALE process on biochemical compositions of *Chlorella* cells were also investigated. This study proved that ALE was an effective approach to improve high CO_2 tolerance of *Chlorella* sp.

SERIE DOMANDE N. 2 – BUSTA ESTRATTA

- Il/La candidato/a descriva la propria esperienza accademica e scientifica in relazione alla tematica oggetto del bando, specificando in che modo tali competenze possano contribuire allo sviluppo e agli obiettivi del progetto GoodByO.
- Il/La candidato/a spieghi che cosa si intende per coltivazione mixotrofica delle microalghe e quali sono i principali vantaggi rispetto alla coltivazione fotoautotrofica. Descriva un caso pratico o di studio di una applicazione della modalità di coltivazione mixotrofica nell'ottica dell'economia circolare.

Conoscenza dell'informatica di base

- Come si crea un grafico o una tabella in un foglio di calcolo, ad esempio Excel, assicurandone la chiarezza e la leggibilità?

Conoscenza della lingua inglese

Engineering a marine microalga *Chlorella* sp. as the cell factory

Abstract

The use of marine microalgae in industrial systems is attractive for converting CO_2 into value-added products using saline water and sunlight. The plant nature and demonstrated industrial potential facilitate *Chlorella* spp. as excellent model organisms for both basic research and commercial application. However, the transformation method has not been developed in marine *Chlorella* spp., thus genetic engineering is hindered in exploiting the industrial potentialities of these strains. In this study, we provided a transformation protocol for the marine *Chlorella* strain MEM25, which showed robust characteristics, including high production of proteins and polyunsaturated fatty acids in multiple cultivation systems over various spatial–temporal scales. We showed that transformants could be obtained in a dramatically time-saving manner (comparable to *Saccharomyces cerevisiae*) with four functional proteins expressed properly. The transgenes are integrated into the genome and can be successfully inherited for more than two years. The development of a marine *Chlorella* transformation method, in combination with the complete genome, will greatly facilitate more comprehensive mechanism studies and provide possibilities to use this species as chassis for synthetic biology to produce value-added compounds with mutual advantage in neutralization of CO_2 in commercial scales.

Keywords Marine microalgae, *Chlorella*, Transformation, Cell factory

SERIE DOMANDE N. 3 – BUSTA ESTRATTA

- Il/La candidato/a analizzi criticamente la propria esperienza formativa e professionale in rapporto alla tematica del bando, evidenziando punti di forza, competenze distintive e possibili contributi specifici al progetto GoodByO.
- Il/La candidato/a illustri come si può orientare il metabolismo microalgale per incrementare la produzione di una o più molecole target?

Conoscenza dell'informatica di base

- Descriva cosa si intende per *backup* dei file e perché è importante.

Conoscenza della lingua inglese

Adaptive Laboratory Evolution of Microalgae: A Review of the Regulation of Growth, Stress Resistance, Metabolic Processes, and Biodegradation of Pollutants

Adaptive laboratory evolution (ALE) experiments are a serviceable method for the industrial utilization of the microalgae, which can improve the phenotype, performance, and stability of microalgae to obtain strains containing beneficial mutations. In this article, we reviewed the research into the microalgae ALE test and assessed the improvement of microalgae growth, tolerance, metabolism, and substrate utilization by ALE. In addition, the principles of ALE and the key factors of experimental design, as well as the issues and drawbacks of the microalgae ALE method were discussed. In general, improving the efficiency of ALE and verifying the stability of ALE resulting strains are the primary problems that need to be solved in future research, making it a promising method for the application of microalgae biotechnology.

Keywords: adaptive laboratory evolution, microalgae, growth, metabolic regulation, biodegradation of pollutants, stress resistance

SERIE DOMANDE N. 4 – BUSTA ESTRATTA

- Il/La candidato/a illustri il proprio percorso formativo e professionale, evidenziando in che modo esso risulti pertinente alla tematica del bando e quale valore aggiunto possa apportare al progetto GoodByO.
- Il/La candidato/a discuta quali sono i principali vantaggi e le criticità dell'utilizzo di microalghe rispetto ad altri organismi (batteri, lieviti, piante) nei processi di biotecnologia ambientale o industriale.

Conoscenza dell'informatica di base

- Come si possono verificare la sicurezza e l'affidabilità di un sito web o di un *link* ricevuto via e-mail?

Conoscenza della lingua inglese

Chlorella zofingiensis as an Alternative Microalgal Producer of Astaxanthin: Biology and Industrial Potential

Abstract: Astaxanthin (3,3′-dihydroxy-β,β-carotene-4,4′-dione), a high-value ketocarotenoid with a broad range of applications in food, feed, nutraceutical, and pharmaceutical industries, has been gaining great attention from science and the public in recent years. The green microalgae *Haematococcus pluvialis* and *Chlorella zofingiensis* represent the most promising producers of natural astaxanthin. Although *H. pluvialis* possesses the highest intracellular astaxanthin content and is now believed to be a good producer of astaxanthin, it has intrinsic shortcomings such as slow growth rate, low biomass yield, and a high light requirement. In contrast, *C. zofingiensis* grows fast phototrophically, heterotrophically and mixtrophically, is easy to be cultured and scaled up both indoors and outdoors, and can achieve ultrahigh cell densities. These robust biotechnological traits provide *C. zofingiensis* with high potential to be a better organism than *H. pluvialis* for mass astaxanthin production.

SERIE DOMANDE N. 5 – BUSTA NON ESTRATTA

- Il/La candidato/a descriva in che modo il proprio background risulta coerente con la tematica del bando e quale impatto concreto può avere sul raggiungimento degli obiettivi principali del progetto GoodByO.
- Il/La candidato/a illustri quali sono i principali limiti attuali nella scala industriale dei processi biotecnologici basati su colture microalgali e quali strategie possono essere adottate per superarli?

Conoscenza dell'informatica di base

- Spieghi come si può condividere un file di grandi dimensioni con un collega in modo sicuro ed efficiente.

Conoscenza della lingua inglese

Review

Carotenoid Production from Microalgae: Biosynthesis, Salinity Responses and Novel Biotechnologies

Abstract: Microalgae are excellent biological factories for high-value products and contain biofunctional carotenoids. Carotenoids are a group of natural pigments with high value in social production and human health. They have been widely used in food additives, pharmaceutics and cosmetics. Astaxanthin, β-carotene and lutein are currently the three carotenoids with the largest market share. Meanwhile, other less studied pigments, such as fucoxanthin and zeaxanthin, also exist in microalgae and have great biofunctional potentials. Since carotenoid accumulation is related to environments and cultivation of microalgae in seawater is a difficult biotechnological problem, the contributions of salt stress on carotenoid accumulation in microalgae need to be revealed for large-scale production. This review comprehensively summarizes the carotenoid biosynthesis and salinity responses of microalgae. Applications of salt stress to induce carotenoid accumulation, potentials of the Internet of Things in microalgae cultivation and future aspects for seawater cultivation are also discussed. As the global market share of carotenoids is still ascending, large-scale, economical and intelligent biotechnologies for carotenoid production play vital roles in the future microalgal economy.

Keywords: carotenoids; microalgae; salt stress; seawater cultivation; Internet of Things

SERIE DOMANDE N. 6 – BUSTA ESTRATTA

- Il/La candidato/a racconti il proprio percorso formativo e professionale e spieghi come le esperienze maturate finora possano integrarsi con la tematica del bando e supportare le attività previste dal progetto GoodByO.
- Il/La candidato/candidata descriva un esempio pratico o un caso di studio riguardante l'implementazione di una bioraffineria microbica in un sistema di economia circolare.

Conoscenza dell'informatica di base

- Spieghi brevemente cosa sono i *malware* e come possono essere prevenuti o rimossi.

Conoscenza della lingua inglese

Article

Coelastrella terrestris for Adonixanthin Production: Physiological Characterization and Evaluation of Secondary Carotenoid Productivity

Abstract: A novel strain of *Coelastrella terrestris* (Chlorophyta) was collected from red mucilage in a glacier foreland in Iceland. Its morphology showed characteristic single, ellipsoidal cells with apical wart-like wall thickenings. Physiological characterization revealed the presence of the rare keto-carotenoid adonixanthin, as well as high levels of unsaturated fatty acids of up to 85%. Initial screening experiments with different carbon sources for accelerated mixotrophic biomass growth were done. Consequently, a scale up to 1.25 L stirred photobioreactor cultivations yielded a maximum of 1.96 $mg \cdot L^{-1}$ adonixanthin in free and esterified forms. It could be shown that supplementing acetate to the medium increased the volumetric productivity after entering the nitrogen limitation phase compared to autotrophic control cultures. This study describes a promising way of biotechnological adonixanthin production using *Coelastrella terrestris*.

Keywords: microalgae; stirred photobioreactor; nutrient starvation; osmotic stress; unsaturated fatty acids; astaxanthin; canthaxanthin

SERIE DOMANDE N. 7 – BUSTA ESTRATTA

- Il/La candidato/a esponga in che modo il proprio background formativo, professionale e/o di ricerca rappresenti un valore aggiunto per il progetto GoodByO e contribuisca al raggiungimento dei suoi obiettivi.
- Il/La candidato/candidata descriva quali strumenti di ingegneria genetica sono ad oggi disponibili per le microalghe, e quali sono le principali sfide tecniche e regolatorie legate al loro impiego.

Conoscenza dell'informatica di base

- Quali strumenti o *software* si possono utilizzare abitualmente per la gestione delle citazioni bibliografiche?

Conoscenza della lingua inglese

Review

Microalgae-Derived Pigments for the Food Industry

Abstract: In the food industry, manufacturers and customers have paid more attention to natural pigments instead of the synthetic counterparts for their excellent coloring ability and healthy properties. Microalgae are proven as one of the major photosynthesizers of naturally derived commercial pigments, gaining higher value in the global food pigment market. Microalgae-derived pigments, especially chlorophylls, carotenoids and phycobiliproteins, have unique colors and molecular structures, respectively, and show different physiological activities and health effects in the human body. This review provides recent updates on characteristics, application fields, stability in production and extraction processes of chlorophylls, carotenoids and phycobiliproteins to standardize and analyze their commercial production from microalgae. Potential food commodities for the pigment as eco-friendly colorants, nutraceuticals, and antioxidants are summarized for the target products. Then, recent cultivation strategies, metabolic and genomic designs are presented for high pigment productivity. Technical bottlenecks of downstream processing are discussed for improved stability and bioaccessibility during production. The production strategies of microalgal pigments have been exploited to varying degrees, with some already being applied at scale while others remain at the laboratory level. Finally, some factors affecting their global market value and future prospects are proposed. The microalgae-derived pigments have great potential in the food industry due to their high nutritional value and competitive production cost.

Keywords: microalgae; carotenoids; phycobiliproteins; food colorant; stability

SERIE DOMANDE N. 8 – BUSTA NON ESTRATTA

- Il/La candidato/a descriva le esperienze precedenti, sia accademiche che professionali, che ritiene più significative rispetto alla tematica del bando e spieghi in che modo queste possano contribuire alla realizzazione del progetto GoodByO.
- Il/La candidato/a descriva sinteticamente quali principali tecniche di ingegneria genetica e genomica vengono oggi applicate per ottimizzare le prestazioni metaboliche di microrganismi, microalghe o piante a uso biotecnologico.

Conoscenza dell'informatica di base

- Quali sono le principali estensioni di file che incontra più frequentemente nel suo lavoro e a cosa corrispondono?

Conoscenza della lingua inglese

Article

Combination of Exhaust Gas Fermentation Effluent and Dairy Wastewater for Microalgae Production: Effect on Growth and FAME Composition of *Chlorella sorokiniana*

Abstract: Microalgae cultivation in wastewater is a promising strategy for reducing nutrient loads and generating biomass that can be further exploited. Although microalgae grown under such conditions are not suitable for high-value applications, the resulting biomass can still be valuable for uses such as biofuels, biofertilizers, or animal feed. In this study, *Chlorella sorokiniana* was cultivated in dairy wastewater and, to the best of our knowledge, for the first time in a spent effluent from gas fermentation, to assess its potential as a sustainable growth medium. Growth kinetics and biomass productivity were evaluated at different dilution ratios, and it was found that high concentrations of ammonium and hexanol in undiluted effluents were inhibitory, while an optimized 50:50 dilution led to the highest biomass accumulation (1.96 g L^{-1}) and productivity (0.5 g L^{-1} d^{-1}) of *C. sorokiniana*. This strategy significantly reduced the nitrogen (100%), phosphate (100%), sulfate (68%), and carbon (61%) contents, demonstrating effective bioremediation activity. Furthermore, the fatty acid profile revealed an increased polyunsaturated fatty acid fraction, enhancing the potential of *C. sorokiniana* biomass as a feed supplement. Overall, contributing to the circular bioeconomy, this approach is scalable and cost-effective, reducing freshwater and chemical dependency in microalgae biomass production.

SERIE DOMANDE N. 9 – BUSTA NON ESTRATTA

- Il/La candidato/a illustri come il proprio percorso formativo e professionale, anche in prospettiva interdisciplinare, possa integrarsi efficacemente con la tematica del bando e favorire la collaborazione nel progetto GoodByO.
- Il/La candidato/a descriva quali sono i composti ad elevato valore aggiunto ottenibili da microalghe e quali condizioni di coltivazione ne favoriscono la sintesi?

Conoscenza dell'informatica di base

- In che cosa consiste la differenza tra un file compresso e un file originale? Quando è utile comprimere o decomprimere file?

Conoscenza della lingua inglese

Turning a green alga red: engineering astaxanthin biosynthesis by intragenic pseudogene revival in *Chlamydomonas reinhardtii*

Summary

The green alga *Chlamydomonas reinhardtii* does not synthesize high-value ketocarotenoids like canthaxanthin and astaxanthin; however, a β-carotene ketolase (*Cr*BKT) can be found in its genome. *Cr*BKT is poorly expressed, contains a long C-terminal extension not found in homologues and likely represents a pseudogene in this alga. Here, we used synthetic redesign of this gene to enable its constitutive overexpression from the nuclear genome of *C. reinhardtii*. Overexpression of the optimized *Cr*BKT extended native carotenoid biosynthesis to generate ketocarotenoids in the algal host causing noticeable changes the green algal colour to reddish-brown. We found that up to 50% of native carotenoids could be converted into astaxanthin and more than 70% into other ketocarotenoids by robust *Cr*BKT overexpression. Modification of the carotenoid metabolism did not impair growth or biomass productivity of *C. reinhardtii*, even at high light intensities. Under different growth conditions, the best performing *Cr*BKT overexpression strain was found to reach ketocarotenoid productivities up to 4.3 mg/L/day. Astaxanthin productivity in engineered *C. reinhardtii* shown here might be competitive with that reported for *Haematococcus lacustris* (formerly *pluvialis*) which is currently the main organism cultivated for industrial astaxanthin production. In addition, the extractability and bio-accessibility of these pigments were much higher in cell wall-deficient *C. reinhardtii* than the resting cysts of *H. lacustris*. Engineered *C. reinhardtii* strains could thus be a promising alternative to natural astaxanthin producing algal strains and may open the possibility of other tailor-made pigments from this host.

IL PRESIDENTE

Signature Not Verified
Firmato digitalmente da Cinzia Comino
Data: 17.11.2025 13:06:14 CET

IL SEGRETARIO

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