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Abbreviations

BA – Blood Agar

CIESM - Mediterranean Science Commission

CL - Cardiolipin

CP – Casein Peptone

CycloBFA – Cyclopropyl Branched Fatty Acids

FAs – Fatty Acids

FAMEs – Fatty Acids Methyl Esters

FDA – Food and Drugs Administration

FTIR - Fourier transform infrared spectroscopy

GC-MS – Gas Chromatography – Mass Spectrometry

Gly – Glycerol

HSFA – Hydroxisubstituted Fatty Acids

K_d – cellular death, (h^{-1})

k_La – Oxygen transfer Coefficient

LB – Luria-Bertani Broth

MA – Marine Agar

MB – Marine Broth

MBFA – Methyl Branched Fatty Acids

MH – Mueller Hinton Broth

MM – Mineral medium

MiM – Minimal medium

MP – Meat Peptone

MTBE – Methyl *Tert*-Butyl Ether

MTP – Microtiter Plates

MUFA – Mono Unsaturated Fatty Acids

μ_{max} – Maximum cellular growth, (h^{-1})

NMR – Nuclear Magnetic Resonance

NO_2^- - Nitrites

OD – Optical Density

OECD - Organization for Economic Cooperation and Development

PE - Phosphatidylethanolamine

PG - Phosphatidylglycerol

PS - Polystyrene

PUFA – Polyunsaturated Fatty Acids

rRNA – Ribosomal ribonucleic acid

RNA – Ribonucleic Acid

SBFA – Saturated Branched Fatty Acids

SSFA – Saturated Sheath Fatty Acids

SP – Soy Peptone

TB – Terrific Broth

TMAO - Trimethylamine *N*-oxide

Tryp - Tryptone

TSA – Triptic Soy agar

TSB – Tryptic Soy Broth

UV-VIS – Ultraviolet - Visible

X_0 - Biomass at t=0, (g/L)

X - Biomass at t=n, (g/L)

YE – Yeast Extract

Y_{PX} – Product per biomass Yield

Vvm – Volume flow of air per volume of liquid in minutes

ZP – Zeta Potential

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1. Abstract

Marine biodiscovery is a promising field for novel compounds and materials. For the application of blue biotechnology, the development of new strategies is paramount to shorten the pipeline between enzyme and metabolite discovery and application, i.e. to increase of efficiency of the bioprocess to obtain industrial relevant yields. By understanding the mechanisms and stress conditions in the marine environment that favoured the production of a given compound by marine bacteria, high production levels of commercially interesting compounds should be achieved. The goal of this thesis was to develop a bioprocess, at laboratory scale, using marine bacteria for the production of prodiginine compound(s), which have applications in different fields including the pharmaceutical and food industries. The selected strain and product(s) were characterized using, respectively, i) 16S rRNA and lipid profile, and ii) UV-VIS, NMR, FTIR, and GC-MS techniques. Through an intense screening process in shaken flasks, using culturomics and lipidomics approaches, the growth conditions leading to the highest yields were assessed. Several parameters were evaluated, including e.g. nature of the carbon and nitrogen sources, presence of metals ions, light exposure, temperature, pH, agitation, and oxygen concentration. Additionally, the effect of specific stresses such as nutrient depletion and the growth of the cells in biofilms were also evaluated. Once the best medium composition and cultivation conditions were determined, the bioprocess was scaled up to 2 L bioreactors for further improvement of the bioprocess. The maintenance of the oxygen transfer coefficient (k_La) was used as scale-up criterion, and Büch's, Doran's, Michels and Miller's Equations were firstly employed for the determination of k_La. The effect of stirring conditions, medium composition and k_La on biomass and prodiginine production was further assessed at this scale. It was found that supplementation of marine broth (MB) with appropriate nitrogen sources and metal ions increased to 200 mg/(L.h) both biomass and prodiginine productivities.

Keywords: Bioprocess, Bioprospecting, Fermentation, Marine bacteria, Prodiginine compounds, Scale-up, Screening

2. Resumo

A descoberta de novos compostos e materiais de origem marinha é um promissor campo de investigação. Para que possa haver a aplicação da biotecnologia azul a nível industrial torna-se fundamental o desenvolvimento de novas estratégias para diminuir o tempo entre a descoberta e a aplicação destes novos compostos, e para aumentar a eficiência do processo para rendimentos suficientemente altos para aplicação industrial. A compreensão dos mecanismos, e das condições de stress a que as bactérias marinhas estão sujeitas e que as levam a produzir um determinado composto, permite que este composto possa ser produzido em larga escala. O objectivo desta tese foi o desenvolvimento de um bioprocesso à escala laboratorial usando bactérias marinhas para a produção de prodigininas. Estes produtos têm várias aplicações em diferentes sectores industriais, tais como a indústria farmacêutica e a alimentar. A estirpe seleccionada e o produto por ela produzido foram caracterizados por i) 16S rRNA e pelo seu perfil lipídico, e por ii) UV-VIS, NMR, FTIR, e GC-MS, respectivamente. Através de um intenso processo de análise em balão Erlenmeyer, e usando técnicas de culturomica e lipidomica, as condições de crescimento que levam a elevados rendimentos de biomassa e de produto foram determinados. Os parâmetros avaliados foram vários, e incluíram o tipo de fontes de carbono e azoto, a presença de iões metálicos, presença de luz, temperatura, pH, agitação e concentração de oxigénio no meio. Condições específicas de stress, como baixas concentrações de nutrientes, e o crescimento celular em biofilme foram avaliadas. Após a selecção da composição do meio e das melhores condições de crescimento, realizou-se o aumento de escala do bioprocesso para fermentadores de 2 L. O coeficiente de transferência de oxigénio (k_La) foi escolhido como critério de aumento de escala, e os modelos de Büch's, Doran's, Michels e Miller foram aplicados na sua determinação. O efeito da agitação, composição do meio e do k_La na concentração de biomassa e produto foi estudado a esta escala. Determinou-se que através do enriquecimento de meio marinho (MB) com fontes de azoto e de iões metálicos apropriados, a produtividade em biomassa e prodigininas aumentou para aproximadamente 200 mg/(L.h).

Palavras chave: Aumento de escala, Bactérias marinhas, Bioprocesso, Bioprospecção, Fermentação, Prodigininas, Selecção

Note: The remaining sections of the Thesis will not be made public during the Confidentiality Period.