BIOPROSPECTING MICROALGAE AS POTENTIAL SOURCES OF "GREEN ENERGY "-CHALLENGES AND PERSPECTIVES (REVIEW)

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Microalgae and cyanobacteria are potential foods, feeds, sources of high-value bioactive molecules and biofuels, and find tremendous applications in bioremediation and agriculture. Although few efforts have been undertaken to index the microalgal germplasm available in terms of lipid content, information on suitability of strains for mass multiplication and advances in development of methods for extraction and generating biofuel are scarce. Our review summarizes the potential of microalgae, latest developments in the field and analyzes the "pitfalls" in oversimplification of their promise in the years to come. Microalgae represent "green gold mines" for generating energy; however, the path to success is long and winding and needs tremendous and concerted efforts from science and industry, besides political will and social acceptance for overcoming the limitations. The major advantages of second generation biofuels based on microalgal systems, include their higher photon conversion efficiency, growth all around the year, even in wastewaters, and production of environment friendly biodegradable biofuels.

MICROALGAE BIOFUEL POTENTIALS (REVIEW)

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With the decrease of fossil based fuels and the environmental impact of them over the planet, it seems necessary to seek the sustainable sources of clean energy. Biofuels, is becoming a worldwide leader in the development of renewable energy resources. It is worthwhile to say that algal biofuel production is thought to help stabilize the concentration of carbon dioxide in the atmosphere and decrease global warming impacts. Also, among algal fuels' attractive characteristics, algal biodiesel is non toxic, with no sulfur, highly biodegradable and relatively harmless to the environment if spilled. Algae are capable of producing in excess of 30 times more oil per acre than corn and soybean crops. Currently, algal biofuel production has not been commercialized due to high costs associated with production, harvesting and oil extraction but the technology is progressing. Extensive research was conducted to determine the utilization of microalgae as an energy source and make algae oil production commercially viable.

ФЕРМЕНТАТИВНЫЙ СИНТЕЗ ЭЛЕКТРОПРОВОДЯЩИХ БИОКОМПОЗИТОВ НА ОСНОВЕ ДНК И ОПТИЧЕСКИ АКТИВНОГО ПОЛИАНИЛИНА

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Методом окислительной полимеризации анилина с использованием двух различных биокатализаторов: пероксидазы из корней хрена и биомиметика - микропероксидазы-11 синтезированы электропроводящие интерполимерные комплексы полианилина на матрице ДНК. Исследованы спектральные характеристики и морфология полученных биокомпозитов. Показано различие стереоспецифичности получаемых образцов интерполимерных комплексов в зависимости от используемого биокатализатора. Полученные результаты свидетельствуют о важной роли биокатализатора в формировании направления закручивания спирали электропроводящего полимера на матрице ДНК, т.е. оптическая активность получаемых образцов полимеров, по-видимому, связана со свойствами биокатализатора.

ENZYMATIC MODIFICATION OF CHITOSAN WITH QUERCETIN AND ITS APPLICATION AS ANTIOXIDANT EDIBLE FILMS

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Quercetin, rutin, naringin, hesperidin and chrysin were tested as substrates for cloroperoxidase to produce reactive quinones to graft onto chitosan. Quercetin and rutin quinones were successfully chemically attached to low molecular weight chitosan. The quercetin-modified chitosan showed an enhancement of plastic, antioxidant and antimicrobial properties as well as of thermal degradability. Finally, chitosan-quercetin films visibly decreased enzymatic oxidation when applied to *Opuntiaficus indica* cladodes.

ENZYMATIC SYNTHESIS OF L-TRYPTOPHAN FROM D,L-2-AMINO-Δ2-THIAZOLINE-4-CARBOXYLIC ACID AND INDOLE BY *Pseudomonas* sp. TS1138 L-2-AMINO-Δ2-THIAZOLINE-4-CARBOXYLIC ACID HYDROLASE, S-CARBAMYL-L-CYSTEINE AMIDOHYDROLASE, AND *Escherichia coli* L-TRYPTOPHANASE

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L-Tryptophan (L-Trp) is an essential amino acid. It is widely used in medical, health and food products, so a low-cost supply is needed. There are 4 methods for L-Trp production: chemical synthesis, extraction, enzymatic synthesis, and fermentation. In this study, we produced a recombinant bacterial strain pET-tnaA of *Escherichia coli* which has the L-tryptophanase gene. Using the pET-tnaA *E. coli* and the strain TS1138 of *Pseudomonas* sp., a one-pot enzymatic synthesis of L-Trp was developed. *Pseudomonas* sp. TS1138 was added to a solution of D,L-2-amino-Δ2-thiazoline-4-carboxylic acid (DL-ATC) to convert it to L-cysteine (L-Cys). After concentration, *E. coli* BL21 (DE 3) cells including plasmid pET-tnaA, indole, and pyridoxal 5'-phosphate were added. At the optimum conditions, the conversion rates of DL-ATC and L-Cys were 95.4% and 92.1 %, respectively. After purifying using macroporous resin S8 and NKA-11, 10.32 g of L-Trp of 98.3% purity was obtained. This study established methods for one-pot enzymatic synthesis and separation of L-Trp. This method of producing L-Trp is more environmentally sound than methods using chemical synthesis, and it lays the foundations for industrial production of L-Trp from DL-ATC and indole.

PROLINE DEHYDROGENASE FROM *Pseudomonas fluorescence:* GENE CLONING, PURIFICATION, CHARACTERIZATION AND HOMOLOGY MODELING

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The gene encoding proline dehydrogenase (ProDH) from *Pseudomonas fluorescence* was isolated using PCR amplification and cloned into pET23a expression vector. The expression of the recombinant target enzyme was induced by addition of IPTG. The produced His-fusion enzyme was purified and its kinetic properties were studied. The 3D structure modeling was also performed to identify key amino acids involved in FAD-binding and catalysis. The PCR product contained a 1033 bp open reading frame encoding 345 amino acid residue polypeptide chain. SDS-PAGE analysis revealed a MWof 40 kDa, whereas the native enzyme exhibited a MW of 40 kDa suggesting a monomeric protein. The K_m and K_{max} values of the *P. fluorescence* ProDH were estimated to be 35 mM and 116 μmol/min, respectively. ProDH activity was stable at alkaline pH and the highest activity was observed at 30°C and pH 8.5. The modeling analysis of the three dimensional structure elucidated that Lys-173 and Asp-202, which were oriented near

the hydroxyl group of the substrate, were essential residues for the proDH activity. This study, to our knowledge, is the first data on the cloning and biochemical and structural properties of *P. fluorescence* ProDH.

ISOLATION AND CHARACTERIZATION OF FEATHER DEGRADING ENZYMES FROM *Bacillus megaterium* SN1 ISOLATED FROM GHAZIPUR POULTRY WASTE SITE

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The SN1 strain of *Bacillus megaterium,* isolated from soil of Ghazipur poultry waste site (India) produced extracellular caseinolytic and keratinolytic enzymes in basal media at 30°C, 160 rpm in the presence of 10% feather. Feathers were completely degraded after 72 h of incubation. The caseinolytic enzyme was separated from the basal media following ammonium sulphate precipitation and ion exchange chromatography. We report 29.3-fold purification of protease after Q Sepharose chromatography. The molecular weight of this enzyme was estimated to be 30 kDa as shown by SDS-PAGE and zymography studies. Protease activity increased by 2-fold in presence of 10 mM Mn^{2+} whereas Ba^{2+} and Hg^{2+} inhibited it. Ratio of milk clotting activity to caseinolytic was found to be 520.8 activity for the 30—60% ammonium sulphate fraction in presence of Mn^{2+} ion suggesting potential application in dairy industry. Keratinase was purified to 655.64 fold with specific activity of 544.7 U/mg protein and 12.4% recovery. We adopted the strategy of isolating the keratinolytic and caseinolytic producing microorganism by its selective growing in enriched media and found that feather protein can be metabolized for production of animal feed protein concentrates.

EFFECT OF PARTIAL PRESSURE OF CO_2 ON THE PRODUCTION OF THERMOSTABLE α-AMYLASE AND NEUTRAL PROTEASE BY *Bacillus caldolyticus*

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Controlling the concentration of dissolved oxygen is a standard feature in aerobic fermentation processes but the measurement of dissolved CO_2 concentrations is often neglected in spite of its influence on the cellular metabolism. In this work room air and room air supplemented with 5% and 10% carbon dioxide were used for aeration during the cultivation of the thermophilic microorganism *Bacillus caldolyticus* (DSM 405) on starch to produce α-amylase (E.C. 3.2.1.1)

and neutral protease (E.C. 3.4.24.27/28). The increased CO_2 concentrations resulted in a 22% raise in activity of secreted α-amylase and a 43% raise in protease activity when compared with aeration with un-supplemented room air. There was no effect on the final biomass concentration. Furthermore, the lag-phase of fermentation was reduced by 30%, further increasing the productivity of α-amylase production. Determinations of dissolved CO_2 in the culture broth were conducted both in situ with a probe as well as using exhaust gas analysis and both the methods of quantification showed good qualitative congruence.

RHAMNOLIPID PRODUCTION BY *Pseudomonas aeruginosa* ENGINEERED WITH THE *Vitreoscilla* HEMOGLOBIN GENE

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The potential of *Pseudomonas aeruginosa* expressing the *Vitreoscilla* hemoglobin gene *(vgb)* for rhamnolipid production was studied. *P. aeruginosa* (NRRL B-771) and its transposon mediated *vgb* transferred recombinant strain, PaJC, were used in the research. The optimization of rhamnolipid production was carried out in the different conditions of cultivation (agitation rate, the composition of culture medium and temperature) in a time-course manner. The nutrient source, especially the carbon type, had a dramatic effect on rhamnolipid production. The PaJC strain and the wild type cells of *P. aeruginosa* started producing biosurfactant at the stationary phase and its concentration reached maximum at 24 h (838 mg l^{-1}) and at 72 h (751 mg l^{-1}) of the incubation respectively. Rhamnolipid production was optimal in batch cultures when the temperature and agitation rate were controlled at 30°C and 100 rpm. It reached 8373 mg l^{-1} when the PaJC cells were grown in 1.0% glucose supplemented minimal media. Genetic engineering of biosurfactant producing strains with *vgb* may be an effective method to increase its production.

ПРОЛОНГИРОВАННОЕ КУЛЬТИВИРОВАНИЕ АНАЭРОБНОГО СООБЩЕСТВА БАКТЕРИЙ, ПРОДУЦИРУЮЩЕГО ВОДОРОД

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Исследованы различные способы длительного поддержания процесса выделения водорода при выращивании анаэробного сообщества бактерий на крахмалсодержащей среде. При культивировании в режиме отъемно-доливной ферментации в течение 72 сут образовывалось от 0.10 до 0.23 л H_2/л среды/сут. Режим регулярных пересевов продолжался более 100 сут с образованием в среднем 0.81 л H_2/л среды/сут. Выявлены достоинства и недостатки различных способов микробиологического получения водорода

в темновом процессе сбраживания крахмала. Из сформированного H_2-образующего сообщества микроорганизмов выделена анаэробная спорообразующая бактерия, штамм BF. Филогенетический анализ последовательности гена 16S рРНК нового штамма показал, что по своим генотипическим свойствам он относится к виду *Clostridium butyricum.*

ВЛИЯНИЕ ЭКЗОГЕННЫХ ЖИРНЫХ КИСЛОТ НА РОСТИ ПРОДУКЦИЮ ЭКЗОПОЛИСАХАРИДА ОБЛИГАТНОЙ МЕТИЛОТРОФНОЙ БАКТЕРИИ *Methylophilus quaylei*

© 2012 С. А. М. Отман, А. Б. Пшеничникова, В. И. Швец

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Обнаружен эффект ускорения роста и увеличения продукции экзополисахарида облигатной метилотрофной бактерии *Methylophilus quaylei* в присутствии жирных кислот C_{12}—C_{18}, добавленных в питательные среды. Наилучшим ростовым фактором оказался олеат натрия. На основании данных о составе фракции свободных жирных кислот в клетках, величиин ζ-потенциала и анизотропии флуоресценции целых клеток высказано предположение о включении жирных кислот в состав наружной мембраны бактерии *M. quaylei.*

ОТНОШЕНИЕ [^{13}C]/[^{12}C] КАК ПОКАЗАТЕЛЬ ДЛЯ ЭКСПРЕСС-ОЦЕНКИ УГЛЕВОДОРОДОКИСЛЯЮЩЕГО ПОТЕНЦИАЛА МИКРОБИОТЫ В ПОЧВЕ, ЗАГРЯЗНЕННОЙ СЫРОЙ НЕФТЬЮ

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Исследован углеводородокисляющий потенциал почвенной микробиоты и интродуцированных в почву углеводородокисляющих микроорганизмов на основе количественных и изотопных характеристик углерода продуктов, образующихся при микробной деградации нефти. Из сравнения скоростей продукции CO_2 в нативной почве и почве, загрязненной сырой нефтью, обнаружено, что интенсивность микробной минерализации почвенного органического вещества **(ПОВ)** в присутствии нефти выше по сравнению с незагрязненной почвой, т.е., обнаруживается затравочное влияние (прайминг-эффект) углеводородов нефти. Показано, что количество углерода вновь синтезированных органических продуктов за счет потребленной нефти (биомасса клеток и экзометаболиты) значительно превосходит количество ПОВ, израсходованное на продукцию CO_2. Обнаружено, что в результате микробиологических процессов в почве, загрязненной нефтью, наблюдается мощный поток углекислоты, поступающей в атмосферу.

CONSTRUCTION OF THE INDUSTRIAL ETHANOL-PRODUCING STRAIN OF *Saccharomyces cerevisiae* ABLE TO FERMENT CELLOBIOSE AND MELIBIOSE

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The gene *mel 1*, encoding α-galactosidase in *Schizosaccharomyces pombe*, and the gene *bgl2*, encoding and β-glucosidase in *Trichoderma reesei*, were isolated and co-expressed in the industrial ethanol-producing strain of *Saccharomyces cerevisiae*. The resulting strains were able to grow on cellobiose and melibiose through simultaneous production of sufficient extracellular α-galactosidase and β-glucosidase activity. Under aerobic conditions, the growth rate of the recombinant strain GC1 co-expressing 2 genes could achieve 0.29 OD_{600} h^{-1} and a biomass yield up to 7.8 g $Г^{1}$ dry cell weight on medium containing 10.0 g 1^{-1} cellobiose and 10.0 g 1^{-1} melibiose as sole carbohydrate source. Meanwhile, the new strain of *S. cerevisiae* CG1 demonstrated the ability to directly produce ethanol from microcrystalline cellulose during simultaneous saccharification and fermentation process. Approximately 36.5 g 1^{-1} ethanol was produced from 100 g of cellulose supplied with 5 g I^{-1} melibose within 60 h. The yield (g of ethanol produced/g of carbohydrate consumed) was 0.44 g/g, which corresponds to 88.0% of the theoretical yield.

РАЗРАБОТКА И ОПТИМИЗАЦИЯ ИММУНОХРОМАТОГРАФИЧЕСКИХ ТЕСТОВ ДЛЯ ВЫЯВЛЕНИЯ БОТУЛИНИЧЕСКИХ ТОКСИНОВ

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Разработаны иммунохроматографические монопараметрические тесты для выявления ботулинических токсинов типов А, В, а также мультипараметрический тест для одновременного выявления ботулинических токсинов типов А и В. Показано, что на чувствительность тестов влияют размеры наночастиц коллоидного золота, использованных в качестве маркеров антител, величины нагрузки антител на наночастицы коллоидного золота в конъюгатах, тип аналитических мембран, а также химический состав буферных растворов для хранения конъюгата и проведения иммунохроматографического анализа. Предел обнаружения монопараметрических иммунохроматографических тестов составляет 0.5 нг/мл, а мультипараметрических — 5.0 нг/мл. Разработанные иммунохроматографические тесты могут быть использованы для экспресс-анализа качества продуктов питания, контроля содержания ботулинических токсинов в фармацевтических препаратах, контроля окружающей среды.