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Biotechnology of Algae: A Bibliography
Biotechnology of Algae: A Bibliography

A selected bibliography of research and product development using genetic engineering and molecular biology techniques and species of fresh water and marine algae.

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# Contents

Preface ................................................................. v

Availability of Cited Documents ...................................... vii

List of Citations

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>General Interest</td>
<td>1</td>
</tr>
<tr>
<td>Culture</td>
<td>3</td>
</tr>
<tr>
<td>Gene Expression and Sequencing Studies</td>
<td>6</td>
</tr>
<tr>
<td>Products and Product Development</td>
<td>29</td>
</tr>
<tr>
<td>Bioremediation Using Algae</td>
<td>47</td>
</tr>
</tbody>
</table>

Author Index .......................................................... 56
Preface

The field of biotechnology continues to expand rapidly with innovative research, new technologies, and the development of beneficial products for animals and humans. The many disciplines using genetic engineering and molecular biology techniques attest to the suggestion that biotechnology is truly a multidisciplinary field. In addition, the species used as investigative tools are as wide ranging and varied as the biological sciences themselves. For example, in agriculture, virtually all of the major plant commodities and animal species such as swine, cattle, sheep, and fish are the subject of biotechnology research, non-photosynthetic microorganisms such as Bacillus thuringiensis, yeasts, and Rhizobium spp. play a critical role in biotechnology and even numerous species of fresh and marine water algae contribute to both basic and applied research and product development in biotechnology.

The use of algae in biotechnology research and in the biotechnology industry is significant. Algae play critical roles as bioreactors for the production of food, chemicals, and fuels. They are becoming extremely important in the development of solar energy technology and in biodegradation and bioremediation programs, and their importance in the ever-expanding domestic and international aquaculture industry cannot be over-emphasized.

Because of the economic importance of this diverse group of organisms, NAL's Biotechnology Information Center, in conjunction with its Aquaculture Information Center, has compiled this bibliography of basic and applied research on algae and biotechnology. The citations listed herein represent research that was selected for its creativity, innovation, and timeliness. The bibliography will be invaluable to researchers, industry representatives, government officials, environmental groups, the interested public, and others interested in algae and biotechnology.

This bibliography has been sub-divided into several sections representing the major efforts in algal biotechnology research. The first section represents literature of a general nature followed by sections on the specific topics of culture, gene expression and sequencing information, products and product development, and finally, bioremediation and biodegradation. An author index follows the bibliographic text.

The citations included in this bibliography were taken from the NAL AGRICOLA database and from BIOSIS Previews. In addition to the title, author, source, and, where available, an abstract, each citation also includes key descriptors and the NAL Call Number if the material is part of the NAL collection.

For directions regarding document delivery of the listed citations, please consult the information sheet entitled "Availability of Cited Documents" in this publication.

ROBERT D. WARM BRODT
COORDINATOR, BIOTECHNOLOGY INFORMATION CENTER
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Biotechnology of Algae: A Bibliography

General Interest

1
*Introduction to Applied Phycology*
Akatsuka, I.
*Descriptors:* book; commercial uses; genetic engineering; toxicity; tissue culture

*Abstract:* This reference work presents the latest research into the commercial use of algae. Techniques and practical applications are described. The topics covered include biotechnology, genetic engineering, tissue culture, pollution and toxicity. The text is supplemented by diagrams, graphs, tables, chemical compounds diagrams, photographs, an author index and a subject index.

2
*Algal and Cyanobacterial Biotechnology*
Cresswell, R.C.; Rees, T.A.V.; Shah, N., editors
*Descriptors:* algae-biotechnology; cyanobacteria-biotechnology
*DNAL Call No.:* TP248.27.A46A44

3
*Seaweed Biotechnology Current Status and Future Prospects*
Evans, L.V. and Butler, D.M.
*Descriptors:* agar; alginic acid; carrageenan; biotechnology; protoplasts; seaweeds; reviews; in vitro culture; seaweed products; enteromorpha; porphyra; gracilaria; fucus

4
*Yeasts Molds and Algae*
Jacobson, G.K. and Jolly, S.O.
*Descriptors:* review; food; bioconversion; baking industry; brewing industry; dairy industry; biomass conversion; wastewater treatment; soil fertilizers; fine chemicals; genetic engineering; biotechnology
*DNAL Call No.:* QR53.B52

5
*The Biotechnology of Microalgae and Cyanobacteria*
Kerby, N.W. and Stewart, W.D.P.
Descriptors: biomass; ammonia; amino acids; pigments; toxins; animal; cell growth; stimulants; lipids; fatty acids; antibiotics; plant growth stimulants; restriction endonucleases
DNAL Call No.: QK898.T4E26

6
Comparative Physiology and Biochemistry of Chlorella Species as the Basis for their Taxonomy and for their Utilization in Research and Biotechnology
Kessler, E.
Descriptors: Chlorella-fusca; Chlorella-vulgaris; Chlorella-sorokiniana; Chlorella-saccharophila; Chlorella-zofingiensis; Chlorella-minutissima; Chlorella-homosphaera; Chlorella-kessleri; Chlorella-luteoviridis; Chlorella-protothecoides

7
Phycotechnology Yesterday Today and Tomorrow Maybe
Lewin, R.A.
Descriptors: abstract; spirulina; dunaliella; protozoa contamination

8
Bioconversion of Seaweeds
Morand, P.; Carpentier, B.; Charlier, R.H.; Maze, J.; Orlandini, M.; Plunkett, B.A.; De Waart, J.
Descriptors: marine algae; seaweed cultivation; fuel; biomass; bioengineering; Europe
DNAL Call No.: SH390.7.S44

9
Permeabilized Cyanobacteria: A Model System for Photosynthetic and Biotechnological Studies
Papageorgiou, G.C.
Descriptors: cyanobacteria; biotechnology; permeability; photosynthesis; ultrastructure; electron microscopy; literature reviews
DNAL Call No.: QH301.N32

10
Seaweeds and Biotechnology Inseparable Companions
Renn, D.W.
Descriptors: polysaccharides; alginate; carrageenan; agar; agarose; separation techniques
DNAL Call No.: 410 H992

11
Recent Advances in Microalgal Biotechnology
Vonshak, A.
Descriptors: spirulina; dunaliella; chlorella haematococcus; algae; biotechnology industry; biomass conversion
DNAL Call No.: TP248.2.B562
Culture

12
*The Biotechnology of Cultivating the Halotolerant Alga Dunaliella*
Ben-Amotz, A.; Avron, M.
Descriptors: dunaliella; biotechnology; algae culture; plant products; salt tolerance
DNAL Call No.: TA166.T72

13
*Effects of Salinity Increase on Carotenoid Accumulation in the Green Alga Dunaliella-salina*
Borowitzka, M.A.; Borowitzka, L.J.; Kessly, D.
Descriptors: food industry; lutein; beta carotene; alpha carotene; shock; osmotic stress; biotechnology
DNAL Call No.: QK564.J68
Abstract:
The effect of sudden salinity increases on the kinetics of growth and carotenogenesis was studied in three geographically diverse isolates of Dunaliella salina. A sudden increase in salinity results in a lag phase in growth and the length of this lag phase is dependent on the final salinity and the magnitude of the salinity change (no lag at 10-15% w/v NaCl, 4-day lag at 30% NaCl). There is also a lag before an increase in the total carotenoid content can be measured following the salinity up-shock, and the length of the lag depends largely on the initial salinity and the magnitude of the salinity up-shock, whereas the rate of carotenogenesis and the final carotenoid content reached depend on the final salinity. The increase in total carotenoid content is mainly due to beta.-carotene. Following the salinity up-shock (especially from 10% to 20% NaCl) the proportion of lutein as a percentage of total carotenoids decreases, whereas zeaxanthin increases. This suggests that the pathway synthesising lutein is more sensitive to salt or osmotic stress and is inhibited at higher salinities thus leading to beta.-carotene formation. The proportion of alpha.-carotene does not change.

14
*On-Line Optimization of Biotechnological Processes I. Application to Open Algal Pond*
Guterman, H. and Ben-Yaakov, S.
Descriptors: algae; biotechnology; production; ponds; mathematical models
DNAL Call No.: 381 J8224
Abstract:
A new on-line optimization and control procedure applicable to biotechnological systems for which a precise mathematical model is unavailable has been developed and tested. The proposed approach is based on an on-line search for optimum conditions by an automatic system using a modified simplex algorithm to which several features have been added to permit real time operation. The simplex algorithm is the upper level of a hierarchical software package in which the other levels are cost evaluation, control, data acquisition, and signal processing. The optimization method was tested in a laboratory minipond for the cultivation of Spirulina platensis. The controlled parameters were light intensity, optical density, pH, and temperature. The proposed optimization method can be applied to other biological processes provided that the pertinent variables can be measured and controlled and the cost function can be defined mathematically.
15
The Mass Culture of Dunaliella-viridis volvocales chlorophyta for Oxygenated Carotenoids Laboratory and Pilot Plant Studies
Moulton, T.P. and Burford, M.A.
Source: HYDROBIOLOGIA 204-205(0):401-408 (1990).
Descriptors: dunaliella-salina; beta carotene; biotechnology
DNAL Call No.: 410 H992

16
Effects of Light Intensity on the Growth Rate of the Red Alga Porphyridium-cruentum
Sada, E.; Katoh, S.; Kheirolomoon, A.; Yokoi, H.
Descriptors: batch fermentation; continuous fermentation; arachidonic acid; yield; biotechnology industry; pharmaceuticals
DNAL Call No.: QP601.A1J6
Abstract:
The red alga, Porphyridium cruentum, which is one of the potential sources of arachidonic acid, was cultured in batch and continuous vessels. The growth rates in batch cultures were correlated to the mean light intensity in the vessels, and the cell concentrations in continuous cultures were estimated by those results. The yield of arachidonic acid was about 1.2 g per 1012 cell at cell concentrations ranging from 0.5 to 1.5 times. 1010 cell/l and independent of the mean light intensity.

17
Macroalgal Strain Selection and Improvement in Japan
Saga, N.
Descriptors: abstract; food; energy source; chemical production; breeding; cultivation; biotechnology

18
Mass Culture of Spirulina-fusiformis and its Nutritional and Toxicological Evaluation
Seshadri, C.V.
Descriptors: abstract; algae; food; protein; vitamin bioavailability; mineral bioavailability; biotechnology
DNAL Call No.: TP248.65.F66P66

19
Growth Chemical Composition of Cyanobacteria Spirulina-maxima in Batch Cultures
Tadros, M.; Tadros, S.; Smith, W.; Mbuthia, P.; Joseph, B.
Source: ABSTR ANNU MEET AM SOC MICROBIOL 90(0):233.
DNAL Call No.: 448.39.S012A

20
Porphyra Cell Cultures Isolation Growth and Polysaccharide Production
Tait, M.I.; Milne, A.M.; Grant, D.; Somers, J.A.; Staples, J.; Long, W.F.; Williamson, F.B.; Wilson, S.B.
Descriptors: bioengineering; nutrients
DNAL Call No.: QK564.J68
Abstract:
A range of cell lines was isolated from Porphyra umbilicalis L. (Rhodophyta) tissue using a variety of methods, the most successful involving exposure to a limpet acetone powder enzyme extract for 24 h, homogenisation and filtration through a series of polyester meshes. All established lines grew as 0.1-5 mm diameter aggregates in liquid culture; most were stable and have been grown in shake-flask or air-lift culture for periods in excess of 1 yr without reverting to the foliose growth form. An investigation of the medium used to grow these lines indicated that it was not nitrogen-deficient and that the sodium chloride concentration was optimal. The addition of an organic buffer increased the final cell yield. None of these cell lines grew heterotrophically in medium supplemented with a range of fixed carbon sources. The infrared spectra of polysaccharides isolated from Porphyra aggregates and from tissue grown under identical conditions indicated that the structures of the two isolates were analogous.

21
Absorption of Carbon Dioxide in Algal Mass Culture Systems a Different Characterization Approach
Talbot, P.; Gortares, M.P.; Lencki, R.W.; De la Noue, J.
Source: BIOTECHNOLOGY AND BIOENGINEERING 37(9):834-842
Descriptors: algae; microorganism; biotechnology industry; mass transfer; kinetics; photosynthesis; bioreactor
DNAL Call No.: 381 J8224
Abstract:
For the characterization of CO2 absorption in aerated microalgal culture systems, a different approach based on KLa(02) determination and transformation was studied. To confirm the validity of this method, the influence of reactions between CO2 and compounds (OH-, H2O, and NH3) present in the culture medium upon the absorption mechanism was evaluated under different physical and chemical culture conditions. Under these conditions, knowledge of the relative magnitudes of the diffusion and reaction kinetics permitted the evaluation of their relative importance. For the determination of the parameters required for the calculation of the CO2 absorption constant, empirical correlation calculations for KLO and a were used that had been previously verified with experimental data for O2 absorption. Since, for the conditions studied, the absorption rate was shown to be independent of the chemical reactions taking place in the liquid phase, the KLa for CO2 could be directly related to the KLa for O2 by a simple factor that took into account the difference in aqueous diffusivity of the two gases. Thus, using methods developed for determining O2 absorption in gas-liquid contactors, it is possible to adequately characterize CO2 absorption for laboratory and pilot scale algal production systems.

22
Effect of Low-Dose Ultrasonic Treatment on Growth Rates and Biomass Yield of Anabaena-flos-aquae and Selenastrum-capricornutum
Thomas, B.J.; McIntosh, D.; Taylor, S.R.; Francko, D.A.; Ownby, J.
Descriptors: chlorophyta; biomass production; growth rate; yields; ultrasonic treatment; cell culture; biotechnology
DNAL Call No.: TP248.24.B55
Abstract:
Major project tasks included assembly of an ultrasonic treatment array and measurement of the cell culture growth rate as a function of ultrasonic frequency, and ultrasonic power level and dosage. Growth rates for Anabaena flos-aquae were increased with both single or multiple ultrasonic dosages and were over and above that obtained with vigorous
mechanical stirring. Selenastrum capricornutum growth rates were decreased by ultrasonic treatment. The results were also shown to be independent of the degree of cell agglomeration. Collectively, the data support the conclusion that low-dose, short duration ultrasonic treatment induces changes in culture growth rates in both algal species examined.

23
Phototropism in Dunaliella and its Application in a Harvesting Device
Toha, J.; Soto, M.A.; Contreras, S.
Descriptors: algae; methods; equipment; biotechnology; large scale culture
DNAL Call No.: TP248.24.B55
Abstract:
The influence of wavelength, light intensity and algal concentration on the phototropism of Dunaliella sp. is described. A practical device for harvesting the alga, based in this effect is shown.

24
Biotechnology Studies on the Breeding of Porphyra-yezoensis ueda
Wang, S.; Zhou, Y.; He, P.
Descriptors: abstract; somatic cells; growth; development; spore yield; mariculture
DNAL Call No.: QK564.J6

Gene Expression and Sequencing Studies

25
Characterization of the IS895 Family of Insertion Sequences from the Cyanobacterium Anabaena sp. strain PCC 7120
Alam, J.; Vrba, J.M.; Cai, Y.; Martin, J.A.; Weislo, L.J.; Curtis, S.E.
Descriptors: anabaena; strains; transposable elements; nucleotide sequences; amino acid sequences
DNAL Call No.: 448.3 J82
Abstract:
A family of repetitive elements from the cyanobacterium Anabaena sp. strain PCC 7120 was identified through the proximity of one element to the psbA1 gene. Four members of this seven-member family were isolated and shown to have structures characteristic of bacterial insertion sequences. Each element is approximately 1,200 bp in length, is delimited by a 30-bp inverted repeat, and contains two open reading frames in tandem on the same DNA strand. The four copies differ from each other by small insertions or deletions, some of which alter the open reading frames. By using a system designed to trap insertion elements, one of the elements, denoted IS895, was shown to be mobile. The target site was not duplicated upon insertion of the element. Two other filamentous cyanobacterial strains were also found to contain sequences homologous to IS895.

26
Evolution of the Rubisco Operon from Prokaryotes to Algae: Structure and Analysis of the rbcS Gene of the Brown Alga Pylaella littoralis
Assali, N.E.; Martin, W.F.; Sommerville, C.C.; Loiseaux-de Goer, S.
Anabaena

Different Fates of the Chloroplast tufA Gene Following its Transfer to the Nucleus in Green Algae
Baldauf, S.L.; Manhart, J.R.; Palmer, J.D.
Descriptors: algae; chloroplasts; dna; evolution; genetic code; genetics
DNAL Call No.: 500 N21P

Characterization of an Insertion Sequence (IS891) of Novel Structure from the Cyanobacterium Anabaena sp. Strain M-131
Bancroft, I. and Wolk, C.P.
Descriptors: anabaena; strains; nucleotide sequence; characterization
DNAL Call No.: 448.3 J82
Abstract:
When recombinant plasmids that here transferred to the cyanobacterium Anabaena sp. strain M-131 were transferred back to Escherichia coli, some of the transformants contained inserts. One of the insertion sequences (ISs) as characterized by sequencing. This 1,351-base-pair IS contained an open reading frame that was capable of encoding a peptide of 310 amino acids and had terminal sequences with distinctive structures, but it lacked terminal inverted repeats and did not duplicate target DNA upon insertion. The element bore no significant sequence homology to any sequence stored in the GenBank data base. Restriction analysis of the genomes of Anabaena sp. strain M-131 and Anabaena sp. strain PCC 7120 showed those strains to be closely related. Sequences homologous to the IS element were also present in the DNA of Anabaena strain PCC 7120, but the copy numbers and chromosomal locations or such sequences differed in the two strains. The largest visualized plasmid was 425 kilobases (kb) in M-131 and 410 kb in PCC 7120; at least the former plasmid contained multiple copies of the element, as did a 115-kb plasmid in M-131.
29

*Studies on Chlamydomonas Chloroplast Transformation: Foreign DNA can be Stably Maintained in the Chromosome*

Blowers, A.D.; Bogorad, L.; Shark, K.B.; Sanford, J.C.


**Descriptors:** chlamydomonas reinhardtii; chloroplast genetics; genetic transformation; homologous recombination; dna; dna hybridization; repetitive dna; restriction mapping; messenger rna; northern blotting; gene expression; chimeras

**DNAL Call No.:** QK725.P532

30

*Transcriptional Analysis of Endogenous and Foreign Genes in Chloroplast Transformants of Chlamydomonas*

Blowers, A.D.; Ellmore, G.S.; Klein, U.; Bogorad, L.


**Descriptors:** chlamydomonas reinhardtii; chloroplast genetics; genetic transformation; chloroplasts; genes; beta-glucuronidase; reporter genes; transcription; promoters; genetic regulation; gene expression; deletions; mutagenesis; nucleotide sequences; homologous recombination

**DNAL Call No.:** QK725.P532

31

*Characterization of Insertion Sequence IS892 and Related Elements from the Cyanobacterium Anabaena sp. Strain PCC 7120*

Cai, Y.


**Descriptors:** anabaena; strains; transposable elements; nucleotide sequences; amino acid sequences

**DNAL Call No.:** 448.3 J82

**Abstract:**

IS892, one of the several insertion sequence (IS) elements discovered in Anabaena sp. strain PCC 7120 (Y. Cai and C.P. Wolk, J. Bacteriol. 172:3138-3145, 1990), is 1,675 bp with 24-bp near-perfect inverted terminal repeats and has two open reading frames (ORFs) that could code for proteins of 233 and 137 amino acids. Upon insertion into target sites, this IS generates an 8-bp directly repeated target duplication. A 32-bp sequence in the region between ORF1 and ORF2 is similar to the sequence of the inverted termini. Similar inverted repeats are found within each of those three segments, and the sequences of these repeats bear some similarity to the 11-bp direct repeats flanking the 11-kb insertion interrupting the nifD gene of this strain (J.W. Golden, S.J. Robinson, and R. Haselkorn, Nature [London] 314:419-423, 1985). A sequence similar to that of a binding site for the Escherichia coli integration host factor is found about 120 bp from the left end of IS892. Partial nucleotide sequences of active IS elements IS892N and IS892T, members of the IS892 family from the same Anabaena strain, were shown to be very similar to the sequence of IS892.

32

*Use of a Conditionally Lethal Gene in Anabaena sp. Strain PCC 7120 to Select for Double Recombinants and to Entrap Insertion Sequences*

Cai, Y. and Wolk, C.P.

**Source:** JOURNAL OF BACTERIOLOGY 172 (6):3138-3145 (1990).

**Descriptors:** anabaena; strains; lethals; recombination

**DNAL Call No.:** 448.3 J82

**Abstract:**

Use of the sacB gene (J.L. Ried and A. Collmer, Gene 57:239-246, 1987) provides a
Herbicide-Resistance Expression

Abstract:

Chungjatupornchai, Campbell, Codon Call Usage plants preferentially versus PLANT intergenic monocot more codons ending mainly encoding and comparison the plants, algae. Anabaena wild-type algae. The conditionally lethal nature of the sacB gene was also used to detect insertion sequences from this Anabaena strain. Sucrose-resistant colonies derived from cells bearing a sacB-containing autonomously replicating plasmid were analyzed. Five different, presumed insertion sequences were found to have inserted into the sacB gene of the plasmids in these colonies. One of them, denoted IS892, was characterized by physical mapping. It is 1.7 kilobases in size and is present in at least five copies in the genome of Anabaena sp. strain PCC 1720.

Codon Usage in Higher Plants, Green Algae, and Cyanobacteria

Campbell, W.H. and Gowri, G.
Descriptors: cyanobacteria; chlorophyceae; plant breeding; protein synthesis; genetic code; codon; genome analysis
DNAL Call No.: 450 P692
Abstract:

Codon usage is the selective and nonrandom use of synonymous codons by an organism to encode the amino acids in the genes for its proteins. During the last few years, a large number of plant genes have been cloned and sequenced, which now permits a meaningful comparison of codon usage in higher plants, algae, and cyanobacteria. For the nuclear and organellar genes of these organisms, a small set of preferred codons are used for encoding proteins. Codon usage is different for each genome type with the variation mainly occurring in choices between codons ending in cytidine (C) or guanosine (G) versus those ending in adenosine (A) or uridine (U). For organellar genomes, chloroplastic and mitochondrial proteins are encoded mainly with codons ending in A or U. In most cyanobacteria and the nuclei of green algae, proteins are encoded preferentially with codons ending in C or G. Although only a few nuclear genes of higher plants have been sequenced, a clear distinction between Magnoliopsida (dicot) and Liliopsida (monocot) codon usage is evident. Dicot genes use a set of 44 preferred codons with a slight preference for codons ending in A or U. Monocot codon usage is more restricted with an average of 38 codons preferred, which are predominantly those ending in C or G. But two classes of genes can be recognized in monocots. One set of monocot genes uses codons similar to those in dicots, while the other genes are highly biased toward codons ending in C or G with a pattern similar to nuclear genes of green algae. Codon usage is discussed in relation to evolution of plants and prospects for intergenic transfer of particular genes.

Expression of the Mosquitocidal-Protein Genes of Bacillus thuringiensis subsp. israelensis and the Herbicide-Resistance Gene Bar in Synechocystis PCC6803

Chungjatupornchai, W.
Descriptors: bacillus thuringiensis subsp. israelensis; cyanobacteria; bacterial proteins; genes; gene transfer; genetic transformation; promoters; marker genes; gene expression; insecticidal action; culicidae; biological control agents; biological control; herbicide resistance; genetic models
DNAL Call No.: QR1.C78

35
Group II Twintron: an Intron within an Intron in a Chloroplast Cytochrome b-559 Gene
Copertino, D.W. and Hallick, R.B.
Descriptors: euglena; chloroplasts; cytochromes; genetic code; photosystem ii; introns; transposable elements; gene mapping
DNAL Call No.: QH506.E46
Abstract:
The psbF gene of chloroplast DNAs encodes the beta-subunit of cytochrome b-559 of the photosystem II reaction center. The psbF locus of Euglena gracilis chloroplast DNA has an unusual 1042 nt group H intron that appears to be formed from the insertion of one group II intron into structural domain V of a second group II intron. Using both direct primer extension cDNA sequencing and cDNA cloning and sequencing, we have determined that a 618 nt internal intron is first excised from the 1042 nt intron of psbF pre-mRNA, resulting in a partially spliced pre-mRNA containing a 424 nt group II intron with a spliced domain V. The 424 nt intron is then removed to yield the mature psbF mRNA. Therefore, the 1042 nt intron of psbF is a group II intron within another group II intron. We use the term 'twintron' to define this new type of genetic element. Intermediates in the splicing pathway were detected by northern hybridization. Splicing of both the internal and external introns occurs via lariat intermediates. Twintron splicing was found to proceed by a sequential pathway, the internal intron being removed prior to the excision of the external intron. A possible mechanism for twintron formation by intron transposition is discussed.

36
Amplified Expression of Ribulose Bisphosphate Carboxylase/Oxygenase inpBR322-Transformants of Anacystis nidulans
Daniell, H.; Torres-Ruiz, J.A.; Inamdar, A.; McFadden, B.A.
Descriptors: anacystis nidulans; enzyme activity; ribulose-bisphosphate carboxylase; plasmids; recombination; chromosomes
DNAL Call No.: 442.8 AR26

37
A Transposon with an Unusual LTR Arrangement from Chlamydomonas reinhardtii Contains an Internal Tandem Array of 76 bp Repeats
Day, A. and Rochaix, J.D.
Descriptors: chlamydomonas reinhardtii; transposable elements; nucleotide sequences
DNAL Call No.: QD341.A2N8
Abstract:
TOC1, a transposable element from Chlamydomonas reinhardtii, is 5662 bases long. The 217 and 237 base long terminal repeat sequences of TOC1 are unusually arranged around the 4600 and 123 base unique regions: [217]-4600-[237][217]-123-[237]. Although TOC1 contains long terminal repeats and most TOC1 elements are complete, features shared
with virus-like retroposons, its unique 4600 base region is more similar to the structure of the L1 family of non-virus retroposons: first, 11 3/4 tandemly repeated copies of a 76 base repeat are found 813 bases from the left end of TOC1, and second using the universal genetic code large open reading frames were not found in TOC1. The relationship between TOC1, virus-like retroposons and the L1 family of non-virus retroposons is unclear and may be very distant since only poor similarity was found between the TOC1 encoded ORFs and retrovirus polypeptides. The length of the tandem array of 76 base repeat sequences was conserved in most TOC1 elements and solo 76 base repeat sequences were not found outside TOC1 elements in the C. reinhardtii genome. Nucleotide substitutions allow all copies of the 76 base repeat to be distinguished from one another.

38  Structure and Inheritance of Sense and Anti-sense Transcripts from a Transposon in the Green Alga Chlamydomonas reinhardtii
Day, A. and Rochaix, J.D.
Descriptors: chlamydomonas reinhardtii; strains; transposable elements; transcription; patterns; antisense rna; inheritance; progeny; strain differences; molecular mapping; genetic analysis
DNAL Call No.: 442.8 J8224
Abstract:
We have studied the transcription pattern of a 5700 base-pair transposon (TOC1) in Chlamydomonas reinhardtii. Northern blotting and nuclease S1 protection experiments define three classes of major TOC1 RNAs that accumulate to different levels in a number of strains and segregate independently in the progeny of crosses: class 1 RNAs are unstable near full-length sense transcripts whose 5' end maps to the left 217 base-pair repeat of TOC1, class 2 and class 3 RNAs are large, discrete chimaeric transcripts containing full-length sense (class 2) and anti-sense (class 3) copies of TOC1. Sequence motifs common to the 5' non-transcribed regions of C. reinhardtii genes were found upstream from the putative initiation site of class 1 transcripts. A functional polyadenylation site was located in the far-right 237 base-pair repeat of TOC1. Class 1 TOC1 transcripts are initiated, and probably terminated, within the terminal repeats of TOC1 and may represent retrotransposition intermediates. Class 2 and 3 TOC1 transcripts co-segregate with specific TOC1 elements identified on Southern blots. The loci that control the production of high levels of class 1 transcripts could correspond to specific TOC1 elements, i.e. only a few TOC1 elements are transcribed, or to a regulatory locus. The accumulation of an 11,500 to 12,000 base sense transcript (class 2) is reduced two- to fourfold by the presence of a 9500 to 9700 base anti-sense transcript (class 3). In contrast, the accumulation of the 5' ends of class 1 transcripts are unaffected by the anti-sense TOC1 transcript.

39  Genetic Analysis of a 9 kDa Phycocyanin-Associated Linker Polypeptide
De Lorimier, R.; Bryant, D.A.; Stevens, S.E. Jr.
Descriptors: synechococcus; strains; gene mapping; genetic analysis; genetic code; molecular genetics; mutations; nucleotide sequences; polypeptides; amino acid sequences; cyanin
DNAL Call No.: 381 B522
Abstract:
The gene encoding LR9, a 9kDa phycocyanin-associated linker polypeptide, was cloned from the cyanobacterium Synechococcus sp. PCC 7002 (Agmenellum quadruplicatum PR-
6). This gene, termed cpcD was located immediately 3' to cpcC, a gene which encodes another phycocyanin-associated linker, LR33. Mutation of cpcD by insertion led to the loss of LR9 as the only detectable change in phycobilisome composition. Cells and isolated phycobilisomes from the cpcD strain did not detectably differ from the wild-type in absorption or steady-state fluorescence emission. Purified phycobilisomes from the wild-type and cpcD strains were compared by electron microscopy. The number of phycocyanin discs in the rod substructures of the mutant was more variable than in the wild-type. Hence, one function of LR9 may be to minimize the heterogeneity of rod length, possibly by binding to the core-distal face of phycocyanin-LR33 complexes to prevent the tandem joining of such units. A mutant in which cpcD and cpcC-cpcD intergenic sequences are deleted shows a partial loss of LR33. Inverted repeats in this intergenic region may be required for optimal stability of the cpcC transcript.

40

Molecular and Biophysical Analysis of Herbicide-Resistant Mutants of Chlamydomonas reinhardtii: Structure-Function Relationship of the Photosystem II D1 Polypeptide

Erickson, J.M.; Pfister, K.; Rahire, M.; Togasaki, R.K.; Mets, L.; Rochaix, J.D.


Descriptors: chlamydomonas reinhardtii; genes; photosystem ii; polypeptides; nucleotide sequences; mutants; herbicide resistance; atrazine; diuron; bromacil; binding site; amino acid sequences; chlorophyll, fluorescence; electron transfer

DNAL Call No.: QK725.P532

41

Characterization of a Chlamydomonas Transposon, Gulliver, Resembling those in Higher Plants

Ferris, P.J.


Descriptors: chlamydomonas reinhardtii; chromosome analysis; linkage maps; molecular genetics; cloning; deletions; chromosome maps; nucleotide sequence

DNAL Call No.: 442.8 G28

Abstract:

While pursuing a chromosomal walk through the mt(+) locus of linkage group VI of Chlamydomonas reinhardtii, I encountered a 12-kb sequence that was found to be present in approximately 12 copies in the nuclear genome. Comparison of various C. reinhardtii laboratory strains provided evidence that the sequence was mobile and therefore a transposon. One of two separate natural isolates interfertile with C. reinhardtii, C. smithii (CC-1373), contained the transposon, but at completely different locations in its nuclear genome than C. reinhardtii; and a second, CC-1952 (sl-C5) lacked the transposon altogether. Genetic analysis indicated that the transposon was found at dispersed sites throughout the genome, but had a conserved structure at each location. Sequence homology between the termini was limited to an imperfect 15-bp inverted repeat. An 8-bp target site duplication was created by insertion; transposon sequences were completely removed upon excision leaving behind both copies of the target site duplication, with minor base changes. The transposon contained an internal region of unique repetitive sequence responsible for restriction fragment length heterogeneity among the various copies of the transposon. In several cases it was possible to identify which of the dozen transposons in a given strain served as the donor when a transposition event occurred. The transposon often moved into a site genetically linked to the donor, and transposition appeared to be nonreplicative. Thus the mechanism of transposition and excision of the transposon, which I have named Gulliver, resembles that of certain higher plant transposons, like the Ac transposon of maize.
The 5'-Flanking Region of the Gene Encoding the Large Subunit of Ribulose-1,5-bisphosphate Carboxylase/oxygenase is Crucial for Growth of the Cyanobacterium Synechococcus sp. Strain PCC 7942 at the Level of CO2 in Air

Friedberg, D.; Kaplan, A.; Ariel, R.; Kessel, M.; Seijffers, J.


Descriptors: synechococcus; strains; growth; carbon dioxide; ribulose-bisphosphate carboxylase; mutants; nucleotide sequence
DNAL Call No.: 448.3 J82

Abstract:

Transformation of the high-CO2-requiring mutants (hcr) 0221 and E1 derived from the cyanobacterium Synechococcus sp. strain PCC 7942 by a wild-type DNA library restored their ability to grow at the level of CO2 in air. A plasmid (pE12) containing a 10-kilobase DNA insert was rescued from a 0221 heterogenote and proved to transform both 0221 and E1 to the wild-type phenotype. The capacity of the pE12 subclones to confer the wild-type phenotype to 0221 transformants enabled the mapping of the mutation in 0221 (designated hcr0221) within a 232-base-pair PstI-BstXI DNA restriction fragment. Sequence analysis revealed two open reading frames (ORFs) at positions -1745 to -1262 (ORFI) and -1218 to -393 (ORFII) upstream of the rbcL gene. A 3-kilobase PstI fragment of 0221 was cloned, and hcr0221 was found to be a point mutation within the PstI-BstXI region -1309 nucleotides upstream of the rbcL gene. The significance of this flanking region for adaptation to air levels of CO2 was further demonstrated by the generation of new hcr mutants following insertional inactivation of wild-type DNA in the BstXI site. Electron microscopy revealed aberrant carboxysome structures in growing cells of the hcr mutants, a defect that was possibly related to the mutation, since transformation with pE12 derivatives restored the carboxysome structure to normal.

Red Algal Plasmids

Goff, L.J. and A.W. Coleman


Descriptors: rhodophyta; plasmids; nucleotide sequences; amino acid sequences; dna; biotechnology
DNAL Call No.: QH426.C8

Transgenic Expression of Aminoglycoside Adenine Transferase in the Chloroplast a Selectable Marker for Site-Directed Transformation of Chlamydomonas

Goldschmidt-Clermont, M.


Descriptors: chlamydomonas reinhardtii; bacterial aada gene transformation; vectors; transcription; translation
DNAL Call No.: AD341.A2N8

Abstract:

Expression vectors for Chlamydomonas reinhardtii chloroplast transformation have been constructed with transcription and translation signals from chloroplast genes. The bacterial aadA sequence coding for aminoglycoside 3′ adenylation transferase was inserted in these vectors and introduced into the C. reinhardtii chloroplast by particle gun transformation. The stable transgenic expression of this foreign protein in the chloroplast confers spectinomycin and streptomycin resistance to the transformed cells. This new marker can be used as a reporter of gene expression, and as a portable selectable cassette for chloroplast reverse genetics. Targeted gene disruption mutants of loci required for
photosynthesis, tscA and psaC, were thus obtained. A gene disruption of an unidentified open reading frame, ORF472, remained heteroplasmic, suggesting that it has a vital function.

45
Trans-splicing Mutants of Chlamydomonas reinhardtii
Goldschmidt-Clermont, M.; Girard-Bascou, J.; Choquet, Y.; Rochaix, J.D.
Descriptors: chlamydomonas reinhardtii; chloroplasts; genomes; plant proteins; photosystem i; genes; introns; exons; loci; messenger rna; alternative splicing; mutants; deletions; restriction mapping; northern blotting; segregation; recombination; complementation
DNA Call No.: 442.8 Z34

46
Gabaculine-Resistant Glutamate 1-Semialdehyde Aminotransferase of Synechococcus. Deletion of a Tripeptide Close to the NH2 Terminus and Internal Amino Acid Substitution.
Grimm, B.; Smith, A.J.; Kannangara, C.G.; Smith, M.
Descriptors: synechococcus; aminotransferases; glutamic acid; aminolevulinic acid; mutants; genes; cloning; nucleotide sequences; aminolevulinic acid; mutants; genes; cloning; nucleotide sequences
DNAL Call No.: 381 J824
Abstract:
Glutamate 1-semialdehyde aminotransferase (GSA-AT) is the last enzyme in the C5 pathway converting glutamate into the tetrapyrrole precursor delta-aminolevulinate in plants, algae, and several bacteria. Sequence analysis of the genes encoding GSA-AT in barley, Synechococcus, and Escherichia coli revealed 50-70% similarity in the primary structures of the proteins. The enzyme is inhibited rapidly by gabaculine when added in approximately stoichiometric amounts with the enzyme. A gabaculine-tolerant Synechococcus strain, GR6, was found to produce a GSA-AT less sensitive to the inhibitor. Accordingly, the mutant gene was isolated and sequenced. In comparison with the wild-type gene it contains a deletion of nine nucleotides (position 12-20) and a guanine to adenine substitution (position 743). This resulted in the loss of the amino acids serine, proline, and phenylalanine (position 5-7) close to the NH2 terminus of the enzyme and an exchange of Met-248 for isoleucine in the middle of the polypeptide chain. Wild-type and mutant GSA-AT were expressed in E.coli and purified close to homogeneity. Although the specific activity of the mutant GSA-AT was only one-fifth of the wild type, it displayed a 100-fold increased resistance to gabaculine. Peaks in the absorption spectrum of the purified recombinant GSA-ATs at 335 and 417 nm are typical of a transaminase containing a B6 cofactor. Incubation with substrate and with inhibitor induced spectral changes characteristic of other gabaculine-sensitive, B6-requiring enzymes.

47
Escherichia-coli and Anacystis-nidulans Plasmid Shuttle Vectors Containing the P-L Promoter from Bacteriophage Lambda
Gruber, M.Y.; Glick, B.R.; Thompson, J.E.
Descriptors: temperature-sensitive; CI857 repressor gene; genetic engineering; temperature regulated; foreign gene expression
DNAL Call No.: QR1.C78
Abstract:
Escherichia coli-Anacystis nidulans shuttle vectors pHIX14, pSMG1, and pANH1,
containing the leftward promoter, PL, of bacteriophage lambda and the gene for the temperature-sensitive repressor, cl857, were constructed and used to transform A. nidulans. The transformation efficiencies and restriction endonuclease maps of these plasmids are reported. The use of these shuttle vectors should allow temperature regulation of foreign gene expression in A. nidulans.

48

Self-splicing of the Chlamydomonas Chloroplast psbA Introns
Herrin, D.L.; Bao, Y.; Thompson, A.J.; Chen, Y.F.
Descriptors: chlamydomonas; introns; alternative splicing; transcription; photosystem ii; plant proteins; genes; chloroplast genetics; nucleotide sequences; chloroplasts; genomes
DNAL Call No.: QK725.P532
Abstract:
We used alpha-(32)P-GTP labeling of total RNA preparations to identify self-splicing group I introns in Chlamydomonas. Several RNAs become labeled with alpha-(32)P-GTP, a subset of which is not seen with RNA from a mutant that lacks both copies of the psbA gene. Hybridization of the GTP-labeled RNAs to chloroplast DNA indicates that they originate from the psbA and rrrn 23s genes, respectively, the only genes known to contain group I introns in this organism. Introns 1, 2, and 3 of psbA (with flanking exon sequences) were subcloned and transcribed in vitro. The synthetic RNAs were found to self-splice; splicing required Mg2+, GTP, and elevated temperature. In addition, the accuracy of self-splicing was confirmed for introns 1 and 2, and intermediates in the splicing reactions were detected. These results, together with our recent data on the 23S intron, indicate that the ability to self-splice is a general feature of Chlamydomonas group I introns. These findings have significant implications for the mechanism of group I intron splicing and evolution in Chlamydomonas and other chloroplast genomes.

49

RNA Splicing in Chlamydomonas Chloroplasts. Self-splicing of 23 S preRNA
Herrin, D.L.; Chen, Y.F.; Schmidt, G.W.
Descriptors: chlamydomonas reinhardtii; chloroplast genetics; rna; precursors; introns; gene mapping; transcription
DNAL Call No.: 381 J824

50

Cloning and Expression of the Chloroplast-Encoded rbcL and rbcS Genes from the Marine Diatom Cylindrotheca sp. strain N1
Hwang, S.R. and Tabita, F.R.
Descriptors: bacillariophyta; escherichia coli; anacystis nidulans; multiple genes; ribulose-bisphosphate carboxylase; genomes; chloroplast genetics; gene expression; cloning; gene mapping; restriction mapping; recombinant dna; gene splicing; enzyme activity
DNAL Call No.: QK710.P62
Abstract:
Both the rbcL and rbcS genes, encoding the large and small subunits, respectively, of ribulose 1,5-bisphosphate carboxylase/oxygenase, have been found to be encoded by chloroplast DNA in the marine diatom Cylindrotheca sp. N1. The rbcS gene in this diatom was found to be adjacent to the rbcL gene by a combination of: (i) Southern-
blotting analyses, using heterologous probes; (ii) examination of recombinant proteins synthesized in Escherichia coli, directed by cloned rbcL/rbcS genes; and (iii) synthesis of enzymatically active heterologous Rubisco protein in vivo by recombinant DNA procedures using large subunits of Anacystis nidulans and small subunits of Cylindrotheca sp. N1. It appears that two copies of rbcL and rbcS genes are encoded by the chloroplast DNA of this diatom.

51
Cloning of the psbK Gene from Synechocystis sp. PCC 6803 and Characterization of Photosystem II in Mutants Lacking PSII-K
Descriptors: cyanobacteria; mutants; photosystem ii; genetic engineering; plant proteins; cloning; nucleotide sequences
DNAL Call No.: 381 J824
Abstract:
We cloned and sequenced the psbK gene, coding for a small photosystem II component (PSII-K), from the transformable cyanobacterium, Synechocystis sp. PCC 6803, and determined the N-terminal sequence of mature PSII-K. The psbK gene product is processed by cleaving off eight amino acid residues from the N terminus. A mutant lacking psbK was constructed; this mutant grew photoautotrophically, but its growth rate was reduced. The number of photosystem II reaction centers on a chlorophyll basis was decreased by less than a factor of 2 in the psbK-deletion mutant. In Synechocystis sp. PCC 6803, the psbK gene is transcribed as a single gene and is not part of an operon. Single-site mutations were introduced into psbK leading to early termination or deletion of the presequence. The phenotype of these mutants strongly resembles that of the psbK deletion mutant, indicating that indeed the change in phenotype in the deletion mutant is directly correlated with PSII-K. PSII-K is not essential for photosystem II assembly or activity but is needed for optimal photosystem II function.

52
Splice Site Selection and Role of the Lariat in a Group II Intron
Jacquier, A. and Jacquesson-Breuleux, N.
Descriptors: fungi; algae; plants; rna; molecular conformation; introns; catabolism; hydrolysis; mutants
DNAL Call No.: 442.8 J8224
Abstract:
The structural elements involved in 5' and 3' splice site (SS) selection in a group II intron were analyzed. While 5' SS selection appears to be defined by only one element, the EBS1-IBS1 pairing, four distinct structural components contribute to 3' SS selection, one of which being analogous to the "internal guide sequence" described for group I introns. Moreover, some of the mutants analyzed during this study induce efficient 5' SS hydrolysis and suggest how 5' SS transesterification is selected against hydrolysis. Finally, the lariat structure was found to accelerate both steps of splicing, suggesting that is "locks" the ribozyme in an active configuration.

53
Transient Expression of Firefly Luciferase in Protoplasts of the Green Alga Chlorella-ellipsoidea
Jarvis, E.E. and Brown, L.M.
Descriptors: dna; transformation; genetic engineering
Abstract:
We report here on the development of a transient expression system for Chlorella ellipsoida using a heterologous gene, firefly luciferase. Cells of this unicellular green alga were converted to protoplasts and treated with plasmid pD0432, which bears luciferase under the control of the CaMV 35s promoter. This treatment resulted in detectable luciferase activity in cell extracts. Expression required Cellulysin treatment, active cell metabolism, and the addition of carrier DNA and polyethylene glycol. Linearization of the luciferase plasmid did not significantly alter the activity. A time course of expression showed that luciferase is made rapidly, within about 7 h after addition of DNA, but that the activity disappears over the course of a few days. These experiments represent an important first step in the development of a Chlorella transformation system.

Molecular Studies of Linkage Group XIX of Chlamydomonas reinhardtii: Evidence Against a Basal Body Location
Johnson, D.E. and Dutcher, S.K.
Descriptors: chlamydomonas reinhardtii; dna; linkage groups; restriction fragment length polymorphism; organelles; flagella; repetitive dna; transposable elements; dna hybridization
DNAL Call No.: 442.8 J828
Abstract:
Linkage group XIX (also known as the UNI linkage group) in the green alga, Chlamydomonas reinhardtii, exhibits a number of unusual properties that have lead to the suggestion that it represents a basal body-associated chromosome. To begin a molecular analysis of this linkage group, we have identified DNA sequences from it and used them to determine the copy number of linkage group XIX within the cell. We find that linkage group XIX is present in the same copy number per cell as nuclear linkage groups in both haploid and diploid strains. We also find that the copy number of linkage group XIX is unchanged in mutants lacking basal bodies. We conclude that there is no convincing evidence that linkage group XIX localizes to the basal bodies of Chlamydomonas reinhardtii cells.

Expression of Salmon Growth Hormone in the Cyanobacterium Agmenellum-quadruplicatum
Kawata, Y.; Yamano, N.; Kojima, H.; Itoh, S.
Descriptors: escherichia-coli; bacteria; microorganism; fish genetics; gene transfer; TRP promoter; total cell protein; aquaculture; biotechnology industry
DNAL Call No.: QR53 B56
Abstract:
The salmon growth hormone gene was introduced into the cyanobacterium Agmenellum quadruplicatum PR-6 by plasmid transformation. The gene expressed the hormone under the trp promoter of Escherichia coli. The amount was estimated to be approximately 0.1% of the total cell protein.

Engineering the Chloroplast Genome: Techniques and Capabilities for Chloroplast Transformation in Chlamydomonas reinhardtii
Kindle, K.L.; Richards, K.L.; Stern, D.B.
Source: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE
Chloroplast transformation of Chlamydomonas reinhardtii has been accomplished by agitating cell wall-deficient cells in the presence of glass beads and DNA. By using the atpB gene as the selected marker and cells grown in 0.5 mM 5-fluorodeoxyuridine, we have recovered up to 50 transformants per micogram of DNA. This method is easy and does not require specialized equipment, although it is not as efficient as the tungsten particle bombardment method [Boynton, J.E., Gillham, N.W., Harris, E.H., Hosler, J.P., Johnson, A.M., Jones, A.R., Randolph-Anderson, B.L., Robertson, D., Klein, T.M., Shark, K.B. and Sanford, J.C. (1988) Science 240, 1534-1537]. By using particle bombardment, we have developed a cotransformation approach in which spectinomycin-resistant 16S rRNA-encoding DNA is the selected marker, and we have demonstrated that cotransformation of an unselected marker on an independent replicon is very efficient. We have used this strategy (i) to recover transformants with partially deleted atpB genes that could not otherwise have been selected since they did not restore photosynthetic capability to a recipient carrying a more extensive atpB deletion and (ii) to generate specific deletion mutations in a wild-type recipient. This methodology should allow the introduction of any desired change into the chloroplast genome, even in the absence of phenotypic selection, and thus a detailed functional analysis of any chloroplast DNA sequence should be possible.
Kraus, M.; Gotz, M.; Loffelhardt, W.
Descriptors: algae; genes; ribosomes; plant proteins; cloning; nucleotide sequences; organelles; phylogeny; amino acid sequences; transcription; messenger rna
DNAL Call No.: QK710.P62

Abstract:
The str operon containing the genes for the ribosomal proteins S12 (rps12) and S7 (rps7) and for the elongation factors G ( fus ) and Tu ( tufA ) has been characterized for some cyanobacteria and chloroplasts from algae and higher plants. In the case of plastids a stepwise reduction by one and two genes, respectively, has been observed due to gene transfer to the nuclear genome. The nucleotide sequence of the str operon on the cyanelle genome from Cyanophora paradoxa was determined as a first example for a chlorophyll b-less plastid. It comprises rps12, rps7 and tufA which are closely linked and not interrupted by introns. Transcript analysis revealed cotranscription of the two ribosomal protein genes whereas tufA gave rise to a monocistronic mRNA. Phylogenetic studies using these three different traits allowed an assessment of the position of Cyanophora paradoxa among oxygenic photoautotrophs.

59
Conjugative Transfer and Autonomous Replication of a Promiscuous IncQ Plasmid in the Cyanobacterium Synechocystis PCC 6803
Kreps, S.; Ferino, F.; Mosrin, C.; Gerits, J.; Mergeay, M.; Thuriaux, P.
Descriptors: cyanobacteria; escherichia coli; saccharomyces cerevisiae; plasmids; genetic transformation; cloning; photosynthesis
DNAL Call No.: 442.8 Z34

60
Developmental Rearrangement of Cyanobacterial nif Genes: Nucleotide Sequence, Open Reading Frames, and Cytochrome P-450 Homology of the Anabaena sp. Strain PCC 7120 nifD Element
Lammers, P.J.; McLaughlin, S.; Papin, S.; Trujillo-Provencio, C.; Ryncarz, A.J. II
Descriptors: anabaena; strains; genes; nucleotide sequences; amino acid sequences; cytochrome p-450
DNAL Call No.: 448.3 J82

Abstract:
An 11-kbp DNA element of unknown function interrupts the nifD gene in vegetative cells of Anabaena sp. strain PCC 7120. In developing heterocysts the nifD element excises from the chromosome via site-specific recombination between short repeat sequences that flank the element. The nucleotide sequence of the nifH-proximal half of the element was determined to elucidate the genetic potential of the element. Four open reading frames with the same relative orientation as the nifD element-encoded xisA gene were identified in the sequenced region. Each of the open reading frames was preceded by a reasonable ribosome-binding site and had biased codon utilization preferences consistent with low levels of expression. Open reading frame 3 was highly homologous with three cytochrome P-450 omega-hydroxylase proteins and showed regional homology to functionally significant domains common to the cytochrome P-450 superfamily. The sequence encoding open reading frame 2 was the most highly conserved portion of the sequenced region based on heterologous hybridization experiments with three genera of heterocystous cyanobacteria.
61
Genomic Structure of Chlamydomonas caltractin. Evidence for Intron Insertion Suggests a Probable Genealogy for the EF-Hand Superfamily of Proteins
Lee, V.D.; Stapleton, M.; Huang, B.
Descriptors: chlamydomonas reinhardtii; genes; calcium binding proteins; genome analysis; introns; exons; nucleotide sequences; amino acid sequences; evolution; ancestry; structure; molecular conformation
DNAL Call No.: 442.8 J8224
Abstract:
A clone containing the gene locus for Chlamydomonas caltractin, a 20,000 Mr calcium-binding protein that is a member of the EF-hand superfamily of calcium-modulated proteins, was isolated and the structural organization of the gene was determined. The intron-exon organization was resolved by direct comparison of the genomic sequence with a caltractin cDNA. The promoter region does not contain the typical TATA or CCAAT boxes, but the sequences at the splice junctions are similar to those of other eukaryotes. The positions of the six introns in the caltractin gene do not typically define unit structures, nor do they coincide with those in genes for other members of the EF-hand superfamily. An analysis of exon sequences at the splice junctions in the genes of this multigene family was undertaken; evidence was obtained that supports the hypothesis that introns arose at protossplice sites. A probable evolutionary history for the EF-hand superfamily based on intron insertion is offered.

62
Recombination of Chlamydomonas Chloroplast DNA Occurs more Frequently in the Large Inverted Repeat Sequence than in the Single-copy Regions
Lemieux, B.; Turmel, M.; Lemieux, C.
Descriptors: chlamydomonas; hybrids; chloroplast genetics; dna; recombination; genetic code; nucleotide sequence; inheritance; genetic polymorphism; gene mapping
DNAL Call No.: 442.8 Z8

63
Homologues of the Green Algal gidA Gene and the Liverwort fixC Gene are Present on the Chloroplast Genomes of Conifers
Lidholm, J. and Gustafsson, P.
Descriptors: pinus contorta; picea abies; chlamydomonas reinhardtii; marchantia polymorpha; genomes; dna; chloroplasts; nucleotide sequences; dna hybridization; southern blotting; genes; transfer rna; asparagine; amino acid sequences; chlorophyll; biosynthesis
DNA Call No.: QK710.P62
Abstract:
Strong hybridization signals were obtained from total DNA of two conifers, lodgepole pine (Pinus contorta) and Norway spruce (Picea abies), in a Southern blot analysis using a probe derived from the chloroplast gidA gene of the green alga Chlamydomonas reinhardtii. The pine fragments detected by the probe were found to originate from the chloroplast genome and, as judged by the signal intensity, this was also true for the spruce fragments. Sequence analysis of the hybridizing pine chloroplast DNA region revealed an open reading frame potentially encoding a 459 amino acid polypeptide, highly homologous to that deduced from the algal gene and to ORF:465 of liverwort chloroplast DNA. Upstream of the gidA sequence, we found a trnN(GUU) gene and an open reading frame
of 291 codons which was 78% identical to the frxC gene of liverwort. Since ORF465 is located immediately downstream of trnN and frxC in liverwort, the genetic organization of this region is very similar in the two plants. In contrast, neither the gidA nor the frxC gene is present in the chloroplast DNA of tobacco or rice. It was recently reported that deletions in the gidA region of the chloroplast genome of Chlamydomonas reinhardtii abolish the light-independent pathway of chlorophyll synthesis which exists in many algae and lower plants. The presence of the gidA gene on the chloroplast genomes of conifers may therefore be of significance with respect to the ability of these plants to synthesize chlorophyll in the dark.

64

Structural Features of the Plastid Ribosomal RNA Operons of Two Red Algae: Antithamnion sp. and Cyanidium caldarium
Maid, U. and Zetsche, K.
Descriptors: rhodophyta; ribosomal rna; ribosomal dna; nucleotide sequences; chloroplasts; evolution; chemotaxonomy; genes; transfer rna; isoleucine; alanine
DNAL Call No.: QK710.P62
Abstract:
The nucleotide sequences of the plastid 16S rDNA of the multicellular red alga Antithamnion sp. and the 16S rDNA/23S rDNA intergenic spacers of the plastid DNAs of the unicellular red alga Cyanidium caldarium and of Antithamnion sp. were determined. Sequence comparisons support the idea of a polyphyletic origin of the red algal and the higher-plant chloroplasts. Both spacer regions include the unsplit tRNA (Ile) (GAU) and tRNA (Ala) (UGC) genes and so the plastids of both algae form a homogeneous group with those of chromophytic algae and Cyanophora paradoxa characterized by 'small-sized' rDNA spacers in contrast to green algae and higher plants. Nevertheless, remarkable sequence differences within the rRNA and the tRNA genes give the plastids of Cyanidium caldarium a rather isolated position.

65

Characterization of Cryptic Plasmids from Marine Cyanobacteria and Construction of a Hybrid Plasmid Potentially Capable of Transformation of Marine Cyanobacterium Synechococcus-sp and its Transformation
Matsunaga, T.; Takeyama, H.; Nakamura, N.
Descriptors: anacystis-nidulans; escherichia-coli; electroporation; genetic engineering; biotechnology
DNAL Call No.: QD415.A1J62

66

Dynamic Interplay between two Copper-Titrating Components in the Transcriptional Regulation of cyt c6
Merchant, S.; Hill, K.; Howe, G.
Descriptors: chlamydomonas reinhardtii; transcription; gene expression; genetic regulation; cytochrome c; genes; copper; binding proteins; messenger rna; plastocyanins
DNAL Call No.: QH506.E46
Abstract:
The algal plastidic cytochrome c (cyt c6) is a biochemical equivalent of the copper-containing protein plastocyanin in photosynthetic electron transfer. But generally, cyt c6 accumulates and functions only under conditions (e.g. Cu-deficiency) where plastocyanin cannot be synthesized. In studying the regulation of Chlamydomonas reinhardtii cyt c6 expression by Cu we have determined that repression of cyt c6 accumulation occurs at the transcriptional level, and specifically in response to Cu as the metal ion regulator. Complete and sustained repression of cyt c6 transcription requires approximately 9 X 10(6) Cu ions in the medium/cell. Based on the estimated plastocyanin content of algal cells (8 X 10(6) molecules/cell) and the observation that lower ratios of Cu per cell result in only transient repression of cyt c6 transcription, we propose that Cu-dependent transcriptional repression of the gene encoding cyt c6 requires a Cu-binding factor which is titrated by Cu only after the alternate electron transfer catalyst, plastocyanin, has accumulated to the stoichiometry required for photosynthesis. The precise and highly metal-specific, autoregulatory control of cyt c6 levels--directly by Cu, and indirectly by holoplastocyanin--is in keeping with the functional role of cyt c6 as an alternate, although perhaps less preferred, electron transfer catalyst.

Targeted Disruption of Chloroplast Genes in Chlamydomonas reinhardtii

Newman, S.M.; Gillham, N.W.; Harris, E.H.; Johnson, A.M.; Boynton, J.E.


Descriptors: chlamydomonas reinhardtii; chloroplast genetics; genetic transformation; plasmids; gene transfer; mutations; genes; direct dna uptake; targeted mutagenesis

DNAL Call No.: 442.8 Z34

Abstract:

We have developed an efficient procedure for the disruption of Chlamydomonas chloroplast genes. Wild-type C. reinhardtii cells were bombarded with microprojectiles coated with a mixture of two plasmids, one encoding selectable, antibiotic-resistance mutations in the 16S ribosomal RNA gene and the other containing either the atpB or rbcL photosynthetic gene inactivated by an insertion of 0.48 kb of yeast DNA in the coding sequence. Antibiotic-resistant transformants were selected under conditions permissive for growth of non-photosynthetic mutants. Approximately half of these transformants were initially heteroplasmic for copies of the disrupted atpB or rbcL genes integrated into the recipient chloroplast genome but still retained photosynthetic competence. A small fraction of the transformants (1.1% for atpB; 4.3% for rbcL) were nonphotosynthetic and homoplasmic for the disrupted gene at the time they were isolated. Single cell cloning of the initially heteroplasmic transformants also yielded nonphotosynthetic segregants that were homoplasmic for the disrupted gene. Polypeptide products of the disrupted atpB and rbcL genes could not be detected using immunoblotting techniques. We believe that any nonessential Chlamydomonas chloroplast gene, such as those involved in photosynthesis, should be amenable to gene disruption by cotransformation. The method should prove useful for the introduction of site-specific mutations into chloroplast genes and flanking regulatory sequences with a view to elucidating their function.

Transformation of Chloroplast Ribosomal RNA Genes in Chlamydomonas: Molecular and Genetic Characterization of Integration Events

Newman, S.M.; Boynton, J.E.; Gillham, N.W.; Randolph-Anderson, B.L.; Johnson, A.M.; Harris, E.H.


Descriptors: chlamydomonas reinhardtii; chlamydomonas; ribosomal dna; ribosomal rna; genes;
chloroplasts; genomes; genetic transformation; induced mutations; direct dna uptake; phenotypes; antibiotics; drug resistance; gene mapping; restriction fragment length polymorphism

**DNAL Call No.: 442.8 G28**

**Abstract:**

Transformation of chloroplast ribosomal RNA (rRNA) genes in Chlamydomonas has been achieved by the biolistic process using cloned chloroplast DNA fragments carrying mutations that confer antibiotic resistance. The sites of exchange employed during the integration of the donor DNA into the recipient genome have been localized using a combination of antibiotic resistance mutations in the 16S and 23S rRNA genes and restriction fragment length polymorphisms that flank these genes. Complete or nearly complete replacement of a region of the chloroplast genome in the recipient cell by the corresponding sequence from the donor plasmid was the most common integration event. Exchange events between the homologous donor and recipient sequences occurred preferentially near the vector:insert junctions. Insertion of the donor rRNA genes and flanking sequences into one inverted repeat of the recipient genome was followed by intramolecular copy correction so that both copies of the inverted repeat acquired identical sequences. Increased frequencies of rRNA gene transformants were achieved by reducing the copy number of the chloroplast genome in the recipient cells and by decreasing the heterology between donor and recipient DNA sequences flanking the selectable markers. In addition to producing bona fide chloroplast rRNA transformants, the biolistic process induced mutants resistant to low levels of streptomycin, typical of nuclear mutations in Chlamydomonas.

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**69**

*A Gene Homologous to the Subunit-2 Gene of NADH Dehydrogenase is Essential to Inorganic Carbon Transport of Synechocystis PCC6803*

Ogawa, T.


**Descriptors:** cyanobacteria; amino acid sequences; chloroplasts; mitochondria; mutants; translocation; carbon; carbon dioxide; genetic transformation; ndh dehydrogenase; nucleotide sequences; respiration; wild strains

**DNAL Call No.: 500 N21P**

**Abstract:**

A clone that transforms the RKa mutant of Synechocystis PCC6803 defective in inorganic carbon (Ci) transport to the wild-type phenotype was isolated from a cyanobacterial genomic library. The clone contained an 11.8-kilobase-pair DNA insert. Sequencing of the insert DNA in the region of the mutation in Rka revealed an open reading frame (designated as ndhB), which showed extensive amino acid sequence homology to the subunit-2 genes of NADH dehydrogenase (EC 1.6.99.3) (ndhB) of chloroplasts and mitochondria. The homology was much stronger with the chloroplast genes. Sequence analysis of the ndhB gene of RKa mutant revealed a G leads to A substitution that results in a Gly lead to Asp substitution in the deduced amino acid. A defined mutant (M55), constructed by inactivating the ndhB gene in wild-type Synechocystis, required high CO2 conditions for growth and was unable to transport CO2 and HCO3- into the intracellular Ci pool. The results indicate that the ndhb gene is required for Ci transport. Dark respiration was also depressed by the inactivation of the ndhB gene. A possible role of the ndhB gene product in the energization of Ci transport is discussed.

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**70**

*Recombination: Recombination of Mobile Genetic Elements from Plants and Cyanobacteria*

Osiewacz, H.D. and Heinen, U.

23
Abstract:
A simple method that combines guanidinium isothiocyanate RNA extraction and probing with antisense and sense RNA probes is described for analysis of microbial gene expression in planktonic populations. Probing of RNA sample extracts with sense-strand RNA probes was used as a control for nonspecific hybridization or contamination of mRNA with target DNA. This method enabled detection of expression of a plasmid-encoded neomycin phosphotransferase gene (nptII) in as few as 105 Vibrio cells per ml in 100 ml of seawater. We have used this method to detect expression of the ribulose-1,5-bisphosphate carboxylase large-subunit gene (rbcL) in Synechococcus cultures and natural phytoplankton populations in the Dry Tortugas, Florida. During a 36-h diel study, rbcL expression of the indigenous phytoplankton was greatest in the day, least at night (1100, 0300, and 0100 h), and variable at dawn or dusk (0700 and 1900 h). These results are the first report of gene expression in natural populations by mRNA isolation and probing. This methodology should be useful for the study of gene expression in microorganisms released into the environment for agricultural or bioremediation purposes and indigenous populations containing highly conserved target gene sequences.

72
Intercistronic Group III Introns in Polycistronic Ribosomal Proteins of Chloroplasts
Stevenson, J.K.; Drager, R.G.; Copertino, D.W.; Christopher, D.A.; Jenkins, K.P.; Yepiz-Plascencia, G; Hallick, R.B.
Descriptors: euglena gracilis; introns; cistrons; genes; ribosomes; plant proteins; transcription; gene expression; messenger rna; nucleotide sequences; amino acid sequences; restriction mapping; chloroplasts; chloroplast genetics
DNAL Call No.: 442.8 Z34
Abstract:
A novel ribosomal protein operon in the Euglena gracilis chloroplast genome was characterized. It encodes the genes for ribosomal proteins S4 and S11 (rps4 and rps11). The coding region of the rps11 gene is interrupted by two introns of 107 and 100 bp. The introns belong to a distinct class known as group III introns. The major transcript from this operon was characterized as a fully spliced dicistronic rps4-rps11 mRNA by RNA blot analysis, primer extension sequencing, and cDNA cloning and sequencing. An additional 95 nucleotide (nt) group III intron was identified in the 123 nt rps4-rps11 intercistronic region. The identification of the intercistronic intron between the rps4 and rps11 genes was unexpected. Other RNA transcripts from regions of the genome that could potentially contain intercistronic introns were re-examined and two other intercistronic, group III introns were found. These are located in a large ribosomal protein operon between the genes for the ribosomal proteins L23 and L2, and between L14 and L5.
There are at least 50 group III introns in the E. gracilis chloroplast genome. All but 6 are found in genes encoding protein components of the transcriptional and translational apparatus. The distribution of group III introns and the unusual location of intercistronic group III introns may reflect some aspect of gene expression, or provide some insight into the mechanism of their splicing.

73
Directed Chloroplast Transformation in Chlamydomonas reinhardtii: Insertional Inactivation of the psaC Gene Encoding the Iron Sulfur Protein Destabilizes Photosystem I
Takahashi, Y.; Goldschmidt-Clermont, M.; Soen, S.Y.; Franzen, L.G.; Rochaix, J.D.
Descriptors: chlamydomonas reinhardtii; chloroplasts; genes; photosystem i; nucleotide sequences; amino acid sequences; binding proteins
DNAL Call No.: QH506.E46
Abstract:
The chloroplast gene psaC encoding the iron sulfur protein of photosystem I (PSI) from the green alga Chlamydomonas reinhardtii has been cloned and characterized. The deduced amino acid sequence is highly, related to that of higher plants and cyanobacteria. Using a particle gun, wild type C. reinhardtii cells have been transformed with a plasmid carrying the psaC gene disrupted by an aadA gene cassette designed to express spectinomycin/streptomycin resistance in the chloroplast. Transformants selected on plates containing acetate as a reduced carbon source and spectinomycin are unable to grow on minimal medium lacking acetate and are deficient in PSI activity. Southern blot analysis of total cell DNA of the transformants shows that the wild type psaC gene has been replaced by the interrupted psaC gene through homologous recombination. While authentic transcripts of the psaC gene are no longer detected, aadA gives rise to a few transcripts in the transformants. Biochemical analysis indicates that neither PSI reaction center subunits nor the seven small subunits belonging to PSI accumulate stably in the thylakoid membranes of the transformants. Pulse-chase labeling of cell proteins shows that the PSI reaction center subunits are synthesized normally but turn over rapidly in the transformants. We conclude that the iron sulfur binding protein encoded by the psaC gene is an essential component, both for photochemical activity and for stable assembly of PSI. The present study suggests that any chloroplast gene encoding a component of the photosynthetic apparatus can be disrupted in C. reinhardtii using the strategy described.

74
Short Leader Sequences may be Transferred from Small RNAs to Pre-mature mRNAs by Trans-splicing in Euglena
Descriptors: euglena gracilis; messenger rna; rna; nucleotide sequences
DNAL Call No.: QH506.E46
Abstract:
Very closely related short sequences are present at the 5' end of cytoplasmic mRNAs in Euglena as evidenced by comparison of cDNA sequences and hybrid-arrested translation experiments. By cloning Euglena gracilis nuclear DNA and isolating the rbcS gene (encoding the small subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase), we have shown that the short leader sequence does not flank the nuclear gene sequence. The leader sequences were found to constitute the 5' extremities of a family of small RNAs. Sequencing six members of this family revealed a striking similarity to vertebrate
UsnRNAs. We propose that a trans-splicing mechanism transfers the spliced leader (SL) sequence from these small RNAs (SL RNAs) to pre-mature mRNAs. Transfer of leader sequences to mRNAs by trans-splicing has been shown only in trypanosomes where cis-splicing is unknown, and in nematodes where not more than 10% of the mRNAs have leader sequences. Our results strongly suggest that Euglena is a unique organism in which both a widespread trans-splicing and a cis-splicing mechanism co-exist.

75
Photosynthetic Electron Transport Controls Nitrogen Assimilation in Cyanobacteria by Means of Posttranslational Modification of the glnB Gene Product
Tsinoremas, N.F.; Castets, A.M.; Harrison, M.A.; Allen, J.F.; Tandeau de Marsac, N.
Descriptors: synechococcus; strains; amino acid sequences; ammonium; cloning; electron transfer; genetic code; molecular genetics; nitrogen metabolism; nucleotide sequences; photosynthesis; polypeptides; restriction mapping; transcription
DNAL Call No.: 500 N21P
Abstract:
A glnB gene is identified in the cyanobacterium Synechococcus sp. PCC 7942, and its gene product is found to be covalently modified as a result of imbalance in electron transfer in photosynthesis, where photosystem II is favored over photosystem I. The gene was cloned and sequenced and found to encode a polypeptide of 112 amino acid residues, whose sequence shows a high degree of similarity to the Escherichia coli regulatory protein, P(II). In E. coli, P(II) is involved in signal transduction in transcriptional and posttranslational regulation of nitrogen assimilation. Increase in ammonium ion concentration is shown to decrease covalent modification of the Synechococcus P(II) protein, as in enteric bacteria. We therefore propose that the photosynthetic electron transport chain may regulate the pathway of nitrogen assimilation in cyanobacteria by means of posttranslational, covalent modification of the glnB gene product. The existence of the glnB gene in different strains of cyanobacteria is demonstrated and its implications are discussed.

76
Six Group I Introns and Three Internal Transcribed Spacers in the Chloroplast Large Subunit Ribosomal RNA Genes of the Green Alga Chlamydomonas eugametos
Turmel, M.; Boulanger, J.; Schnare, M.N.; Gray, M.W.; Lemieux, C.
Descriptors: chlamydomonas eugametos; chloroplast genetics; ribosomal rna; genes; nucleotide sequences; genome analysis; introns; transcription; nucleases
DNAL Call No.: 442.8 J8224
Abstract:
The chloroplast large subunit rRNA gene of Chlamydomonas eugametos and its 5' flanking region encoding tRNA(Ile) (GAU) and tRNA(Ala) (UGC) have been sequenced. The DNA sequence data along with the results of a detailed RNA analysis disclosed two unusual features of this green algal large subunit rRNA gene: (1) the presence of six group I introns (CeLSU.1-CeLSU.6) whose insertion positions have not been described previously, and (2) the presence of three short internal transcribed spacers that are posttranscriptionally excised to yield four rRNA species of 280, 52, 810 and 1720 nucleotides, positioned in this order (5' to 3') in the primary transcript. Together, these RNA species can assume a secondary structure that is almost identical to that proposed for the 23 S rRNA of Escherichia coli. All three internal transcribed spacers map to variable regions of primary sequence and/or potential secondary structure, whereas all six introns lie within
highly conserved regions. The first three introns are inserted within the sequence encoding the 810 nucleotide rRNA species and map within domain II of the large subunit rRNA structure; the remaining introns, found in the sequence encoding the 1720 nucleotide rRNA species, lie within either domain IV or V, as is the case for all other large subunit rDNA introns that have been documented to date. CeLSU.5 and CeLSU.6 each contain a long open reading frame (ORF) of more than 200 codons. While the CeLSU.6 ORF is not related to any known ORFs, the CeLSU.5 ORF belongs to a family of ORFs that have been identified in Podospora and Neurospora mitochondrial group I introns. The finding that a polymorphic marker showing unidirectional gene conversion during crosses between C. eugametos and Chlamydomonas moewusii is located within the CeLSU.5 ORF makes it likely that this intron is a mobile element and that its ORF encodes a site-specific endonuclease promoting the transfer of the intron DNA sequence.

77
Structural Similarities between psbA Genes from Red and Brown Algae
Descriptors: rhodophyta; phaeophyta; genes; plant proteins; photosystem ii; cloning; nucleotide sequences; plastids; chloroplast genetics; evolution; amino acid sequences; deletions
DNAL Call No.: QH426.C8
Abstract:
The single copy psbA genes from the multicellular red alga Antithamnion spec. and the brown alga Ectocarpus siliculosus have been cloned and sequenced and monocistronic transcripts have been detected. Both genes contain an insertion of 21 bp at the 3' end which was also found in cyanobacteria and which is absent in chloroplasts and the chlorophyll b-containing prochlorophyte Prochlorothrix hollandica. These findings are in agreement with the hypothesis of a polyphyletic origin of plastids. Plastids of red and brown algae appear to be closely related.

78
The Group IIB Intron from the Green Alga Scenedesmus obliquus Mitochondrion: Molecular Characterization of the In Vitro Splicing Products
Winkler, M. and Kuck, U.
Descriptors: scenedesmus; mitochondrial dna; introns; alternative splicing; nucleotide sequences; restriction mapping; molecular conformation; ribosomal rna; genes; ribosomal dna
DNAL Call No.: QH426.C8
Abstract:
In the presence of high molar salt concentrations, the mitochondrial group IIB intron (rH1) from the green alga Scenedesmus obliquus is capable of splicing in vitro. After establishing the optimal conditions for RNA processing the in vitro splicing products were unequivocally identified in self-splicing experiments by Northern hybridization analysis employing 3'end-labelled RNAs or exon-and/or intron-specific probes. Finally, two trans-esterification products were identified by sequencing of the spliced RNA. From our data we conclude that the processing of group II introns from both algal and yeast mitochondria is preceded by identical consecutive trans-esterification steps. The predicted secondary and tertiary structure of intron rH1 of S. obliquus contains all the motifs necessary for optimal self-splicing and which are characteristic of other group IIB introns from different species.

79
Use of a Transposon with Luciferase as a Reporter to Identify Environmentally Responsive Genes in a
Cyanobacterium
Wolk, C.P.; Cai, Y.; Panoff, J.M.
Descriptors: anabaena; algae; cell differentiation; dna libraries; luciferase; mutants; nitrogen; nutrient deficiencies; temperature; transcription
DNAL Call No.: 500 N21P
Abstract:
Anabaena, a filamentous cyanobacterium, is of developmental interest because, when deprived of fixed nitrogen, it shows patterned differentiation of N2-fixing cells called heterocysts. To help elucidate its early responses to a decrease in nitrogen, we used a derivative of transposon Tn5 to generate transcriptional fusions of promoterless bacterial luciferase genes, luxAB, to the Anabaena genome. Genes that responded to removal of fixed nitrogen or to other environmental shifts by increased or decreased transcription were identified by monitoring the luminescence of colonies from transposon-generated libraries. The Tn5 derivative transposed in Anabaena at ca. 1.4 X 10-5 per cell and permitted high-resolution mapping of its position and orientation in the genome and facile cloning of contiguous genomic DNA.

Nostoc Commune UTEX 584 Gene Expressing Indole Phosphate Hydrolase Activity in Escherichia coli
Xie, W.Q.; Whitton, B.A.; Simon, J.W.; Jager, K.; Reed, D.; Potts, M.
Descriptors: nostoc; genes; gene expression; hydrolases; indoles; enzyme activity; phosphatases
DNAL Call No.: 448.3 J82
Abstract:
A gene encoding an enzyme capable of hydrolyzing indole phosphate was isolated from a recombinant gene library of Nostoc commune UTEX 584 DNA in lambda gt10. The gene (designated iph) is located on a 2.9-kilobase EcoRI restriction fragment and is present in a single copy in the genome of N. commune UTEX 584. The iph gene was expressed when the purified 2.9-kilobase DNA fragment, free of any vector sequences, was added to a cell-free coupled transcription-translation system. A polypeptide with an Mr of 74,000 was synthesized when the iph gene or different iph-vector DNA templates were expressed in vitro. When carried by different multicopy plasmids and phagemids (pMP005, pBH6, pBH8) the cyanobacterial iph gene conferred an IpH+ phenotype upon various strains of Escherichia coli, including a phoA mutant. Hydrolysis of 5-bromo-4-chloro-3-indolyl phosphate was detected in recombinant E. coli strains grown in phosphate-rich medium, and the activity persisted in assay buffers that contained phosphate. In contrast, indole phosphate hydrolase activity only developed in cells of N. commune UTEX 584, when they were partially depleted of phosphorus, and the activity associated with these cells was suppressed partially by the addition of phosphate to assay buffers. Indole phosphate hydrolase activity was detected in periplasmic extracts from E. coli (IpH+) transformants.

Repetitive Sequence-Mediated Rearrangements in Chlorella ellipsoidea Chloroplast DNA: Completion of Nucleotide Sequence of the Large Inverted Repeat
Yamada, T.
Descriptors: chlorella ellipsoidea; repetitive dna; chloroplasts; nucleotide sequences; restriction mapping; amino acid sequences; genes; ribosomal rna; ribosomal dna; gene mapping; transfer rna
DNAL Call No.: QH426.C8
Abstract:
A 3454 base pair (bp) sequence of the large inverted repeat (IR) of chloroplast DNA (cpDNA) from the unicellular green alga Chlorella ellipsoidea has been determined. The sequence includes: (1) the boundaries between the IR and the large single copy (LSC) and the small single copy (SSC) regions, (2) the gene for psbA and (3) an approximately 1.0 kbp region between psbA and the rRNA genes which contains a variety of short dispersed repeats. The total size of the Chlorella IR was determined to be 15 243 bp. The junction between the IR and the small single copy region is located close to the putative promoter of the rRNA operon (906 bp upstream of the -35 sequence on each IR). The junction between the IR and the large single copy region is also just upstream of the putative psbA promoter, 218 bp upstream from the ATG initiation codon. A few sets of unique sequences were found repeatedly around both junctions. Some of the sequences flanking the IR-LSC junction suggest a unidirectional and serial expansion of the IR within the genome. The psbA gene is located close to the LSC-side junction and codes for a protein of 352 amino acid residues. A highly conserved C-terminal Gly is absent. Unlike the psbA of Chlamydomonas species, which contains 2-4 large introns, the gene of Chlorella has no introns. The overall gene organization of the Chlorella IR is very different from that of higher plants, but a similar gene cluster of rrn-psbA is also found in the IR of Chlamydomonas species and in a single copy region of some chlorophyll a/c-containing algae, indicating a common evolutionary lineage of these cpDNAs. The origin and evolution of the IR structure are discussed in the light of these observations.

82
Sequences of trnR-ACG and petD that Contain a tRNA-like Element within the Chloroplast Genome of Chlamydomonas reinhardtii
Yu, W. and Spreitzer, R.J.
Descriptors: chlamydomonas reinhardtii; chloroplast genetics; transfer rna; nucleotide sequences
DNAL Call No.: QD341.A2N8

Products and Product Development

83
Glycerol
Agarwal, G.P.
Descriptors: saccharomyces cerevisiae; yeasts; endomycetales; algae; glycerol; biosynthesis; biotechnology; literature review
DNAL Call No.: TP248.3.A38
Abstract:
Glycerol is traditionally produced as a by-product of soap and fatty acids industries. The demand for glycerol has always exceeded the supply from these industries so the excess demand has been met by chemical synthesis from propylene for the last several decades. Though glycerol production has a long history (dating back to World War I) of being produced via a biochemical route, yet it is not sufficiently developed to compete with the chemical route. In the present review a case has been made to produce glycerol via any of the several known biochemical routes: a) Sulfite-Alkali-Steered Yeast Process b)
Bacterial Process c) Osmotolerant Yeast Process d) Algal Cultivation Process. The possible reasons for these processes not being able to compete with chemical processes are critically reviewed. The literature on downstream processing of any of the biochemical processes is quite limited and more investigations are required into this aspect to make these processes viable. The biosynthesis mechanism of glycerol production in the organisms is summarized and the need to look into some of the fundamental aspects of glycerol synthesis in an osmotolerant yeast emphasized. The comparison between the various processes is made wherever possible.

84
Enriching Marine Macroalgae with Eicosatetraenoic Arachidonic and Eicosapentaenoic Acids by Chilling
Al-Hasan, R.H.; Hantash, F.M. Radawan, S.S.
Descriptors: algae; methods; lipids; fatty acids; biotechnology industry
DNAL Call No.: QR1.E9
Abstract:
Twelve macroalgae belonging to the Chlorophyta, Phaeophyta and Rhodophyta were collected from the Arabian Gulf. Field samples and samples that were first incubated at 5 degree. C and 24 degree. C in the light for 1 week were analysed for lipids and fatty acids. The lipid contents varied according to the macroalga and, within the Chlorophyta and Phaeophyta, some representatives accumulated more lipids at 5 degree. C and others at 24 degree. C. All samples of algae had similar lipid composition with only quantitative differences. The temperature did not have a common effect on the lipid composition of representative algae, although changes in the relative concentration of specific classes were recorded. The Phaeophyta and Rhodophyta were as a rule richer than the Chlorophyta in eicosatetraenoic (20:4) and eicosapentaenoic (20:5) but poorer in linolenic (18:3) acids. In most of the algae, incubation at 5 degree. C was associated with lowering the proportion of palmitic acid (16:0) in the total lipids, and, but only in the Phaeophyta and Rhodophyta, increasing the concentration of 20:4 and 20:5. These polyunsaturated fatty acids occurred in high levels in monogalactosyldiacylglycerols (MGDG) and digalactosyldiacylglycerols (DGDG) of the Phaeophyta and Rhodophyta but not the Chlorophyta, the MGDG and DGDG of which were rich in 18:3 and hexadecatrienoic acid (16:3).

85
Biosynthesis of 130-Kilodalton Mosquito Larvicide in the Cyanobacterium Agmenellum-quadruplicatum PR-6
Angsuthanasombat, C. and Panyim, S.
Source: APPLIED AND ENVIRONMENTAL MICROBIOLOGY
Descriptors: bacillus thuringiensis; genes; genetic transformation; cyanobacteria; ovicides and larvicides; biological control; diptera
DNAL Call No.: 448.3 AP5
Abstract:
The 130 kilodalton mosquito larvicidal gene, cloned from Bacillus thuringiensis var. israelensis, was introduced into the cyanobacterium Agmenellum quadruplicatum PR-6 by plasmid transformation. Transformed cells synthesized 130-kilodalton delta-endotoxin protein and showed mosquito larvicidal activity. Results demonstrate a potential use of a cyanobacterium for biological control of mosquitoes.

86
Anaerobic Digestion of Seaweed for Biogas a Kinetic Evaluation

30
Abstract:
A kinetic study of biogas production in batch digesters by anaerobic digestion of seaweed, Sargassum tenerrimum, with a mixed bacterial culture consisting of methanogenic bacteria and an algin-degrading bacterial strain was carried out at different concentrations of dry total solids. Specific rate constants of biogas production during the lag, exponential and monomolecular (stationary) phases of bacterial growth were determined. About half the total volume of biogas was generated during the exponential phase irrespective of the concentration of seaweed in the digesters. The specific rates of substrate destruction and biogas generation in the stationary phase decreased with increasing substrate concentration. The yield of biogas per gram dry total solids of seaweed was about the same at all concentrations, but with a marked decline at 12% (w/v) total solids. The maximum destruction of volatile solids effected was about 63% over a period of 72 days.

87
Agar and Agarose Biotechnological Applications
Armisen, R.
Descriptors: agar; agarose; bacteriological agar; chromatography; electrophoresis
DNAL Call No.: 410 H992

88
Biotechnology for Rural Nutrition an Economic Evaluation of Algal Protein Supplements in South India
Babu, S.C. and Rajasejaren, B.
Descriptors: algae; human; developing countries; food policy; costs
DNAL Call No.: HD9000.1.F66
Abstract:
This article evaluates the introduction of algal biotechnology as a nutrition intervention in rural south India in terms of its benefits, costs and acceptability to the tastes and income of rural households. Data were collected from a whole-village study, and developments in the theory of the economics of tastes are used to analyze the socioeconomic acceptance of algal supplements among the study households. The results indicate that the algal food supplement is highly cost effective in providing adequate protein and other micronutrients. However, the absolute deviation between actual and optimal intake of algal food increased with higher income class. Several policy implications for food and nutrition interventions in developing countries are derived.

89
A Marketing Approach to Agar
Becker, K.J. and Rotmann, K.W.G.
Descriptors: consumer awareness; thickener; laxative; packaging; biotechnology; far east; japan; germany; islamic countries
DNAL Call No.: QK564.J68
Abstract:
Agar has, with the exception of certain retail markets in the Far East, specifically Japan,
traditionally been sold to the industrial user. Small quantities are consumed in Islamic countries during Ramadan and in the Germanic countries as a food thickener and a laxative. However, outside of Japan, no significant marketing effort has ever been undertaken with a view to increase the demand for agar by consumers. A marketing plan is suggested to change this situation. All possible uses for agar by the consumer have been identified and studied. The special features of the product, together with certain packaging, are highlighted. Potential markets for these features are identified. Strategies for the development of these markets have been developed. The overall plan is now in a state of final review and just prior to implementation. The product launch should generate a significant consumer awareness which will translate into demand, thereby increasing the market for agar in various forms, formulations and packagings.

90
The Biotechnology of Cultivating Dunaliella for Production of Beta-Carotene Rich Algae
Ben-Amotz, A.; Shaish, A.; Avron, M.
Descriptors: dunaliella; algae culture; beta-carotene; biosynthesis; light intensity; growth inhibitors; isomerization; phytoene; food biotechnology; mass cultivation; salt tolerance; glycerol
DNAL Call No.: TD930.A32
Abstract:
Dunaliella accumulates massive amounts of beta-carotene when cultivated under high light intensity and growth-limiting conditions. The pathway for biosynthesis of beta-carotene was elucidated by analysis of the effect of selected inhibitors. The presence of the inhibitors elicited the accumulation of the following intermediates: beta-zeacarotene, lycopene, beta-carotene, phytofluene, phytoene and a few unidentified long-chain isoprenoids. Each of the accumulated intermediates was composed of about equal amounts of two stereoisomers, as is the case for beta-carotene in the untreated algae. It is deduced, therefore, that the isomerization reaction occurs early in the pathway of beta-carotene biosynthesis, at or before phytoene. The unique carotenogenesis properties of Dunaliella led to the development of a new biotechnological process for mass-cultivation of the alga. Commercial production facilities for beta-carotene rich Dunaliella exist today in Israel, USA, Australia, Spain and China. Recent developments, which indicate that the stereoisomeric mixture of beta-carotene present in Dunaliella is preferentially absorbed in animal tissues, coupled with new evidence for the efficacy of beta-carotene in reducing the incidence of cancer, have opened new vistas of potential markets for the high beta-carotene algae.

91
Microbial Production of Hydrocarbons
Birch, L.D. and Bachofen, R.
Descriptors: review; algae; cyanobacteria; bacteria; trichomonads; hydrogenases; nitrogenases; formate; hydroxynylase; biotechnology
DNAL Call No.: QR53.B52

92
Elucidation and Optimization of the Medium Constituents Controlling Antibiotic Production by the Cyanobacterium Nostoc-muscorum
Bloor, S. and England, R.R.
Abstract:
A study has been made to determine which nutrient factors control antibiotic production by the cyanobacterium, Nostoc muscorum. A two-phase approach was employed using a factorial method to explore the response surface and a steepest ascent method to climb the response surface to the region of the optimum. It was found that nitrate and iron were the factors significantly affecting antibiotic production; 26.4 mM nitrate and 6.μM iron were the optimal concentrations for maximizing antibiotic production by N. muscorum.

93
Development of Western Biotechnology’s Algal Beta-carotene Plant
Borowitzka, L.J.
Descriptors: algae culture; dunaliella; industrial methods; beta-carotene; food colorants
DNAL Call No.: TD930.A32
Abstract:
In the past ten years, laboratory studies and open pond experiments at Hutt Lagoon, in Western Australia, have developed a commercial process, for extracting the food colouring, .beta.-carotene, from algal cultures. The hypersaline microscopic alga, Dunaliella salina, is grown in 50 ha of open ponds, harvested, and the .beta.-carotene concentrated and packaged as 2% and 20% suspensions in vegetable oil.

94
Algal Biotechnology Products and Processes Matching Science and Economics
Borowitzka, M.A.
Descriptors: abstract; carotenoids; beta carotene; phycocyanin; culture systems; harvesting processing
DNAL Call No.: QK564.J6

95
Hydrogen Production by Eukaryotic Algae
Brand, J.J.; Wright, J.N.; Lien, S.
Descriptors: fermentation; biotechnology industry
DNAL Call No.: 381 J8224

96
Environmental Control of Lipid and Biomass Production in Two Diatom Species
Chelf, P.
Descriptors: chaetoceros-muelleri-var-subsalsum; navicula-saprophila; nitrogen biotechnology
DNAL Call No.: QK564.J68
Abstract:
Biomass and neutral lipid accumulation were examined in Chaetoceros muelleri var. subsalsum and Navicula saprophila using a multivariate, fractional factorial design. Variables included were conductivity, temperature, nitrogen concentration, silicon concentration, time (culture age), and alkalinity. Measured characteristics included nile red fluorescence (as a measure of neutral lipid content) and ash-free dry weight (AFDW). Nitrogen concentration was the variable with the greatest effect on neutral lipid and ash-
free dry weight accumulation over the ranges tested. Increasing conductivity in the range examined had a significant, negative impact on neutral lipid accumulation in both of these strains, while increasing alkalinity had a positive effect on lipid and ash-free dry weight in both strains. Experimental designs such as those described here have great potential utility in biological systems with complex interactions.

97

Biosynthesis of High Concentrations of an Exopolysaccharide during the Cultivation of the Microalga Botryococcus-braunii
Fernandes, H.L.; Tome, M.M.; Lupe, F.M.; Fialho, A.M.; Sa-Correia, I.; Novais, J.M.
Descriptors: fermentation; biotechnology industry
DNAL Call No.: QR53.B56
Abstract:
A non-axenic strain of the microalga Botryococcus braunii Kutzing, isolated from a small lake in Portugal, when cultured at 25. C in mineral medium and under continuous illumination, showed a poor production of hydrocarbons (5% of the dry biomass) but excreted remarkably high quantities of an exopolysaccharide (4-4.5 g/l) into the medium. The production of soluble polysaccharide with galactose, fucose and uronic acid residues, follows growth. The role of the mucoid contaminating bacteria in polysaccharide production in the mixed culture was unproven.

98

Analysis of the Biomass Quality and Photosynthetic Efficiency of a Nitrogen-Fixing Cyanobacterium Grown Outdoors with Two Agitation Systems
Fontes, A.G.; Moreno, J.; Vargas, M.A.
Descriptors: biotechnology industry; airlift; paddlewheel; productivity; protein; energy conversion efficiency; nitrogen fixation
DNAL Call No.: 381 J8224
Abstract:
The efficiency of two different agitation systems (airlift and paddlewheel) in the biomass photoproduction of a nitrogen-fixing filamentous blue-green alga was evaluated outdoors, and the elemental and molecular composition of the cells grown with each system was analyzed. With the paddlewheel system, the productivity values achieved were over 30% higher than with the airlift system, both in summer and winter. In this last season, a conversion efficiency of total solar energy into stored biomass energy of 3.3% was estimated for the paddlewheel system. Moreover, the algal cells grown with this system exhibited a higher net protein (58.9% of dry weight) and nitrogen (11.3%) content than those grown with the airlift device, with an estimated nitrogen fixation rate of more than 2 g N m^-2 day^-1. These advantages of the paddlewheel system make this procedure more appropriate for the large-scale photoproduction of nitrogen-fixing blue-green algae outdoors.

99

Effect of Low-dose Ultrasonic Treatment on Physiological Variables in Anabaena Flos-aquae and Selenastrum-capricornutum
Francko, D.A.; Taylor, S.R.; Thomas, B.J.; McIntosh, D.
Descriptors: anabaena flos-aquae; algae; ultrasonics; biomass accumulation; chlorophyll; alkaline phosphatase; growth rate; biotechnology
DNAL Call No.: QR53.B56
Abstract:

Cell protein content in two species of cultured algae Anabaena flos-aguae and Selenastrum capricornutum, was markedly enhanced by low-dose, short-duration ultrasonic treatment. Chlorophyll a levels and 14C-bicarbonate uptake rates were not affected by ultrasonic treatment in either species. Ultrasonically-activated Anabaena cultures placed in media deficient in nitrogen and phosphorus produced more biomass per unit time, exhibited less cell-surface alkaline phosphatase activity per cell, and had a higher heterocyst frequency than non-sonicated, nutrient-deficient cultures. In contrast, sonicated, nutrient-deficient Selenastrum cultures grew more slowly and had higher alkaline phosphatase activity than non-sonicated variants. Collectively, the data support that key metabolic variables may be altered by ultrasonic treatment in algal cultures and that the magnitude and direction of change may be species specific.

Hydrocarbon Recovery and Biocompatibility of Solvents for Extraction from Cultures of Botryococcus-braunii

Frenz, J.; Largeau, C.; Casadevall, E.; Kollerup, F.; Daugulis, A.J.
Descriptors: fermentation; biotechnology; yield; cell viability; cell wall; chromatography
DNAL Call No.: 381 J8224
Abstract:

Various water-immiscible solvents were tested for biocompatibility and hydrocarbon recovery under different contact conditions with the hydrocarbon-rich microalga Botryococcus braunii. Eighteen solvents were first selected from a data base of 1500 compounds (compiled for solvent selection for ethanol recovery from Saccharomyces cerevisiae fermentation). Nine of these candidate solvents were shown to be biocompatible with B. braunii following short contact times. This biocompatibility tends to be associated with high molecular weights and high boiling points but strongly depends on solvent chemical structure. A low polarity is essential to biocompatibility and calculated octanol-water partition coefficients, or capacity factors determined by reversed-phase high-performance liquid chromatography (HPLC), are suitable predictors of biocompatibility with B. braunii. High recoveries of hydrocarbons directly from the algal culture require relatively polar solvents and are, therefore, inimical with maintenance of cell viability. The inaccessibility of weakly polar solvents to the cell surface appears to protect the algae but also prevents substantial recovery of the hydrocarbons stored in B. braunii outer walls. In order to achieve a high recovery, contact with the solvent must be carried out on algae concentrated by filtration. Then, a large fraction of B. braunii hydrocarbons can be recovered, after a short contact time, without impairing cell viability. Under these conditions, the pertinent solvent property is affinity for the nonpolar hydrocarbons, and the highest recovery yield, approx. 70% after contact for 30 min, is achieved with hexane.

Hydrocarbon Recovery by Extraction with a Biocompatible Solvent Form Free and Immobilized Cultures of Botryococcus-braunii

Frenz, J.; Largeau, C.; Casadevall, E.
Descriptors: algae; biotechnology industry; growth; bioreactor; colony size; alginate beads; yield
DNAL Call No.: TP248.E5E565
Abstract:

Recovery of a substantial fraction of B. braunii hydrocarbons was achieved via short
contact with hexane of algae concentrated by filtration. Growth and hydrocarbon production during subsequent cultures were not impaired, even after repeated extractions. In fact, the hydrocarbon content of the cultures derived from treated algae tends to be higher than in controls. Recovery yields can be influenced by the physiological stage of the extracted culture. In addition, algae corresponding to the early exponential stage afford higher recoveries when grown under air-lift conditions relative to standard conditions; this likely originates from the smaller average size of colonies in the former cultures. The scale-up of extraction indicates that the recovery yield falls off when relatively large amounts of algae are contacted with hexane (large clump formation due to sharp polarity contrast between wet cells and the nonpolar solvent). Immobilization, via entrapment in alginate beads and adsorption on polyurethane foams, was used to overcome this problem. Contact with hexane does not affect subsequent growth and hydrocarbon production of immobilized cultures. Recovery yields are markedly increased, relative to free cells, especially in the case of polyurethane foams.

101

Production of Bioflavor by Regeneration from Protoplasts of Ulva-pertusa ulvales Chlorophyta
Fujimura, T. and Kajiwara, T.
Source: HYDROBIOLOGIA 204-205(0):143-150 (1990).
Descriptors: cappa carrageenan; agarose; agar; polymers; long chain aldehydes; marine biotechnology
DNAL Call No.: 410 H992

102

Actual Potential and Speculative Applications of Seaweed Cellular Biotechnology some Specific Comments of Gelidium
Garcia-Reina, G.; Gomez-Pinchetti, J.L.; Robledo, D.R.; Sosa, P.
Descriptors: callus; cell culture; domestication; protoplast; tissue culture; organogenesis; method; application
DNAL Call No.: 410 H992

103

Growth and Nitrogen Fixation by Immobilized Cyanobacteria
Gendel, S.M. and Nohr, R.S.
Descriptors: nostoc muscorum; nitrogen fixation; photosynthesis; growth rate; immobilization
DNAL Call No.: QR1.E9
Abstract:

The nitrogen-fixing photosynthetic cyanobacteria have significant potential for utilization as a biological system for the production of reduced nitrogen compounds, either by industrial fermentation or in the environment as soil inocula. In either system, the ability to immobilize cyanobacteria on the external surface of fibrous substrata would significantly improve the ease of manipulation of the cells, control of growth, and product recovery without the complications inherent in immobilization by entrapment. We have shown that the filamentous heterocystous species Nostoc muscorum is naturally able to attach to a variety of different fibres, both natural and artificial. Attached cells are able to grow and fix nitrogen in both liquid and plate culture. Nitrogen-fixing cells attach to the fibres much more readily than do non-fixing cells, suggesting that the physiological and morphological changes accompanying heterocyst differentiation result in the production of specific attachment sites. Scanning electron microscopy of attached cells shows that heterocysts act as attachment sites and that the external cell wall material specifically
Microbial manganese oxidation was demonstrated at high Mn2+ concentrations (5 g/liter) in bacterial cultures in the presence of microalga. The structure of the oxide produced varied depending on the bacterial strain and mode of culture. A nonaxenic, acid-tolerant microalga, a Chlamydomonas sp., was found to mediate formation of manganite (\(\gamma\)-MnOOH). Bacteria isolated from associations with crude cultures of this alga grown in aerated bioreactors formed disordered \(\gamma\)-MnO2 from Mn2+ at concentrations of 5 g/liter over 1 month, yielding 3.3 g of a semipure oxide per liter. All algal-bacterial cultures removed Mn2+ from solution, but only those with the highest removal rates formed an insoluble oxide. While the alga was an essential component of the reaction, a Pseudomonas sp. was found to be primarily responsible for the formation of a manganese precipitate. Medium components, algal biomass and urea, showed optima at 5.7 and 10 g/liters, respectively. The scaled-up culture (50 times) gave a yield of 22.3 g (53 mg/liter/day from a 15-liter culture) of semipure disordered \(\gamma\)-MnO2, identified by X-ray diffraction and Fourier transform infrared (FTIR) spectroscopy, and had a manganese oxide O/Mn ratio of 1.92. The Mn(IV) content in the oxide was low (30.5%) compared with that of mined or chemically formed \(\gamma\)-MnO2 (ca. 50%). The shortfall in the bacterial oxide manganese content was due to biological and inorganic contaminants. FTIR spectroscopy, transmission electron microscopy, and electron diffraction studies have identified manganite as a likely intermediate product in the formation of disordered \(\gamma\)-MnO2.

In mass algal cultures, some form of agitation is usually provided, which amongst others, moves the organisms through an optically dense profile. During this transport, fluctuations in the light energy supply are perceived by the algae, which are of the order
of 1 Hz and less. Additional to these variations the cultures are subject to diurnal, seasonal and climatic light variations. It has been suggested that turbulence with the resultant light/dark cycles enhances their productivity. However, turbulence has two major influences on an organism, i.e. it facilitates fluctuating light regimes and decreases the boundary layer which results in an increased exchange rate between the organism and its environment. With the aid of oxygen liberation measurements, the influence of fluctuating light regimes on productivity was measured. No simple relation existed, but no enhancement of productivity could be shown at cycles of 1-0.0038 Hz. Short term physiological changes were found to influence productivity severely.

107 
Preparation of Protoplasts from the Carrageenophyte Gigartina corymbifera (Kutz.) J. Ag. (Rhodophyta)
Gross, W.
Descriptors: rhodophyta; protoplasts; viability; isolation techniques; carrageenan; enzymes; mixtures; pseudomonas; cell walls; genetic engineering; genetic improvement
DNAL Call No.: QR65.J68
Abstract:
Protoplasts were isolated with high yield from the carrageenophyte Gigartina corymbifera (Kutz.) J. Ag. by using the enzyme carrageenase in combination with cell wall-digesting enzymes. The enzyme mixture consisted of 5 U carrageenase.ml-1, 2% cellulase, 2% Macerozyme R-10, and 0.2% Pectolyase Y-23 dissolved in 60% seawater containing 0.7 M mannitol, 5mM CaCl2, and 40 mM Tris-HCL, pH 7. Carrageenase was prepared from cultures of the marine bacterium Pseudomonas carrageenovora. Protoplasts from G. corymbifera were spherical, dark red-colored and very uniform in size (approximately 17 micrometer); they originated solely from the epidermal tissue and > 95% of the protoplasts were viable. Freshly prepared protoplasts lack cell walls and polysaccharide sheaths, as demonstrated by electron microscopy and specific staining methods. Spontaneous cell fusion was observed on several occasions. These protoplasts could serve as a useful tool in crop improvement of this important carrageenan-producing alga.

108 
Chemical and Physical Properties of Algal Polysaccharides used for Cell Immobilization
Guiseley, K.B.
Descriptors: review; microorganisms; enzymes; biotechnology industry; crosslinking; algin; agar; agarose; carrageenan
DNAL Call No.: TP248.E5E565

109 
Automatic On-line Growth Estimation Method for Outdoor Algal Biomass Production
Guterman, H.; Ben-Yaakov, S.; Vonshak, A.
Descriptors: oxygen; production rate; photosynthetic respiration; industrial application; wastewater treatment; automation; biotechnology industry
DNAL Call No.: 381 J8224
Abstract:
An on-line measuring procedure for estimating productivity in outdoor algal cultures was developed and tested experimentally. The procedure is based on a previously described method for on-line measuring net O2 production rate (OPR). The data obtained by this method was found to correlate well with the conventional procedures for estimation
productivity by measuring the changes in biomass concentration in the culture. The new procedure seems to be superior to the latter since it can be carried out in an almost continuous way and can give immediate indication on the productivity. OPR could be used to monitor on-line the photosynthetic and/or respiration activity in small research fermentors or in large-scale open systems outdoors.

110

**Gamma Linolenic Acid Production by Microalgae**

Hirano, M.; Mori, H.; Miura, Y.; Matsunaga, N.; Nakamura, N.; Matsunaga, T.


*Descriptors*: spirulina-platensis; fermentation biotechnology

*DNAL Call No.:* QD415.A1J62

111

**Effect of Light and Carbon Dioxide on Biopolymer Production by the Unicellular Red Alga Porphyridium-Cruentum**

Iqbal, M.; Stepan-Sarkissian, G.; Grey, D.; Fowler, M.W.


*Descriptors*: abstract; algae; food industry use; biotechnology

*DNAL Call No.:* TP248.65.F66F66

112

**Screening Test for Deodorizing Substance from Marine Algae and Identification of Phlorotannins as the Effective Ingredients in Eisenia-bicyclis**

Kita, N.; Fujimoto, K.; Nakajima, I.; Hayashi, R.; Shibuya, K.


*Descriptors*: eisenia-bicyclis; ecklonia-cava; ecklonia-kurome; mercaptan; trapping effect; chlorophyll; sodium; copper; chlorophyllin; biotechnology

*DNAL Call No.:* QK564.J68

*Abstract:*

Aqueous extracts from 33 species of marine algae were assessed for their methyl mercaptan-trapping activity by gas chromatography to search for novel natural oral deodorants. Brown algae belonging to the Laminariales such as Eisenia bicyclis, Ecklonia cava and Ecklonia kurome were found to show remarkable deodorizing action against methyl mercaptan. The effective components in Eisenia bicyclis were identified as a phlorotannin, a group of molecules which are characteristic components of Laminariales. In addition phlorotannins extracted from E. bicyclis were more effective at reducing methyl mercaptan than conventional natural deodorants such as chlorophyll and sodium copper chlorophyllin.

113

**Microbial Production of Hydrogen**

Kosaric, N. and Lyng, R.P.


*Descriptors*: review; algae; cyanobacteria; bacteria; trichomonads; hydrogenases; nitrogenases; formate; hydrogenylase; biotechnology

*DNAL Call No.:* QR53.B52
**Abstract:**
The kinetics of the reduction of externally added 2-hydroxy-1,4-naphthoquinone by blue-green algae of the strains Anabaena PCC 7120 and Anacystis nidulans PCC 6301 were studied in aqueous cell suspensions by electrochemical monitoring of the concentration of the formed hydroquinone. This reaction is of potential interest for bioelectrochemical fuel cells. The experimental curves obtained could be interpreted by a model that takes into account that both substrate and product have to be transported through the microbial cell walls and that the conversion reaction takes place with first-order kinetics within the microbial cells. No clear evidence was found for the involvement of photosynthesis. It is suggested that the reduction of the quinone probably occurs via the enzyme catalyzed oxidation of endogenous storage product(s), presumably glycogen.

**Market Applications for Microalgae**
Kyle, D.

**Abstract:**
The marine phytoplankter Tetraselmis suecica was grown in shallow outdoor flumes for a period of approximately 6 months at the Natural Energy Laboratory of Hawaii. In full sunlight, gross production rates were 15-20 g C m-2 d-1. The corresponding photosynthetic efficiencies (PE's) were 9-10%. Respiration losses removed about half the gross production. The CO2 utilization efficiencies of 96 ±. 11% were achieved by bubbling CO2 into the culture with the use of a counterflow sump system. Adding the CO2 in the form of carbonated water resulted in utilization efficiencies of 81 ±. 11%.

Archimedes screws proved superior to both paddle wheels and propellers as a means of circulating the water in the flumes. Insertion of foil arrays into the flumes to effect systematic mixing of the culture significantly enhanced production. The enhancement was greater when the foils were oriented at a small angle relative to the horizontal than when they were oriented at the same angle relative to the vertical. Light modulation effect are implicated as the probable cause of most of the enhancement. Substitution of electric power plant stack gases for pure CO2 resulted in no significant change in the production of T. suecica grown in chemostat culture.
117
Protoplast Production in Chondrus-crispus Gametophytes Gigartinales Rhodophyta
Le Gall, Y.; Braud, J.P.; Kloareg, B.
Descriptors: marine bacteria; cellulase; carrageenase; algae regeneration; cell culture; yield; crop industry; biotechnology industry
DNAL Call No.: QK725.P54
Abstract:
Protoplasts were isolated from female gametophytes of Chondrus crispus (Stackh.) using commercial cellulase and various carrageenases prepared from marine bacteria. Depending on the nature of the donor tissue (apices or whole thallus, wild or cultivated strains), yields ranged from 1.0-8.5 \times 10^8 protoplasts per gram of fresh tissue. Preincubating the tissue with a potassium chelator, Kryptofix 222, enhanced protoplast yields by 30-50% based on staining with fluorescein diacetate most protoplasts were viable. A few protoplasts regenerated a cell wall and divided.

118
Glutamate Production from Carbon Dioxide by Marine Cyanobacterium Synechococcus-sp Using a Novel Biosolar Reactor Employing Light-Diffusing Optical Fibers
Descriptors: biotechnology
DNAL Call No.: QD415.A1J62

119
Opportunities and Applications of Biotechnology in the Food Industry
Meek, S.D.
Descriptors: food industry; biotechnology; food quality; ingredients; food processing; cheeses; algal cultures; patents; secondary metabolites; food wastes
DNAL Call No.: TP368.F662

120
Metzger, P.; Largeau, C.; Casadevall, E.
Descriptors: algae; lipogenesis; hydrocarbons; fatty acids; triacylglycerols; sterols; carotenoids; cell walls; polymers; aldehydes; biosynthesis; biotechnology; geochemistry; literature; reviews
DNAL Call No.: 384 F773

121
Mechanism of Adaptation and Hydrogen Photoproduction in a Marine Green Alga Chlamydomonas-sp MGA 161
Miyamoto, K.; Nawa, Y.; Matsuoka, S.; Ohta, S.; Miura, Y.
Descriptors: microorganism; fermentation; biotechnology industry
DNAL Call No.: QP601.A1J6
Abstract:
The adaptation process in a marine green alga, Chlamydomonas sp. MGA 161 was studied together with its light-dependent hydrogen evolution in terms of photosystem involvement and electron donation, and these processes were compared with those in Chlamydomonas reinhardtii. Hydrogen production in the light by MGA 161 was only a little more than that in the dark. Hydrogen metabolism in the illuminated cells of MGA 161 depended not on water but on cellular starch for as electron source.

Construction of Multiple Herbicide Resistant Ammonia Excreting Strains of Cyanobacterium Nostoc muscorum

Descriptors: nostoc muscorum; strains; gloecapsa; herbicides; herbicide resistance; phenotypes; dna; genetic transformation; gene transfer; mutations; ammonia; excretion; photosystem ii; nitrogen fixation
DNAL Call No.: QR53.B56
Abstract:
Machete resistant (Matr), basalin resistant (Basr), 3(3,4 dichlorophenyl)-1,1-dimethyl urea resistant (DCMU(r)), atrazine resistant (Atr(r)) and propanil resistant (Prpr) phenotypes Gloecapsa sp. were contransformed to Nostoc muscorum at high frequency. Spontaneously occurring mutants of the multiple herbicide resistant transformant containing L-methionine-DL-sulfoximine resistant (Msr), ethylene diamine resistant (Edar) of phosphinothricin resistant (Ptr) glutamine synthetase (GS) showed extracellular liberation of ammonia resulting from fixation of N2 under photosynthetic conditions. Results suggest a definite role of GS activity in regulation of extracellular ammonia.

European Bioconversion Projects and Realizations for Macroalgal Biomass St.-Case-La Guildo France Experiment

Morand, P.; Charlier, R.H.; Maze, J.
Descriptors: laminaria ulva; biomass utilization; compost biotechnology
DNAL Call No.: 410 H992

Storm Wrack of Marine Algae and Grasses as Raw Material for Bioconversion

Mun, T.H.; Kondrat'eva, L.M.; Garetova, L.A.
Descriptors: marine areas; algae; grasses; biomass production; raw materials; conversion; biotechnology
DNAL Call No.: TP248.13.S68

The Application of Two-Phase Aqueous Systems to the Purification of Phycoerythrin from Synechococcus-sp DC-2

Niven, G.W.; Smith, S.J.; Andrews, A.T.
Descriptors: algae; marine; cyanobacterium; biotechnology industry
DNAL Call No.: TP248.24.B55
Abstract:
The potential of aqueous two-phase systems for the purification of phycoerythrin from a
marine cyanobacterium was investigated. Purities in excess of 90% total soluble protein were obtained in a single step processes and separation of two polymeric forms of phycoerythrin was achieved.

126
Market Applications for Microalgae
Rattray, J.B.M.
Descriptors: biotechnology industry; fats and oils
DNAL Call No.: 307.8 J82

127
Microbial Production of Glycerol and other Polyols
Rehm, H.J.
Descriptors: review; yeast; bacteria; algae; biotechnology
DNAL Call No.: QR53.B52

128
Coupling of Solar Energy to Hydrogen Peroxide Production in the Cyanobacterium Anacystis-nidulans
Roncel, M.; Navarro, J.A.; De La Rosa, M.A.
Descriptors: anacystis nidulans; hydrogen peroxide; biosynthesis; solar energy; photosynthesis; immobilization
DNAL Call No.: 448.3 AP5
Abstract:
Hydrogen peroxide production by the blue-green algae (cyanobacteria) under photoautotrophic conditions is of great interest as a model system for the bioconversion of solar energy. Our experimental system was based on the photosynthetic reduction of molecular oxygen with electrons from water by Anacystis nidulans 1402-1 as the biophotocatalyst and methyl viologen as a redox intermediate. It has been demonstrated that the metabolic conditions of the algae in their different growth stages strongly influence the capacity for hydrogen peroxide photoproduction, and so the initial formation rate and net peroxide yield became maximum in the mid-log phase of growth. The overall process can be optimized in the presence of certain metabolic inhibitors such as iodoacetamide and p-hydroxymercuribenzoate, as well as by permeabilization of the cellular membrane after drastic temperature changes and by immobilization of the cells in inert supports such as agar and alginate.

129
Potential Production of Protoplasts from Porphyridium-sp Using an Enzymatic Extract of its Predator Gymnodinium-sp
Roth-Bejerano, N.; Van Moppes, D.; Sivan, A.; Arad, S.
Descriptors: algae; species; genetic engineering; protoplasts; production; genetic improvement; gymnodinium; enzymes; extracts; cell walls; degradation; osmotic pretreatment; respiration rate; photosynthesis; growth curve; cell division
DNAL Call No.: TD930.A32
Abstract:
Production of biochemicals from red algae will become an agro-industrial reality only after
improvement of strain through genetic manipulation has been achieved. In the absence of
sexual reproduction, preparation of protoplasts is a pre-requisite for genetic improvement
of new strains. Although preparation of protoplasts from plant cells is a common
technique, its application in red algae was limited. The unicellular alga Porphyridium sp.
is encapsulated in a sulfated polysaccharide, the structure of which is still not fully known.
A crude extract of a dinoflagellate Gymnodinium, a natural predator of Porphyridium cells
in open cultures, was found to degrade Porphyridium sp. polysaccharide enzymatically.
Porphyridium cells treated with the crude Gymnodinium extract were exposed to various
osmotic media (0-1.5 M sucrose), and their volume was measured. Volume increase was
observed in diluted sucrose solutions up to 0.175 M. While further dilution of the
external osmoticum to 0.1 M had little effect, dilution to 0.0 M (distilled water) led to cell
rupture. Elevated concentrations of external osmoticum resulted in shrinkage of the
treated cells. Such osmotic behavior indicates exposure of the cells and thus cleavage of
the capsule. The treatment did not affect the viability of the cells, as evidenced by
fluorescein diacetate (FDA) fluorescence, nor did it affect the respiration rate, but it did
lower the photosynthetic rate to some extent. The growth curves for the treated cells
exhibited a longer lag time than in the non-treated controls. Lowering the NaCl content
in the growth medium resulted in a further increase in the lag time of the treated cells.
These results indicate that the treatment lowers the ability of Porphyridium cells to divide.
Ability to divide is eventually recovered with time, the recovery apparently depending
upon the external osmoticum. The results indicate that Gymnodinium crude extract
degrades Porphyridium cell wall and thus can be used for protoplast production.

130
Ammonium Photoproduction by Free and Immobilized Cells of Chlamydomonas-reinhardtii
Santos-Rosa, F. and Galvan, F.
Descriptors: calcium alginate-entrapped cells; chlorophyll membrane permeability; cell-matrix
interaction; fermentation; biotechnology industry
DNAL Call No.: QR1.E9
Abstract:
Free-living or immobilized Chlamydomonas reinhardtii cells photoproduce ammonium
from nitrite in a medium containing 1 mM of L-methionine-D,L-sulphoximine (MSX). Ammonium is
accumulated in the medium to 8 mM final concentration, which inhibits
nitrite uptake by the MSX-treated cells and consequently the excretion of ammonium is
blocked. However, if ammonium was removed from the medium and nitrite and MSX
periodically restored, the photoproduction process could be maintained over 96 h, with a
final ammonium concentration of about 18 mM for free-living cells and 28 mM for
immobilized ones. The MSX-treated cells showed a photoproduction productivity of 1300
.mu.mol NH4+ .cntdot. mg chlorophyll (Chl)-1, with an average production rate of 14
.mu.mol NH4+ .cntdot. mg chlorophyll Chl-1 per hour, for calcium alginate-entrapped
cells, while the corresponding data for free-living ones was 650 .mu.mol NH4+ .cntdot.
mg Chl-1 and 6.7 .mu.mol NH4 .cntdot. mg Chl-1 per hour, respectively. Immobilized
cells showed a significant increase in the nitrite uptake rate, probably due to a change in
membrane permeability as a consequence of cell-matrix interactions.

131
Biological Viability of Chlamydomonas-reinhardtii Cells Entrapped in Alginate Beads for Ammonium
Photoproduction
Santos-Rosa, F.; Galvan, F.; Vega, J.M.
Descriptors: photosynthesis; biotechnology
DNAL Call No.:
Abstract:
The green alga Chlamydomonas reinhardtii was immobilized by entrapment in Ca2+-alginate gel for ammonium photoproduction. The physical characteristics of the beads, their stability and ammonium-photoproduction capacity were affected by a variety of interactive factors, including cell loading, alginate viscosity and concentration, and the nature of the buffered medium. Electron micrographs show alterations in the morphology of the immobilized cells. Entrapped C. reinhardtii cells retained their biological viability, maintaining normal photosynthetic and respiratory activities, and they grew inside the gel beads, with a generation time of 9 h as compared with the 8 h shown by free cells under similar conditions. The unusually high rates for nitrite uptake and ammonium photoproduction, as well as the nitrate reductase activity shown by alginate-immobilized cells, were related to the changes in the membrane permeability induced by cell-matrix interaction.

132
Photoproduction of Ammonium by Chlamydomonas-reinhardtii Cells Immobilized in Barium Alginate a Reactor Feasibility Study
Santos-Rosa, F.; Galvan, F.; Vega, J.M.
Descriptors: chlamydomonas reinhardtii; ammonia; immobilization; barium; alginites
DNAL Call No.: QR1.E9
Abstract:
Chlamydomonas reinhardtii cells immobilized in Ba-alginate beads provide a stable and effective system for photoproducing ammonium from nitrite in a culture medium containing L-methionine-D,L-sulphoximine. The process was studied in either air-lift, fluidized- or packed-bed reactors, the last one providing the most suitable system with a volumetric activity of 2700 .mu.mol NH4+.cntdot.1-1 per hour.

133
Pharmaceuticals from Cultured Algae
Schwartz, R.E.; Hirsch, C.F.; Sesin, D.F.; Flor, J.E.; Chartrain, M.; Fromtling, R.E.; Harris, G.H.; Salvatore, M.J.; Liesch, J.M.; Yudin, K.
Descriptors: axenic; cyanobacteria; bacteria; microorganism; pachydictyol caulerpenyne hapalindoles; antifungal depsipeptide; biotechnology industry; fermentation
DNAL Call No.: QR53.J68
Abstract:
An alage screening program, including cultured macroalgae, cultured cyanobacteria and cultured eukaryotic microalgae has been undertaken. Methods for the isolation, purification, preservation and cultivation of axenic cyanobacteria and eukaryotic cultures have been developed. Screening of these groups for biologically active components has lead to the isolation of pachydictyol and caulerpenyne from cultured macroalgae, while a series of hapalindoles and an antifungal depsipeptide have been isolated from cyanobacteria.

134
Applications of Some Algal Polysaccharides in Biotechnology
Skjak-Braek, G. and Martinsen, A.
Descriptors: marine; cell culture medium; catalyst; immobilization; food technology; biomedics
The Novel Non-Heme Vanadium Bromoperoxidase from Marine Algae Phosphate Inactivation
Soedjak, H.S.; Everett, R.R.; Butler, A.
Descriptors: industrial; enzyme stability; biotechnology industry
DNAL Call No.: QR35.J68
Abstract:
Vanadium bromoperoxidase is a naturally occurring vanadium-containing enzyme isolated from marine algae. V-BrPO catalyzes the oxidation of halides by hydrogen peroxide which can result in the halogenation of organic substrates. Bromoperoxidase activity is measured by the halogenation of monochlordimedone (2-chloro-5,5-dimethyl-1,3-dimedone, MCD). In the absence of an organic substrate, V-BrPO catalyzes the halide-assisted disproportionation of hydrogen peroxide yielding dioxygen. The dioxygen formed is in the single excited state (1O2). V-BrPO is quite stable to thermal denaturation and denaturation by certain organic solvents which makes V-BrPO an excellent candidate for industrial applications. The stability of V-BrPO in the presence of strong oxidants and in the presence of phosphate is reported. Incubation of V-BrPO in phosphate buffer (1-100 mM at pH 6; 2-10 mM at pH 5) inactivates the enzyme. The inactivity can be fully restored by the addition of vanadate if excess phosphate is removed. The inactivation of V-BrPO by phosphate can be prevented by the presence of H2O2 (4-40 mM). We are currently investigating the mechanism of V-BrPO inactivation by phosphate. V-BrPO was not inactivated by HOCl (1 mM) nor H2O2. In addition V-BrPO was not inactivated under turnover conditions of 1 mM H2O2 with 0.1-1 M Cl- at pH 5 nor 2 mM H2O2 with 0.1 M Br-.

Environmental Control of Lipids and Fatty Acid Production in the Diatom Navicula-saprophila
Sriharan, S.; Bagga, D.; Sriharan, T.P.
Descriptors: navicula; lipids; fatty acids; production; temperature relations; biomass; renewable resources
DNAL Call No.: QD415.A1J62

Biological Active Substances from Algae
Taeymans, D.
Descriptors: phycocolloids; enzymes; vitamins; amino acids; coloring agents; single cell protein; single cell lipid; biotechnology

Glycolate Photoproduction by Free and Alginate-Entrapped Cells of Chlamydomonas-reinhardtii
Vilchez C.; Galvan, F.; Vega, J.M.
Descriptors: algae; immobilization; biotechnology industry
DNAL Call No.: QR1.E9
Abstract:
Chlamydomonas reinhardtii cells provide an effective system for glycolate photoproduction, operative during 30 h when they are growing under low CO2, in the presence of 1 mM aminoxyacetate and 50 .mu.M acetazolamide. Glycolate excretion by
the cells can proceed for about 4 days if every other 12 h a high CO2 level is restored in the culture in the absence of inhibitors. The immobilized system in alginate beads has about a twofold higher glycolate photoproduction rate \((23 \times \mu\text{mol cdot mg chlorophyll (Chl)-1 .cdot mg h-1)}\) than free-living cells \((12 \times \mu\text{mol cdot mg Chl-1 .cdot mg h-1)}\).

139

*Production of L. Leucine from Alpha Ketoisocaproic Acid by Cell-Free Extract of Euglena-gracilis Z.*


**Descriptors:** algae; alpha ketoglutarate decarboxylase; biotechnology industry  
**DNAL Call No.:** QP601.A1J6

**Abstract:**

L-Leucine was produced from .alpha.-ketoisocaproic acid at about 100% conversion with L-glutamate as an amino donor using cell-free extracts of Euglena gracilis Z..alpha.-ketoglutarate decarboxylase in Euglena drives the conversion to completion by removal of .alpha.-ketoglutarate formed during the transamination.

140

*Isolation and Analysis of Nitrate Reductase Deficient Mutants for use in Genetic Engineering of Microalgae for Increased Fuel Production.*

Zeiler, K.G. and Brown, L.M.

**Source:** JOURNAL OF PHYCOLOGY 27(3 suppl.):80 (1991).

**Descriptors:** abstract; monoraphidium-minutum; cyclotella-cryptica  
**DNAL Call No.:** QK564.J6

**Bioremediation Using Algae**

141

*A Bioseparation Process for Removing Lead-II Ions from Waste Water by Using Chlorella-vulgaris.*

Aksu, Z. and Kutsal, T.


**Descriptors:** algae; microorganism; metals; wastewater treatment; biotechnology industry; batch reactor; bioreactor  
**DNAL Call No.:** QR53.J685

**Abstract:**

Biosorption of heavy metals by microbial cells has been recognized as a potential alternative to existing technologies for removing heavy metals from industrial waste waters. Many aquatic microorganisms, such as algae, can take up dissolved metals from their surroundings to their cells. In this study, the adsorption of lead(II) ions was investigated in a single-staged batch reactor. Chlorella vulgaris, a green alga, was used as the sorbent. The sorption phenomenon was expressed by the Freundlich adsorption isotherm and this expression was used for the calculation of residual or adsorbed metal ion concentration at equilibrium \((Ceq \ or \ Cx, eq)\) at a given 'volume of waste water containing heavy metal ion/quantity of alga \((Vo/Xo)' ratio in a single-staged batch reactor. Experimental \(Ceq\) and \(Cx, eq\) values were compared to calculated ones. Applications in waste water treatment for lead (II) removal have been suggested.
142
Review of Biotechnology Applications to Nuclear Waste Treatment
Ashley, N.V. and Roach, D.J.W.
Descriptors: review; algae; bacteria; fungus; metal; radionuclide biosorption; biopolymer; industrial waste treatment
DNAL Call No.: QR53.J685

143
Microbiotests in Aquatic Ecotoxicology Characteristics Utility and Prospects
Blaise, C.
Descriptors: bacteria; protozoa; microalgae; invertebrate; fish cell line; hazard assessment; biotechnology; immunochemistry; cost effectiveness
Abstract:
Small-scale biological tests (microbiotests) have steadily increased in development and application over the last 30 years in the field of aquatic ecotoxicology. Multitrophic level assessment requirements, attractive features of microbiotests, and the constant search for simplicity and cost efficiency of testing are reasons explaining the expanding use of microbiotests. In this article, the major characteristics that advantageously confer popularity on microbiotests are presented and 25 currently applied aquatic toxicity microbiotests are listed. Conducted with bacteria, protozoans, microalgae, small invertebrates, and fish cell lines, these microbiotests represent a realistic cross section of those that are now becoming an essential part of ecotoxicological assessment. Microbiotests can be profitably employed for ranking and screening chemicals, for novel applications enabling rapid detection of ecotoxic effects in complex liquid samples, and for increasing the cost efficiency and diagnostic potential of hazard assessment schemes. Microbiotesting research, development, and applications will continue to surge in the 1990s, driven, among other factors, by the imperative need for cost effectiveness in environmental programs. Research in the fields of ecotoxicology, biotechnology, and immunochemistry should provide interesting breakthroughs to further enhance the specificity and diagnostic value of microbiotests.

144
Selection of Surrogates for a Genetically Engineered Microorganism with Cellulolytic Capability for Ecological Studies in Streams
Bott, T.L. and Kaplan, L.A.
Descriptors: cellulomonas-sp; cellulomonas-uda; cladophora-glomerata; liriodendron-tulipifera; genetic engineering; statistics; biodegradation; flowing-water microcosms
DNAL Call No.: 448.8 C162
Abstract:
Aerobic cellulolytic bacteria were ranked according to ability to degrade cellulose azure and to clear cellulose agar. Cellulomonas uda NRRL B404 and Cellulomonas sp. NRC 2406 showed greater clearing of cellulose agar than other isolates, but differences in cellulose azure decomposition were not statistically significant. Isolates were tested for ability to accelerate decomposition of tulip poplar (Liriodendron tulipifera) leaves and Cladophora glomerata (Chlorophyta) detritus in stream water. There was significantly more cellulose lost from leaves exposed to Cellulomonas flavigena NRC 2403, Cellulomonas fimi NRRL B402, Cellulomonas sp. NRC 2406, and Cellvibrio gilvus ATCC 13127 and NRC 2407 than in the stream-water control, and the weight losses of leaves
exposed to some isolates were significantly greater than in the control. There was significantly more cellulose lost from Cladophora glomerata detritus exposed to these and five other isolates, and there were greater weight losses than in the stream-water control. Cellulomonas uda NRRL B404 was the slowest growing isolate, although growth rates of isolates did not differ statistically. Cellulomonas uda NRRL B404, Cellulomonas sp. NRC 2406, Cellulomonas fimi NRRL B402, Cellulomonas flavigena NRC 2403, and Cellulibrio gilvus ATCC 13127 were selected as the best candidates for larger scale experiments. Persistence of Cellulomonas uda, Cellulomonas sp. NRC 2406, and Cellulomonas sp. CS1-1 in stream-bed sediments was studied in flowing-water microcosms, using fluorescent antibodies and epifluorescence microscopic counts to assess densities of target cells. Isolate densities declined from postinoculation maxima, but organisms were detected 2-4 weeks later in three different experiments.

145
Applied Microbial Processes for Metals Recovery and Removal from Wastewater
Brierley, C.L.; Brierley, J.A.; Davidson, M.S.
Descriptors: review; bacteria; fungi; algae; biotechnology
DNAL Call No.: QR.92.M45M47

146
Options for the Rational Design and Operation of Oxidation Ponds
Carberry, J.B.
Descriptors: incident sunlight; diurnal; pH variation; calcium chloride addition; wastewater treatment; algal bacterial clay treatment system; computer model; methods; biotechnology; microorganism

147
Method for Evaluating Algal Production and Degradation Based on Nitrogen Levels in Particulate Organic Matter
De Casabianca-Chassany, M.L.
Descriptors: ulva-rotundata; algae; free algal biomass; algal growth; carbon level; photosynthesis; coastal lagoon; water pollution; environmental quality; biotechnology industry
DNAL Call No.: TD930.A32
Abstract:
A population of Ulva rotundata (Bliding, C., A critical survey of European taxa in Ulvales. Part I. Opt. Bot. Lund, A8 (3), 1-160) was studied for 7 months in a shallow brackish eutrophic lagoon (Prevost Lagoon, Languedoc, France). Biomass was measured in square-meter quadrates, and algal growth in on-site cages. Carbon and nitrogen levels were spatiotemporally monitored in Ulva thalli and in particulate organic matter. Thalli rapidly degraded into particulate matter from the surface to the bottom, increasing nitrogen concentrations and decreasing carbon concentrations. We propose a method for estimating algal production and possible carbon loss in this type of lagoon, based on nitrogen levels in particulate organic matter. Algal production over the whole period can be evaluated by adding instantaneous biomass levels corresponding to the main particulate nitrogen peaks.

148
Accumulation of Metals by Microorganisms and Algae

49
Removal of Lead from Contaminated Soil and Water Using a Mixed Microbial Ecosystem
Ibeanusi, V. and Archibold, E.
Source: ABSTR ANNU MEET AM SOC MICROBIOL 90(0):327 (1990).
Descriptors: abstract; bacteria; algae pond; biotechnology; bioremediation
DNAL Call No.: 448.39.S012A

Mechanisms of Mixed Microbial Mobilization and Recovery of Heavy Metals in a Simulated Pond System
Ibeanusi, B.; Archibold, E.; Bender, J.; Gould, J.
Source: ABSTR ANNU MEET AM SOC MICROBIOL 89(0):362 (1989).
DNAL Call No.: 448.39.S012A

Accumulation of Cobalt by Marine Alga
Kuyucak, N. and Volesky, B.
DNAL Call No.: 381 J8224

Desorption of Cobalt-Laden Algal Biosorbent
Kuyucak, N. and Volesky, B.
Descriptors: ascophyllum-nodosum accumulation; metals; calcium chloride solution; cellular
Sorption

Descriptors:

Liu desorption

Source:

DNAL

Descriptors:

Kuyucak, 155

The DNAL structure; H-H;

Call SCIENCE

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wall incubated radionuclides occurs mechanism dispersion uptake Nonliving accumulating 60.degree.C. of effect H2S04, nodosum, Following of N. strontium-90 and yttrium-90 by Anacystis Cells

Liu H-H; Chen, W-L; Wu, J-T.


Descriptors: biosorbent; biotechnology; respiration; passive adsorption; light vs. dark acid desorption

Abstract:

A study of the sorption and desorption of strontium-90 and yttrium-90 by Anacystis cells showed a concentration factor of 4 .times. 104 ml g-1. The uptake of both radionuclides by the cells was very rapid and a linear relationship exists between the amount of radionuclides accumulated in the cells and those in the surrounding medium. The results show that the uptake of Sr/Y by the cells is primarily by passive adsorption rather than active absorption. There was no significant difference in the sorption rate between cells incubated in the light and those left in the dark. However, both heat treatment and the addition of a respiration inhibitor reduced the sorption of radionuclides by up to 10%. The radionuclides accumulated in the cell and could readily be desorbed by washing with
acid. Thus, this organism can be used as a biosorbent for concentrating and removal of these two radionuclides.

156

Bioremoval of Copper and Uranium from Solution Using Alginate-Immobilized Microcystis-spp
Lopez, S.L.; Pryfogle, P.A.; Stoner, D.L.; Dugan, P.R.
Source: ABSTR ANNU MEET AM SOC MICROBIOL 90(0):327 (1990).
Descriptors: abstract; biotechnology
DNAL Call No.: 448.39.S012A

157

Evaluation of the Metal Uptake of Several Algae Strains in a Multicomponent Matrix Utilizing Inductively Coupled Plasma Emission Spectrometry
Mahan, C.A.; Majidi, V.; Holcombe, J.A.
Descriptors: chlorella-pyrenoidosa; stichococcus-bacillaris; chlamydomonas-reinhardtii; blue-green algae; iron; lead; copper; analytical preconcentration technique; biotechnology industry
DNAL Call No.: 381 J825A

Abstract:
Three freshwater heat-killed, lyophilized blue-green algae strains have been characterized as to their ability to accumulate heavy metals with a focus on the utilization of these algae as an analytical preconcentration technique. This study examines the metal uptake in several multicomponent mixtures by using inductively coupled plasma optical emission spectrometry (ICP-OES). Six milligrams of a pure strain of algae was added to 20-mL aliquots of buffered (pH 5.5-6.5) multielement solutions containing 0.1, 0.5, 1.0, 2.0, and 4.0 mg/L of K, Mg, Ca, Fe, Sr, Cu, Mn, Ni, V, Zn, As, Cd, Mo, Pb, and Se. All three algae strains exhibit relatively high adsorption affinities for Fe, Pb, and Cu, with uptake between 70 and 98% at the 4 ppm concentration level. Biosorption occurs for essentially every element with the relative affinities decreasing in the order Pb > Fe > Cu > Cd > Zn > Mn > Mo > Sr > Ni > V > Se > As > Co for Chlorella pyrenoidosa at the 4 mg/L concentration level. Although some minor differences were seen, the other algae strains (Stichococcus bacillaris and Chlamydomonas reinhardtii) displayed similar adsorption behavior over the concentration range studied, indicating similar cell wall binding sites. Langmuirian isotherms exhibited a minimum of two slopes over the concentration range of 0.1-4.0 mg/L, indicating the probable existence of a least two adsorption mechanisms.

158

Retardation of Toxic Heavy Metal Dispersion from Nickel-Copper Mine Tailing Sudbury District Ontario Canada Role of Acidophilic Microorganisms II. Structure and Microanalysis of Bioprecipitants
Mann, H.; Tazaki, K.; Fyfe, W.S.; Wiseman, M.
Descriptors: algae; carbon; oxygen; iron; sulfur mineralization; cellular deposits; biotechnology
DNAL Call No.: TA418.74B56

Abstract:
Fe-rich sediments in the tailings effluent of the Sudbury Ni, Cu mining area, northern Ontario, are predominantly magnetite and ferrhydrite, with subordinate akaganeite (\(\gamma\)-FeOOH), goethite (\(\alpha\)-FeOOH), hematite (\(\alpha\)-Fe2O3) and vivianite [\(\text{Fe}_3(\text{PO}_4)2.8\text{H}_2\text{O}\)]. The iron is derived from biologically mediated oxidative breakdown of Fe-sulphide minerals in the mine tailings waste, which generates acidic effluent with enhanced concentrations of aqueous Fe and SO42-. Surface analysis of the sediments by ESCA reveals that C (57 atom %), O (31%) and Fe (6.7%) are most abundant, in accord
with the presence of ubiquitous acidophilic microorganisms and their remnants, and Fe-oxide and oxyhydroxide minerals. The high resolution spectra of S (S2p) indicates partitioning of S between SH (163.4 eV), SO3 (167.3 eV) and SO42- oxidation states, with SO42- the most abundant, as the mineral gypsum (CaSO4). Similarly, carbon (Cls spectrum) is partitioned between hydrocarbon (285 eV) and carbonate (289.2 eV); and iron (Fe2p spectrum) between FeS, Fe(CSH3)(CO)3, FeSO42- and FeOOH. Both C and Fe spectra show a relationship between organic and inorganic compounds, suggesting that Fe-mineralization is linked to the microorganisms. TEM electron micrographs revealed ubiquitous remains of microorganisms in the sediments, heavily encrusted with Fe-oxide and oxyhydroxide minerals. Magnetite, maghemite, ferricydrite, and geothite associated with cell walls, and intracellular sites, were identified from d-spacings of electron diffraction patterns. Nucleation of iron oxide minerals by acidophilic microorganisms appears to be ubiquitous in acidic Fe-rich tailings environments. The precise synthetic pathways of Fe mineral formation are not known.

159
Retardation of Toxic Heavy Metal Dispersion from Nickel-Copper Mine Tailing Sudbury District Ontario Canada Role of Acidophilic Microorganisms I. Biological Pathway of Metal Retardation
Mann, H.; Fyfe, W.S.; Kerrich, R.; Wiseman, M.
Descriptors: euglena; iron; molybdenum; thorium; aluminum; zinc; manganese; cadmium; titanium; sequestration; lake; biotechnology
DNAL Call No.: 159
Abstract:
Leaching of approx. 600 M tonnes of mine tailings waste from the Sudbury nickel-copper mining district, Ontario, by infiltration of precipitation and dewatering of tailings, results in the release of approx. 41,000 kg of Ni and other toxic heavy metals to the environment per year. Tailings effluent waters are variably acidic (pH 3.5-6.3) due to the generation of H2SO4 by the biologically-mediated oxidation of sulphide minerals in the tailings, and contain enhanced levels of Fe (200x), Ni (8300x), Cu (670x), Mo (240x) and Th (1,300x) relative to concentrations in world average river water. Acidophilic microorganisms (Euglena sp.) thrive in the low pH, heavy metal laden discharge, and act as efficient scavengers of the aqueous solutes, such that bulk samples of algae are characterized by abundances of Fe, Al, Zn, Mn, Cd, Ti and Ni 103 to 105 times the aqueous solute concentration (by dry weight). Where tailings discharge is treated with "limestone slurry" to raise the pH, organic-rich sediments in lakes are enriched in Ni, Cu and other heavy metals, endorsing the role of microorganisms in sequestering toxic heavy metals from solutions, thereby diminishing the metal loading, and retarding their dispersion into the natural environment.

160
A Photo-Bioreactor Using Algal Phototaxis for Solids-Liquid Separation
Nakajima, T. and Takahashi, M.
Descriptors: euglena-gracilis; algae culture medium; wastewater treatment; bioengineering
DNAL Call No.: TD420.W3
Abstract:
Using algal-positive photoaxis, the possibility of keeping a high density of algae and separating them from water (i.e. solids-liquid separation) for removal of nutritive substances was investigated. Euglena gracilis shows positive phototaxis. Culture medium containing the alga in a culture vessel (bioreactor) was transferred to a shaded vessel (i.e., photo-clarifier), part of which was exposed to a spotlight. The organisms gathering
around the light were returned to the culture vessel, and the effluent was taken out from the shaded part and discharged out of the system. Two types of bioreactors having different types of photo-clarifiers were employed: vertical form (Type A) and horizontal form (Type B). Densities of E. gracilis in the culture vessel and effluent were examined in both types over the experimental period, and compared with a control system without a photo-clarifier. In both types the E. gracilis density in the effluent was lower than that in the culture vessel, whereas in the control system the density in the effluent was almost identical with that in the culture vessel. Separation efficiency was higher in Type B than in Type A. The obtained results indicate that it is possible to achieve solids-liquid separation by using algal phototaxis, and suggest a possibility of further improvement in the separation efficiency by modifying the structure of the photo-clarifier.

161
*Comparative Water Quality Dynamics in a Recirculating Systems with Solids Removal and Fixed-Film or Algal Biofiltration*
Rakoczy, J.E.; Hargreaves, J.A.; Bailey, D.S.
Descriptors: abstract; oreochromis-niloticus; bacteria; biotechnology
DNAL Call No.: SH138.W62

162
*Phosphorus Uptake Kinetics of Immobilized Chlorella in Batch and Continuous-Flow Culture*
Robinson, P.K.; Reeve, J.O.; Goulding, K.H.
Descriptors: biotechnology; waste disposal reactor; stocking density; process optimization
DNAL Call No.: TP248.E5E565
Abstract:
Study has been made of the uptake of orthophosphate phosphorus (PO4-P) by Chlorella emersonii (CCAP 211/8a) entrapped in 4-mm diameter Ca-alginate beads. In batch culture studies, 100 beads, each stocked with 107 cells, removed all PO4-P from 100 ml synthetic growth medium (i.e. about 10 .mu.mol) in under 24 h. Uptake followed exponential kinetics with medium PO4-P concentration falling by 50% every 2.0 h, when external concentrations were above 6 .mu.M. A stocking density of 107 cell/bead (the highest tested) was found to be suitable for rapid phosphorus removal, though this was not necessarily optimal with respect to cellular efficiency. Small-scale packed-bed reactors containing 10 ml gel were able to remove up to 240 .mu.mol PO4-P from 4-5 l medium over 10-12 day experimental periods. Uptake efficiencies ranged from 29 to 97% and averaged 66% and 44% with synthetic growth medium and secondary treated effluent, respectively. Uptake kinetics are analyzed and results discussed with respect to process optimization.

163
*The Engineering of Microalgae Mass Cultures for Treatment of Agricultural Wastewater, with Special Emphasis on Selenium Removal from Drainage Waters*
Shelef, G.
Source: BIOTREATMENT OF AGRICULTURAL WASTEWATER. Huntley, Mark E., editor.
Descriptors: environmental pollution; agricultural wastes; drainage water; pollutants; selenium; waste water treatment; biotechnology; algae
DNAL Call No.: TD755.B48
164

An Aerobic Piggery Slurry Treatment System with Integrated Heat Recovery and High-Rate Algal Ponds

Svoboda, I.F. and Fallowfield, H.J.


Descriptors: biotechnology industry; metabolic heat; mathematical model; continuous culture reactor; livestock industry; agriculture; wastewater treatment; sewage disposal

165

Waste Water Treatment Using Saline Cultures of Microalgae

Toha, J.; Soto, M.A.; Cuadros, X.


Descriptors: aphanothece-sp; dunaliella-sp; escherichia-coli; microorganism; algae; bacteria; photosynthesis; stabilization pond; wastewater treatment; biotechnology industry

DNAL Call No.: TP248.24.B65

Abstract:

Survival of microorganisms (Escherichia coli has been used as an example) is affected by a combination of salinity and high pH induced by the active photosynthesis of marine microalgae (Aphanotece or Dunaliella sp.). This effect can be applied to create a more efficient wastewater treatment process using algal stabilization ponds.

166

Mercury Accumulation and Volatilization in Immobilized Algal Cell Systems

Wilkinson, S.C.; Goulding, K.H.; Robinson, P.K.


Descriptors: chlorella; algae; wastewater treatment methods; biotechnology

DNAL Call No.: QR53 B56

Abstract:

Rapid removal of mercury from growth medium and its uptake by free and alginentrapped Chlorella has been observed. Immobilized cell systems accumulated more mercury than free cell systems. In addition, both volatilized significant quantities of mercury. Studies show, however, that mercury lost in this way may re-enter the aqueous phase and subsequently be accumulated by immobilized cells.

167

Removal of Organochlorine Compounds in an Upflow Flocculated Algae Photobioreactor

Wu, X. and Kosaric, N.


Descriptors: chlorella scenedesmus; biodegradation; chlorobenzene 2 4 dichlorophenol; wastewater treatment; biotechnology industry; bioreactor
## AUTHOR INDEX

<table>
<thead>
<tr>
<th>Author Name</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.W. Coleman</td>
<td>16</td>
</tr>
<tr>
<td>Agarwal, G.P.</td>
<td>31</td>
</tr>
<tr>
<td>Akatsuka, I.</td>
<td>5</td>
</tr>
<tr>
<td>Akku, Z.</td>
<td>47</td>
</tr>
<tr>
<td>Al-Hasan, R.H.</td>
<td>31</td>
</tr>
<tr>
<td>Alam, J.</td>
<td>10</td>
</tr>
<tr>
<td>Allen, J.F.</td>
<td>27</td>
</tr>
<tr>
<td>Andrews, A.T.</td>
<td>42</td>
</tr>
<tr>
<td>Angsuthanasombat, C.</td>
<td>31</td>
</tr>
<tr>
<td>Anjanevulu, K.</td>
<td>32</td>
</tr>
<tr>
<td>Arad, S.</td>
<td>43</td>
</tr>
<tr>
<td>Archibald, E.</td>
<td>49, 50</td>
</tr>
<tr>
<td>Ariel, R.</td>
<td>16</td>
</tr>
<tr>
<td>Ariura, S.</td>
<td>41</td>
</tr>
<tr>
<td>Armisen, R.</td>
<td>32</td>
</tr>
<tr>
<td>Ashley, N.V.</td>
<td>47</td>
</tr>
<tr>
<td>Assali, N.E.</td>
<td>10</td>
</tr>
<tr>
<td>Avron, M.</td>
<td>6, 33</td>
</tr>
<tr>
<td>Babu, S.C.</td>
<td>32</td>
</tr>
<tr>
<td>Bachofen, R.</td>
<td>33</td>
</tr>
<tr>
<td>Bagga, D.</td>
<td>46</td>
</tr>
<tr>
<td>Bailey, D.S.</td>
<td>53</td>
</tr>
<tr>
<td>Baldauf, S.L.</td>
<td>10</td>
</tr>
<tr>
<td>Bancroft, L.</td>
<td>11</td>
</tr>
<tr>
<td>Bao, Y.</td>
<td>17</td>
</tr>
<tr>
<td>Becker, K.J.</td>
<td>32</td>
</tr>
<tr>
<td>Ben-Amotz, A.</td>
<td>6, 33</td>
</tr>
<tr>
<td>Ben-Yaakov, S.</td>
<td>7, 39</td>
</tr>
<tr>
<td>Bender, J.</td>
<td>50</td>
</tr>
<tr>
<td>Berning, J.L.</td>
<td>40</td>
</tr>
<tr>
<td>Birch, L.D.</td>
<td>33</td>
</tr>
<tr>
<td>Blaise, C.</td>
<td>47</td>
</tr>
<tr>
<td>Bloor, S.</td>
<td>34</td>
</tr>
<tr>
<td>Blowers, A.D.</td>
<td>11</td>
</tr>
<tr>
<td>Bogorad, L.</td>
<td>11</td>
</tr>
<tr>
<td>Borowitzka, L.J.</td>
<td>7, 34</td>
</tr>
<tr>
<td>Borowitzka, M.A.</td>
<td>7, 34</td>
</tr>
<tr>
<td>Bott, T.L.</td>
<td>48</td>
</tr>
<tr>
<td>Boulanger, J.</td>
<td>28</td>
</tr>
<tr>
<td>Boynton, J.E.</td>
<td>24</td>
</tr>
<tr>
<td>Brand, J.J.</td>
<td>34</td>
</tr>
<tr>
<td>Braud, J.P.</td>
<td>41</td>
</tr>
<tr>
<td>Brierley, C.L.</td>
<td>48</td>
</tr>
<tr>
<td>Brierley, J.A.</td>
<td>48</td>
</tr>
<tr>
<td>Brown, L.M.</td>
<td>19, 47</td>
</tr>
<tr>
<td>Bryant, D.A.</td>
<td>14</td>
</tr>
<tr>
<td>Burford, M.A.</td>
<td>7</td>
</tr>
<tr>
<td>Burgess, J.G.</td>
<td>41</td>
</tr>
<tr>
<td>Butler, A.</td>
<td>45</td>
</tr>
<tr>
<td>Butler, D.M.</td>
<td>5</td>
</tr>
<tr>
<td>Cai, Y.</td>
<td>11, 12, 29</td>
</tr>
<tr>
<td>Campbel, W.H.</td>
<td>12</td>
</tr>
<tr>
<td>Carberry, J.B.</td>
<td>48</td>
</tr>
<tr>
<td>Carpentier, B.</td>
<td>6</td>
</tr>
<tr>
<td>Casadevall, E.</td>
<td>36, 41</td>
</tr>
<tr>
<td>Castets, A.M.</td>
<td>27</td>
</tr>
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<td>Chakravarty, K.S.</td>
<td>42</td>
</tr>
<tr>
<td>Chan, R.L.</td>
<td>27</td>
</tr>
<tr>
<td>Charlier, R.H.</td>
<td>6, 42</td>
</tr>
<tr>
<td>Charytain, M.</td>
<td>45</td>
</tr>
<tr>
<td>Chelf, P.</td>
<td>34</td>
</tr>
<tr>
<td>Chen, W.L.</td>
<td>51</td>
</tr>
<tr>
<td>Chen, Y.F.</td>
<td>17, 18</td>
</tr>
<tr>
<td>Choquet, Y.</td>
<td>17</td>
</tr>
<tr>
<td>Christopher, D.A.</td>
<td>26</td>
</tr>
<tr>
<td>Chungjatupornchai, W.</td>
<td>13</td>
</tr>
<tr>
<td>Contreras, S.</td>
<td>9</td>
</tr>
<tr>
<td>Copertino, D.W.</td>
<td>13, 26</td>
</tr>
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<td>Cresswell, R.C.</td>
<td>5</td>
</tr>
<tr>
<td>Cuadros, X.</td>
<td>54</td>
</tr>
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<td>Curtis, S.E.</td>
<td>10</td>
</tr>
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<td>Daniell, H.</td>
<td>13</td>
</tr>
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<td>Daugulis, A.J.</td>
<td>36</td>
</tr>
<tr>
<td>Day, A.</td>
<td>14</td>
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<tr>
<td>De Casabianca-Chassany, M.L.</td>
<td>49</td>
</tr>
<tr>
<td>De la Noue, J.</td>
<td>8</td>
</tr>
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<td>De La Rosa, M.A.</td>
<td>43</td>
</tr>
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<td>De Lorimier, R.</td>
<td>14</td>
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<td>De Waart, J.</td>
<td>6</td>
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<td>Drager, R.G.</td>
<td>26</td>
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<td>Dugan, P.R.</td>
<td>51</td>
</tr>
<tr>
<td>Dutcher, S.K.</td>
<td>19</td>
</tr>
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<td>Eggert, J.</td>
<td>18</td>
</tr>
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<td>Ellmore, G.S.</td>
<td>11</td>
</tr>
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<td>England, R.R.</td>
<td>34</td>
</tr>
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<td>Erickson, J.M.</td>
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<td>Evans, L.V.</td>
<td>5</td>
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<td>45</td>
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<td>Fallowfield, H.J.</td>
<td>54</td>
</tr>
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<td>Ferino, F.</td>
<td>21</td>
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<td>Fernandes, H.L.</td>
<td>35</td>
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<td>35</td>
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<td>Fournier, R.</td>
<td>27</td>
</tr>
</tbody>
</table>

56
<table>
<thead>
<tr>
<th>Name</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kreysa, G.</td>
<td>40</td>
</tr>
<tr>
<td>Kuck, U.</td>
<td>29</td>
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<td>Kutsal, T.</td>
<td>47</td>
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<td>21</td>
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<td>Largeau, C.</td>
<td>36, 41</td>
</tr>
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<td>Laws, E.A.</td>
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<td>Le Gall, Y.</td>
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<td>Lemieux, C.</td>
<td>22, 28</td>
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<td>Liu H-H</td>
<td>51</td>
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<td>37</td>
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<td>51</td>
</tr>
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<td>Maid, U.</td>
<td>23</td>
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<td>51</td>
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<td>10</td>
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<td>Mann, H.</td>
<td>51, 52</td>
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<td>45</td>
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<td>Matsunaga, N.</td>
<td>39</td>
</tr>
<tr>
<td>Matsunaga, T.</td>
<td>23, 39, 41</td>
</tr>
<tr>
<td>Matsuoka, S.</td>
<td>42</td>
</tr>
<tr>
<td>Maze, J.</td>
<td>6, 42</td>
</tr>
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<td>Mbuthia, P.</td>
<td>8</td>
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<td>McFadden, B.A.</td>
<td>13</td>
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<td>McIntosh, D.</td>
<td>9, 35</td>
</tr>
<tr>
<td>McLaughlin, S.</td>
<td>21</td>
</tr>
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<td>Meek, S.D.</td>
<td>41</td>
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<td>Mehta, D.J.</td>
<td>32</td>
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<td>Merchant, S.</td>
<td>23</td>
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<td>41</td>
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<td>39, 42</td>
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<td>42</td>
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<td>42</td>
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<td>6, 42</td>
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<td>35</td>
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<td>21</td>
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<td>Moulton, T.P.</td>
<td>7</td>
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<td>Mun, T.H.</td>
<td>42</td>
</tr>
<tr>
<td>Nakajima, I.</td>
<td>39</td>
</tr>
<tr>
<td>Nakajima, T.</td>
<td>52</td>
</tr>
<tr>
<td>Nakamura, N.</td>
<td>23, 39, 41</td>
</tr>
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<td>Navarro, J.A.</td>
<td>43</td>
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<td>Nawa, Y.</td>
<td>42</td>
</tr>
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<td>Newman, S.M.</td>
<td>24</td>
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<td>Niven, G.W.</td>
<td>42</td>
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<td>Noehr, R.S.</td>
<td>37</td>
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<td>25</td>
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<td>25</td>
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<td>41</td>
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<td>29</td>
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<td>Panyim, S.</td>
<td>31</td>
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<tr>
<td>Papageorgiou, G.C.</td>
<td>6</td>
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<td>21</td>
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<td>51</td>
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<td>31</td>
</tr>
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<td>15</td>
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<td>32</td>
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<td>Rakocy, J.E.</td>
<td>53</td>
</tr>
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<td>Randolph-Anderson, B.L.</td>
<td>24</td>
</tr>
<tr>
<td>Rao, A.K.</td>
<td>42</td>
</tr>
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<td>43</td>
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<td>53</td>
</tr>
<tr>
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<td>43</td>
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<tr>
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<td>6</td>
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<td>20</td>
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<td>47</td>
</tr>
<tr>
<td>Robinson, P.K.</td>
<td>53, 54</td>
</tr>
<tr>
<td>Robledo, D.R.</td>
<td>37</td>
</tr>
<tr>
<td>Rochaix, J.D.</td>
<td>14, 15, 17, 26</td>
</tr>
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<td>Ronceel, M.</td>
<td>43</td>
</tr>
<tr>
<td>Roth-Bejerano, N.</td>
<td>43</td>
</tr>
<tr>
<td>Rotmann, K.W.G.</td>
<td>32</td>
</tr>
<tr>
<td>Ryncarz, A.J. II</td>
<td>21</td>
</tr>
<tr>
<td>Sa-Correia, I.</td>
<td>35</td>
</tr>
</tbody>
</table>
Yu, W. .......................... 30
Yudin, K. .......................... 45
Zeiler, K.G. .......................... 47
Zetsche, K. ......................... 23, 28
Zhou, Y ............................. 9