WELCOME to the latest issue of Mainstream Biocatalysis. When engineered to fit into a process, enzymes can beat chemical catalysts on cost, speed, yield, and purity. Mainstream Biocatalysis helps you mine the rapidly growing body of biocatalysis information for the pharmaceuticals, industrial biotechnology, chemicals, and synthetic biology fields. This free monthly publication includes academic and industry news, descriptions of companies, examples of notable enzymes, and compilations of relevant publications and patent applications in the field. Visit our website at www.bio-catalyst.com to inquire about additional industry information.

News Highlights:

August 1, 2014: Gevo Inc. projected that its newly expanded plant in Luverne, MN will break even in 2014. Isobutanol output is estimated to be 1 million gallons in 2014 and 3 million gallons in 2015, together with 18 million gallons of ethanol, according to Gevo CEO Pat Gruber.

August 4, 2014: The Product design and Management Association awarded its 2014 Outstanding Corporate Innovator (OCI) Award to Novozymes.

August 4, 2014: A test method developed by diagnostic enzyme producer Megazyme International (Republic of Ireland) is being adopted as global method of choice to ensure that dietary fiber is not double counted. Megazyme is currently the only supplier of the reagents needed to run the new test method.

August 5, 2014: Dyadic International, Inc. announced that it has signed a collaboration agreement to commercialize second generation biofuel and bio-based chemical technology with Compagnie Industrielle de la Matière Végétale of France.

August 6, 2014: Codexis released financial projections that were revised to be well ahead of previous expectations, driven by the recently announced collaboration and license agreement with GlaxoSmithKline.

August 6, 2014: Genomatica announced that major nylon intermediates, including hexamethylenediamine, caprolactam and adipic acid, are the focus of its third publicly-disclosed development program.

August 11, 2014: Novozymes reported that its starch processing enzymes will allow ethanol producers to unlock more energy from each corn kernel, producing 5% more fuel while using 8% less energy.
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August 12, 2014: ACIB (Graz, Austria) announced the development of a new enzyme search engine to improve the search for new enzyme functions based on enzyme sequence and structure.

Upcoming Events:


Oct. 7-9. CPhI in Paris, France

Oct. 20-21. “Center for Biocatalysis and Bioprocessing Annual Conference,” Iowa, USA.

Enzyme Profile: Aldolases Part 1

Today’s profile is the first of a two-part profile on aldolases. The basic reaction is a C-C bond forming reaction between a ketone- or aldehyde-containing donor substrate with an aldehyde acceptor. Aldolases are typically rigid with respect to their donor substrate selectivity. The acceptor substrate selectivity, however, is typically much more relaxed and a range of aldehydes are typically accepted into the active site. Aldolases are classified both by their mechanisms (type I or II), and by their donor substrates. Type I aldolases use a lysine in the active site to activate a donor ketone by converting it to the electron rich enamine. This enamine then reacts with an aldehyde, and then the Schiff base is hydrolyzed to release product. Type II aldolases use a divalent metal (typically Zn$^{+2}$) cofactor to activate the ketone and enable it to generate the metal-stabilized enolate, which then reacts with the aldehyde acceptor.

Aldolases are further classified by their donor substrates: the dihydroxyacetone phosphate (DHAP) aldolases, pyruvate aldolases, acetaldehyde aldolases, and the glycine aldolases. The acceptors are invariably aldehydes, though there is substantial variation in the types of aldehydes which serve as substrates.
Deoxyribose-5-phosphate aldolase (DERA) has been one of the most visible aldolases, and is an acetaldehyde aldolase. In nature, the enzyme catalyzes the reaction between acetaldehyde and glyceraldehydes 3-phosphate to generate 2-deoxyribose-5-phosphate. It is a type I enzyme, and structures of DERA from several organisms including *E. coli* have been solved. The *E. coli* DERA has been engineered by groups at DSM and Verenium (now part of BASF) and by the group of Professor Wong. In the Wong lab, DERA was used in two different steps in the convergent synthesis of the anti-cancer natural product, epothilone A.

Verenium and DSM independently identified a route to an intermediate in the process route to the blockbuster drug, atorvastatin. The reaction includes the sequential condensation of first two molecules of acetaldehyde and then a terminal chloroacetaldehyde. Protein engineering was used to improve the reaction, and the improvements included resistance to deactivation caused by the substrates.

DERA enzymes can be purchased from Sigma, GL Syntech, and Chiral Vision. In the next issue, we will turn our focus to some of the other aldolases and their applications as biocatalysts.


**Biocatalyst Company Profile: Biocatalysts Ltd. (Cardiff, Wales, UK)**
Biocatalysts Ltd was founded in 1986, and has grown into a company that distributes in 82 countries and has an office in the US. The company maintains a substantial catalog of enzymes, offers consultancy services, and conducts toll manufacturing and R+D. Biocatalysts’ primary operations are in a facility built in 2004 in the UK, and manufacture is done under numerous accreditations including Kosher, Halal, and GMP. These accreditations have been attained because the primary market for Biocatalysts’ products is in food applications. The company has fermentation capacity up to 20,000 L, though it also offers early-stage research quantities and projects.

Biocatalysts’ catalog offerings include lipases, esterases, proteases, lactases, glucose oxidase, glucanase, catalase, cellulases, amylases, mannitol dehydrogenases, pectinases, phenylalanine dehydrogenase, tannase, and proprietary enzyme mixtures. Though the emphasis is on enzymes for food applications, several products have biocatalytic use. The R+D and toll manufacturing services offered by the company have been utilized by several other sectors, including active pharmaceutical intermediate manufacture, healthcare and diagnostics, and chemical manufacture. As an example, a bacterial dehydrogenase was recombinantly expressed for a biosensor application. The company claims that these projects can be completed in as little as 8-12 weeks. The company manufactures in microbial hosts and emphasizes its expertise in E. coli and Pichia pastoris. Biocatalysts is part of the Centre of Excellence for Biocatalysis (CoEBio3) which has aided the company in establishing associations with academic institutions including the University of Nottingham.

**Recent Publications of Interest**


http://aem.asm.org/content/early/2014/08/12/AEM.01883-14.abstract

http://aem.asm.org/content/early/2014/07/28/AEM.01529-14.abstract

“Structural-functional evaluation of ionic liquid libraries for the design of co-solvents in lipase-catalysed reactions” L. P. N. Rebelo Green Chem.
http://pubs.rsc.org/en/content/articlelanding/2014/gc/c4gc01329h#!divAbstract

“Towards the synthesis of glycosylated dihydrochalcone natural products using glycosyltransferase-catalysed cascade reactions” B. Nidetzky Green Chem.
http://pubs.rsc.org/en/content/articlelanding/2014/gc/c4gc00960f#!divAbstract


http://pubs.rsc.org/en/content/articlelanding/2014/ra/c4ra04625k#!divAbstract

“ADH catalysed hexanol oxidation with fully integrated NADH regeneration performed in microreactors connected in series” B. Zelic RSC Adv.
http://pubs.rsc.org/en/content/articlelanding/2014/ra/c4ra05421k#!divAbstract

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Recently Published Patent Applications

Novel 2-deoxy-scyllo-inosose synthase (ASAHI KASEI CHEMICALS CORP) EP2759594

Process for preparing alpha, omega alkanediols (EVONIK) WO2014114505, EP2759598 terminal alkane hydroxylase
PROCESS FOR PRODUCING NOVEL SIALO-SUGAR CHAIN (WAKAYAMA U) EP2759548 sialic acid-introducing enzyme

NOVEL GLUCOSE OXIDASES DERIVED FROM ASPERGILLUS NIGER (ROCHE) WO2014114810

RECOMBINANT SYNTHESIS OF ALKANES (JOULE) WO2014117084

Methods and compositions for degrading cellulosic material (NOVOZYMES) US20140212932

COMPOSITIONS COMPRISING CELLOBIOSE DEHYDROGENASE FROM Pycnoporus Cinnabarinus and their use for the degradation of lignocellulosic biomass (Institut National de la Recherche Agronomique) US20140212927

Carbohydrate degrading polypeptide and uses thereof (DSM) WO2014118360

Endonuclease for genome editing (University of Western Ontario) WO2014121222

Recombinant microorganisms comprising NADPH dependent enzymes and methods of production thereof (LANZATECH) WO2014120852

Methods, systems, and software for identifying bio-molecules using models of multiplicative form (CODEXIS) WO2014120821

Robust, easy to use immobilized enzyme reactors (PERFINITY BIOSCIENCES) WO2014120890

Diaphorase composition (TOYOBO) WO2014119516

Method of producing nanobiocatalysts (INOFEA GMBH) WO2014118247

TEVL Chimeric endonuclease and their preferential cleavage sites (CELLECTIS) WO2014118719

α-Amylase, gene of α-amylase, engineering bacteria containing the gene, and applications of engineering bacteria (Nanjing Agricultural U) WO2014117472

Enzymatic preparation of indigo dyes and intermediates (NOVOZYMES) WO2014122109

Method for producing fructose (ANNIKKI GMBH) WO2014122167

Polypeptides having protease activity (NOVOZYMES) WO2014122161
ENZYMES THAT SYNTHESIZE ZINGIBERENE (Michigan State U) US20140230101

THERMOPHILIC AND THERMOACIDOPHILIC METABOLISM GENES AND ENZYMES FROM ALICYCLOBACILLUS ACIDOCALDARIUS AND RELATED ORGANISMS, METHODS (BATTLE E ENERGY) US20140227788

NOVEL MANNANASE PRODUCED FROM CELLULOSIMICROBIUM SP. STRAIN HY-13 (Korea Research Institute) US20140227762

OPLOPHORUS-DERIVED LUCIFERASES, NOVEL COELENTERAZINE SUBSTRATES, AND METHODS OF USE (PROMEGA) US20140227759

NOVEL FRUCTOSYL PEPTIDYL OXIDASE (ROCHE) US20140227728

ENZYMES FOR THE TREATMENT OF LIGNOCELLULOSICS, NUCLEIC ACIDS ENCODING THEM AND METHODS FOR MAKING AND USING THEM (BP) US20140223602

FRUCTOSYLATED MANGIFERIN AND PREPARATION METHOD THEREFOR AND USE THEREOF (NANJING UNIVERSITY OF TECHNOLOGY) US20140221643

CYTOCHROME P450S AND USES THEREOF (U KENTUCKY) US20140212939

Dehydrogenase Variants and Polynucleotides Encoding Same (NOVOZYMES) US20140212938

MUTANT GAMMA-GLUTAMYLTRANSFERASE, AND A METHOD FOR PRODUCING GAMMA-GLUTAMYLVALYLGLYCINE OR A SALT THEREOF (Ajinomo) US20140212920

N-DEMETHYASE GENES AND USES THEREOF (U OF IOWA) US20140227729

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