Journal of Pharmacogenomics & Pharmacoproteomics

Gupta, J Pharmacogenomics Pharmacoproteomics
2018, 9:1
DOI: 10.4172/2153-0645.100e159

Editorial Open Access

Biotransformation Serves as an Alternative Tool to the Chemical Synthesis

Meenu Gupta*

Government College of Girls, Gurgaon-Mehraulli Road, Sector 14, Gurugram, Haryana, India

***Corresponding author:** Gupta M, Assistant Professor of Botany, Government College of Girls, Gurgaon-Mehraulli road, Sector 14, Gurugram, Haryana, India, Tel: +91-8010208200; E-mail: meenugupta.999@gmail.com

Received date: January 22, 2018; **Accepted date:** January 29, 2018; **Published date:** January 31, 2018

Copyright: © 2018 Gupta M. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Editorial

Proteases are hydrolytic enzymes with high selectivity which does not need any expensive cofactors and can be used as synthetic tools. Most of the synthetic reactions are carried out in presence of organic solvents. Proteases in organic solvents can catalyze reactions such as esterification and peptide synthesis. Unfortunately most of the protease loses their activity in the presence of organic solvents. Several protein engineering methods have been used to increase stability and activity of enzyme in organic solvents. If the enzyme is naturally organic solvent tolerant then no such modification is needed thus there is a continuous demand of microorganisms that can produce solvent tolerant enzymes.

Microorganisms are good source of enzymes because of short generation time, easy genetic modification which are useful for bulk production [1]. First organic solvent tolerant enzyme has been reported from *Pseudomonas aeruginosa* was a lipolytic enzyme [2]. Many extremophilic bacteria such as thermophiles and halophiles serve as a good source of organic solvent tolerant enzyme besides their counterpart mesophiles [3,4]. Halophiles are adapted to grow at high salt conditions thus the enzymes from halophiles require salt for activity and stability. High salt environments are low water environments thus halophiles are adapted to cope with low water activity.

New application of protease as antifouling agent needs organic solvent tolerance and high activity in saline sea water. Undesirable attachment and accumulation of phytoplankton zooplankton and other microorganisms on ship surface is termed as fouling. Microbial biofouling occur in many steps which firstly involves the formation of a conditional layer than unicellular microorganisms of marine ecosystem attached to it and lastly large multicellular organisms attached to it and cause biofouling [5]. This process is very much similar to bacterial biofouling of implants, when an implant is placed in the body proteins and other macromolecules adsorbed on the surface of implant and forms a conditional layer, eventually this conditional layer is colonized by neutrophills and macrophages. Colonization is followed either by collagen encapsulation or bacterial infection (Figure 1). If bacterial infection takes place before encapsulation than it is impossible to cure infection [6].

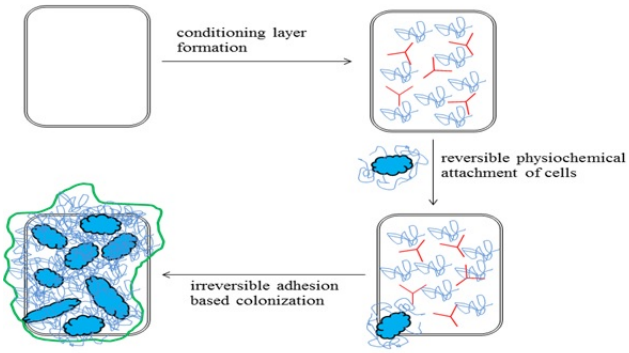

Figure 1: Schematic representation of steps involved in biofouling.

Several antifouling strategies have been used to overcome such problems which include the preparation of anti-adhesion coatings by chemical or physical adsorption of hydrophilic polymer molecules that can work as a steric and/or hydration barrier between the underlying surface and the proteins and/or glycoproteins of the cells thus prevents the initial attachment. Several biomolecules such as Bovine Serum Albumin (BSA), dextran, hyaluronic acid, chitosan, alginate, and mannitol were used as anti- adhesive [7,8]. Besides these polymers such as Poly ethylene glycol (PEG), polyacrylamides were also used as anti-adhesive [9,10]. Several anti fouling coatings were used to stop fouling in marine industry which involves use of tributyltin self-polishing copolymer (TBT-SPC) in paints. Till 2008, Tri-n-butyl tin (TBT) has been extensively used as antibiofouling agent in marine paint industry. TBT has adverse effect on marine ecological diversity. Thus paints containing TBT has been banned. Haloarchaeal proteases serve as a better alternative in antifouling coating. As most of the coating materials are suspended in organic solvent, there is urgent need to have organic solvent compatible protease and other related enzymes. Organic solvents reduce water activity thus most of the salt stable halophilic enzyme remain active and stable in the presence of organic solvents. Besides conventional protease may also work suboptimally in saline water condition which is the most important criteria for application of enzyme as antifoulant. Archaea are also important to understand life as halites have been found from mars also [11].

TBT-SPC was an environmental threat thus it was completely banned from January 2008. Use of enzymes in paints has provided an alternative and environmental friendly way to overcome fouling. Enzymes present in the paint directly interact with glycoproteins of microorganism thus reduces attachment to the ship surface. Organic

solvents are the essential component of paints thus it is mandatory to use an organic solvent stable enzyme in such preparations. Organic solvent tolerant and stable proteases from different bacteria sources have proved beneficial in marine industry to stop fouling [12]. In industrial bio catalysis, cross-linked enzyme aggregates (CLEAs) are very beneficial in terms of economy and environment. CLEAs are easily obtained from crude enzyme thus economic over immobilization through protein engineering. General mode of preparation of CLEAs is given in Figure 2 glutaraldehyde is used as cross-linking agent for decades. Glutaraldehyde bring about inter and intramolecular aldol condensations reaction between the free amino groups of lysine residues, on the surface of neighbouring enzyme molecules which involves schiff's base formation and Michael-type 1,4 addition to a, β-unsaturated aldehyde moieties resulting the formation of CLEAs [13].

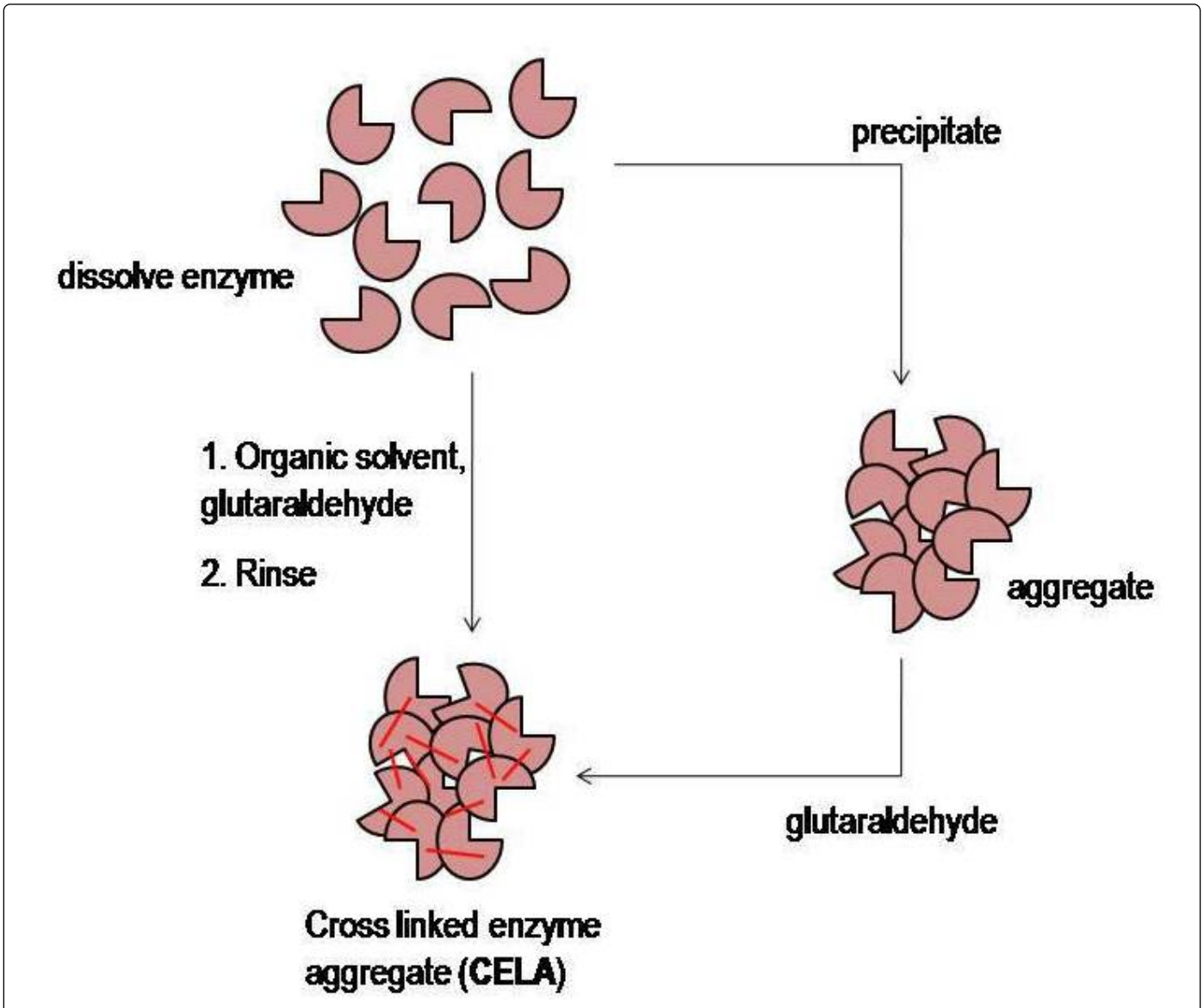

Figure 2: Schematic representation of CLEA preparation [13].

CLEAs can also be prepared by the cross- in the presence of a siloxane e.g. $(MeO)_4Si$, resulting the formation of CLEA- silica composite [14]. Major advantage of using CLEAs is that they can be recycled. Immobilizations of enzyme as CLEAs increase stability at high temperatures [15]. Skovgard et al. stabilized subtilisins, from different bacterial sources, by converting them in cross-linked enzyme aggregates- CLEAs. Protease activity of CLEAs in artificial seawater (ASW) was tested to find out their stability towards marine conditions furthermore they incubated the CLEAs in xylene an important component of ship paint. They found that catalytic activity was increased as compared to the initial catalytic activity in ASW for 7 days. A possible explanation is that continuous hydration of paint increases in seawater which leads to an increased amount of molecules leaching from the paint surface. Silicates can be used as matrices for enzyme immobilization some organic solvent proteases have been summarized in Table 1.

One of the most promising approaches is the use of enzymes which can interact directly with microorganisms on the surface. For successful use in paints enzyme must possess solvent stability and should function in saline conditions. Most of the solvent stable protease show less activity in artificial sea water due to inhibition by NaCl, Mg^{+2} and Ca^{+2}. Most halophilic proteins are not suitable for such environment because they require high sat concentration for activity.

A protease from a moderately halophilic *Bacillus* sp. strain isolated from sea water has maximum activity at pH optimum 9.0, $t_{1/2}$ 190 min at 60ºC and 1% (w/v) NaCl. The protease shows stability in polar and nonpolar solvents at high concentrations [25]. The solvent stability among halophilic enzymes seems a generic novel feature making them potentially useful in non-aqueous enzymology thus there is a continuous demand of microorganisms that can produce solvent tolerant enzymes [20,30].

Source	Incubation Condition	Stability	Unstable in the presence	References
Pseudomonas aeruginosa K	37ºC, 14 days	25% (v/v) Deccane, Octane	5% (v/v) Benzene, Heptane, Xylene	[16]
Pseudomonas aeruginosa PST-01	30ºC, 15 days	25% (v/v) Ethanol, methanol, DMSO, Octanol, Butanol	25% (v/v) Benzene, Haptane, Xylene	[4]
Pseudomonas aeruginosa PseA	30ºC, 72 h	25% (v/v) Benzene, Heptane, Hexane, Toluene	25% (v/v) Butanol	[17]
Pseudomonas aeruginosa PT121	30ºC, 5 or 14 days	50% (v/v) Benzene, Heptane, Hexane, Toluene and DMSO	50% (v/v) DMF, Ethanol,	[18]
Pseudomonas aeruginosa san-ai	30ºC, 10 days	25% (v/v) DMF	25% (v/v) Hexane, Benzene, Acetone	[19]
Bacillus sp. APR-4	4ºC, 24 h	50% (v/v) Ethanol, Methanol Benzene, Butanol	50% (v/v) Acetone	[20]
Bacillus cereus BG1	30ºC, 1–55 days	25% (v/v) DMSO, Ethanol, methanol	25% (v/v) Acetonitrile	[21]
gamma-Proteobacterium	30ºC, 10 days	33% (v/v) Ethanol, Methanol Butanol DMSO, Xylene		[22]
Natrialba magadii	30ºC, 24 h, 1.5 M NaCl	15 % or 30% (v/v) DMSO	15% (v/v) Acetone, Ethanol, Acetonitrile	[23]
Halobacterium sp. SP1	20ºC, 30 min	33%(v/v) Toluene, Xylene		[24]
halophilic Bacillus sp.	30ºC, 24 h	50% (v/v) Ethanol, Methanol	50% (v/v) Benzene, Toluene	[25]
Geomicrobium sp. EMB2	30ºC, 24 h	50% (v/v) Toluene, Butanol, Heptane, Hexane, Benzene		[26]
Staphylococcus aureus strain MSSA 476		Toluene, xylene and cyclohexane		[27]
Halobiforma BNMIITR		DMSO		[28]
Oceanobacillus sp.		Isooctane		[29]

Table 1: Organic solvent tolerant protease from different microorganisms.

References

1. Joo HS, Kumar CG, Park GC, Kim KT, Paik SR, et al. (2002) Optimization of the production of an extracellular alkaline protease from *Bacillus horikoshii*. Process Biochem. 38: 155-159.
2. Ogino H, Miyamoto K, Ishikawa H (1994) Organic-solvent-tolerant bacterium, which secretes organic-solvent-stable lipolytic enzyme, Appl. Environ. Microbiol 60: 3884-3886.
3. Doukyua N, Ogino H (2010) Organic solvent-tolerant enzymes. Biochem Eng J 48: 270-282.
4. Ogino H, Miyamoto K, Yasuda M, Ishimi K, Ishikawa H (1999) Growth of organic solvent-tolerant *Pseudomonas aeruginosa* LST-03 in the presence of various organic solvents and production of lipolytic enzyme in the presence of cyclohexane. Biochem Eng J 4: 1-6.
5. Banerjee I, Pangule RC, Kane RS (2010) Antifouling Coatings: Recent Developments in the Design of Surfaces That Prevent Fouling by Proteins, Bacteria, and Marine Organisms. Advanced Materials 23: 690-718.
6. Castner DG, Ratner BD (2002) Biomedical surface science: Foundations to frontiers. Surface Science 500: 28-60.
7. Bongaerts JHH, Cooper-White JJ, Stokes JR (2009) Low Biofouling Chitosan-Hyaluronic Acid Multilayers with Ultra-Low Friction Coefficients. Biomacromolecules 10: 1287-1294.
8. Luk YY, Kato M, Mrksich M (2000) Self-Assembled Monolayers of Alkanethiolates Presenting Mannitol Groups Are Inert to Protein Adsorption and Cell Attachment. Langmuir 16: 9604-9608.
9. Buddy Ratner AH, Schoen F, Lemons J (1996) Biomaterials Science. An Introduction to Materials in Medicine. San Diego, USA: Acamedic press.
10. Callow JA, Callow ME (2011) Trends in the development of environmentally friendly fouling-resistant marine coatings. Nat Commun 2: 244.
11. Gooding JL (1992) Soil mineralogy and chemistry on Mars: possible clues from salts and clays in SNC meteorites. Icarus 99: 28-41.
12. Yebra DM, Kiil S, Dam-Johansen K (2004) Antifouling technology-past, present and future steps towards efficient and environmentally friendly antifouling coatings. Prog Org Coating 50: 75-104.
13. Migneault I, Dartiquenave C, Bertrand MJ, Waldron KC (2004) Glutaraldehyde: behaviour in aqueous solution, reaction with proteins, and application to enzyme croslinking. Biotechniques 37: 790-802.

14. Schoevaart WRK, Van Langen LM, Van den Dool RTM, Boumans JWL (2006) WO 2006/046865 A2, to CLEA Technologies.
15. Sheldon RA (2011) Characteristic features and biotechnological applications of cross-linked enzyme aggregates (CLEAs). Appl Microbiol Biotechnol 92: 467-477.
16. Geok LP, Razak CNA, Rahman RNZA, Basri M, Salleh AB (2003) Isolation and screening of an extracellular organic solvent-tolerant protease producer. Biochem Eng J 13: 73-77.
17. Gupta A, Roy I, Khare SK, Gupta MN (2005) Purification and characterization of a solvent stable protease from *Pseudomonas aeruginosa*. J Chromatogr A 1069: 155-161.
18. Tang XY, Pan Y, Li S, He BF (2008) Screening and isolation of an organic solvent-tolerant bacterium for high-yield production of organic solventstable protease, Bioresour Technol 99: 7388-7392.
19. Karadzic I, Masui A, Zivkovic LI, Fujiwara N (2006) Purification and characterization of an alkaline lipase from *Pseudomonas aeruginosa* isolated from putrid mineral cutting oil as component of metalworking fluid. J Biosci Bioeng 102: 82-89.
20. Kumar D, Bhalla TC (2005) Microbial proteases in peptide synthesis: approaches and applications, Appl Microbiol Biotechnol 68 : 726-736.
21. Ghorbel B, Sellami-Kamoun A, Nasri M (2003) Stability studies of protease from *Bacillus cereus* BG1. Enzyme Microb Technol 32: 513-518.
22. Sana B, Ghosh D, Saha M, Mukherjee J (2006) Purification and characterization of a salt, solvent, detergent and bleach tolerant protease from a new gammaproteobacterium isolated from the marine environment of the Sundarbans. Process Biochem. 41: 208-215.
23. Ruiz DM, De Castro RE (2007) Effect of organic solvents on the activity and stability of an extracellular protease secreted by the *Haloalkaliphilic archaeon* Natrialba magadii. J Ind Microbiol Biotechnol 34: 111-115.
24. Akolkar AV, Deshpande GM, Raval KN, Durai D, Nerurkar AS, et al. (2008) Organic solvent tolerance of *Halobacterium* sp. SP(1) and its extracellular protease. J Basic Microbiol 48: 421-425.
25. Sinha R, Khare SK (2013). Characterization of detergent compatible protease of a *Halophilic Bacillus* sp. EMB9: Differential role of metal ions in stability and activity. Bioresource Tech 145: 357-361.
26. Karan R, Khare SK (2010) Purification and characterization of a solvent-stable protease from *Geomicrobium* sp. EMB2. Environmental Technology 31: 1061-1072.
27. Nielsen LE, Kadavy DR, Rajagopal S, Drijber R, Nickerson KW (2005) Survey of Extreme Solvent Tolerance in Gram-Positive Cocci: Membrane Fatty Acid Changes in *Staphylococcus haemolyticus* Grown in Toluene. Appl Environ Microbiol 71: 5171-5176.
28. Gupta M, Aggarwal S, Navani NK, Choudhury B (2014) Isolation and characterization of a protease producing novel *Haloalkaliphilic bacterium Halobiforma* sp. strain BNMIITR from Sambhar lake Rajasthan India. Ann Microbiol 65: 677-686.
29. Pandey S, Rakholiya KD, Raval VH, Singh SP (2012) Catalysis and stability of an alkaline protease from a *Haloalkaliphilic bacterium* under non-aqueous conditions as a function of pH, salt and temperature. J Biosci Bioeng 114: 251-256.
30. Serdakowski AL, Dordick JS, (2008) Enzyme activation for organic solvents made easy. Trends Biotechnol 26: 48-54.