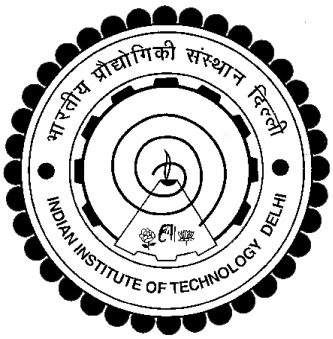
BIODEGRADATION OF 7-KETOCHOLESTEROL AND STUDIES INTO CHOLESTEROL OXIDASE

SHUBHRIMA GHOSH



DEPARTMENT OF CHEMISTRY

INDIAN INSTITUTE OF TECHNOLOGY DELHI

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by

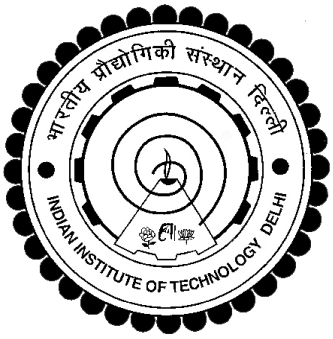
SHUBHRIMA GHOSH

DEPARTMENT OF CHEMISTRY

Submitted in fulfillment of the requirements of the degree of

Doctor of Philosophy

to the



INDIAN INSTITUTE OF TECHNOLOGY DELHI

JULY 2020

Dedicated to my parents and
Dr. A.P.J Abdul Kalam

CERTIFICATE

This is to certify that the thesis entitled "***Biodegradation of 7-Ketocholesterol and studies into cholesterol oxidase***" being submitted by **MS. SHUBHRIMA GHOSH** to the Indian Institute of Technology Delhi for the award of the degree of ***Doctor of Philosophy*** in Chemistry is a record of bonafide research work carried out by her. Ms. Shubhrima Ghosh has worked under my guidance and supervision, and has fulfilled the requirements for the submission of the thesis which, to my knowledge, has reached the requisite standard.

The results contained in this dissertation have not been submitted in part or full to any other University or Institute for the award of any degree or diploma.

Date: July 28, 2020

Place: New Delhi

Dr. S. K. Khare

Professor of Biochemistry

Department of Chemistry

Indian Institute of Technology Delhi

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Shubhrima Ghosh

New Delhi

Abstract

The oxidation of cholesterol results in the formation of oxysterols such as 7-ketocholesterol (7KC), which are implicated in a number of age-related disorders such as atherosclerosis, Alzheimer's disease and macular degeneration. 7KC can be majorly absorbed from animal-origin food products or produced endogenously in the body. Thus, at the very outset of the thesis, Indian milk products were assessed for their 7KC content. According to preliminary studies, 7KC levels were detected to be higher in milk powder samples than in raw milk. However, it was detected in negligible amounts in cheese and ghee samples. Thus, the nature of cooking/processing and temperature involved plays an important role in 7KC formation.

Current modalities against 7KC mediated cytotoxicity use antioxidants and other natural or synthetic molecules to reduce 7KC-induced cytotoxity. The alternative application of enzymes from microbial sources to degrade oxysterols *in vitro* and *in vivo* is an innovative 'Medical Bioremediation' approach. During initial screening, *Pseudomonas aeruginosa* PseA and *Rhodococcus erythropolis* MTCC 3951 were found to be potential degrader strains using 7KC as a sole carbon source. Under optimized conditions, they respective strains were able to degrade 88% (within 25 days) and 91% (within 15 days) of an initial concentration of 1g/L (1000ppm) 7KC. Preliminary *in vitro* studies with extra-cellular extracts showed degradation of the compound, thus reinforcing the occurrence of suitable enzymatic systems involved in the process. The strains produced cholesterol oxidase, dehydrogenase, reductase and lipase in the presence of 7KC, with cholesterol oxidase being reported as the major enzyme involved in the degradation pathway. Some of the intermediates were also identified to predict the degradation pathway. The

extracellular extracts of both the strains decreased the 7KC content when added to milk products, which may form a strategy for dealing with 7KC cytotoxicity at source.

In addition to catalyzing the first step of 7KC degradation, cholesterol oxidase also finds immense industrial and biomedical applications. Thus, further studies were taken up for investigating it. Owing to the low production levels of cholesterol oxidase in *P. aeruginosa* PseA, cloning and overexpression of its gene were attempted. The gene was cloned through pGEM-TEasy vector into *E. coli* XL1 Blue-MRF' strain and was found to be highly conserved in nature with a size of 1794 bp, identical to its homologues in other *Pseudomonas* strains. This sequence was further submitted to GenBank (Accession: KU315227.1 GI: 1031987706). Several structures were predicted *in-silico* using Phyre[2] and I-Tasser, showing high similarity with a solvent tolerant cholesterol oxidase from *Chromobacterium* sp. DS-1 (PDB ID-3js8A) and another cholesterol oxidase from *B. sterolicum* (PDB ID-1i19A). Restriction cloning was done through pET22b(+) vector into *E. coli* Rosetta (DE3) strain. However, activity was only obtained on plates and not in solution. Thus, additional studies are required for obtaining active overexpressed cholesterol oxidase.

In case of cholesterol oxidase from *R. erythropolis* MTCC 3951, cloning was not attempted due to the less-conserved nature of the gene. Process optimization accomplished high yield of the enzyme. The protein was then purified to homogeneity in a three-step process, involving 10 kDa molecular weight cut-off, Q Sepharose Anion Exchange Chromatography followed by Phenyl Sepharose Hydrophobic Interaction Chromatography. A novel monomeric 35 kDa protein, which is uncommon in *Rhodococcus* strains, was obtained in SDS-PAGE and confirmed through activity staining in Native-PAGE. The enzyme was further structurally and biochemically characterized. pH 7.5 and temperature 30 °C were found to be optimum for the enzyme activity, while stability was observed in the pH range (4.0-9.0) and temperature range (30-50 °C). The K_m was found to

be 22.75 mM while V_{max} was obtained as 9.21 mM/min. Structural studies using fluorescence and Far-UV CD-spectroscopy revealed 36.3% helix, 0.9% antiparallel and 6% parallel β-sheets, 8.5 % turns and 18.3% other structures (3_{10}-helix) in the protein.

In order to increase the applicability, cholesterol oxidase (ChOx) enzyme from *P. aeruginosa* PseA (ChOxP) and *R. erythropolis* MTCC 3951 (ChOxR) strains and a commercial variant from *Streptomyces* sp. (ChOxS) were immobilized on functionalized magnetic Iron (II, III) oxide (MNP) and Silica nanoparticles (SNP). The MNP-nanobiocatalysts in case of ChOxP, ChOxR and ChOxS, retained 71, 91 and 86% of cholesterol oxidase activity respectively. In case of SNPs, the immobilization efficiency was calculated as 68, 86, 83% for ChOxP, ChOxR and ChOxS respectively. The catalytic efficiency of the immobilized enzyme was found to be almost 2.0 times higher than free enzyme, along with increase in stability over a wide range of temperature (10-70 °C) and pH (4.0-9.0). However, the pH (7.5) and temperature (30 °C) optima were found to remain unchanged. The nanobioconjugates were reusable upto 10^{th} cycle of operation. The immobilization of the enzyme on nanoparticles was confirmed by FTIR, SEM and TEM. Pharmaceutically important molecules, 4-cholesten-3-one and 4-cholesten-3,7-dione, were produced through biotransformation of cholesterol and 7KC respectively using the nanobioconjugates. Thus, immobilization on nanoparticles makes enzymes suitable from the point of view of applications.

The last part of the thesis covers an interesting application of nanoparticles in modulating structure of proteins. Proteins can be denatured by changes in temperature, pH or due to the presence of denaturants. The refolding process involves the reconfiguration of denatured proteins into their native functional state. Nanoparticles act as artificial ‘chaperones’, due to favorable orientation of the proteins on their scaffold which prevents aggregation. In the present study, thermal denaturation of cholesterol oxidases from *P. aeruginosa* PseA, *R. erythropoilis* MTCC 3951 and

Streptomyces sp. were studied at temperatures 50-70 ºC. Further, these thermally denatured proteins were refolded using functionalized MNPs. The activity was recovered by 47, 79 and 29.4% in case of ChOxP, ChOxS and ChOxS respectively. The refolding was confirmed using FTIR, DLS, Zeta Potential Measurements, fluorescence and Far-UV CD spectroscopy. This study proves that magnetic nanoparticles were effective in the refolding of heat denatured ChOx which regain their structure and restore their activity to a great extent.

The current investigation thus encompasses a connection between the multiple perspectives of food cytotoxicity, biodegradation, molecular biology, enzymology, nanotechnology and structural biology.

सार

कॉलस्ट्रोल के ऑक्सीकरण से ऑक्सीस्टेरोल का निर्माण होता है। ये ऑक्सीस्टेरोल जैसे 7–कीटोकोलेस्ट्रोल (7 के.सी.), ऐथेरोस्लेरोसिस, अल्जाइमर तथा पेशीयों के ह्रास जैसे उम्र आधारित रोगों के लिए उत्तरदायी होते हैं। 7 के.सी. को मुख्य रूप से पशु .मूल खाद्य उत्पादों से अवेशोषित किया जा सकता है या शरीर में अंतर्जात रूप से उत्पादित किया जा सकता है। भारतीय दुग्ध व दुग्ध उत्पादों (कच्चे व संसाधित उत्पाद दोनो में) इसके प्रति उपचार पाया गया है। 7 के.सी. सामान्यतः सूखे दुग्ध पाउडर में कच्चे व उबले हुए दूध की तुलना में अधिक मात्रा में पाया जाता है। घी व पनीर में ये (7 के.सी.) बहुत ही नगण्य मात्रा में पाया जाता है। यह बात इसके तापमान सम्बन्धित संवेदनशीलता को भी परिलक्षित करती है।

आमतौर पर अभी तक 7 के.सी. द्वारा जन्य काशिकीय विषाक्तता को एण्टीऑक्सीडेन्ट, संश्लेषित या जैविक तत्वों द्वारा घटाने के प्रयास होता रहा है। इसके अतिरिक्त एन्जाइम व माइक्रोबियल (रोगाणुओं) द्वारा भी ऑक्सीस्टीरोल को कोशिका के भीतर या बाहर दोनों ही तरह से परिवर्तनात्मक विधी द्वारा चिकित्सकीय बायोरेमिडिएशन से घटाने का प्रयास किया गया है। शुरूआती प्रयासों से स्यूडोमोनास एरूजिनोसा PseA व रोडोकोकस इरिथ्रोपॉलिस MTCC 3951, 7 के.सी. के विघटन में सफल पाये गये। 7 के.सी. के 1 ग्राम/लिटर (1000 पीपीम) वाली प्रारम्भिक सांद्रता का पच्चीस दिनों में 88 प्रतिशत व पन्द्रह दिनों में 91 प्रतिशत तक विघटित करने में उक्त जीवाणु (स्यूडोमोनास एरूजिनोसा PseA व रोडोकोकस इरिथ्रोपोलिस MTCC 3951) सफल रहे हैं। शुरूआती शोध में 'इन विट्रो' जीवित कोशिका के

बाहर, बाह्य स्रावित एन्जाइम द्वारा इस 7 के.सी. का विघटन देखा गया। इससे उचित एन्जाइम के कार्य क्षमता का प्रमाण मिला। 7 के.सी. के लिए जीवाणु में कोलेस्ट्रोल ऑक्सीडेंज, लाइपेज, डीहाइड्रोजिनेज व रिडक्टेज पाये जाते हैं जिनमें से कॉलेस्ट्रोल ऑक्सीडेंज सर्वाधिक महत्वपूर्ण विघटन एन्जाइम है। दोनों ही जीवाणु के एन्जाइम दूध उत्पाद में कारगर है तो यह रणनीति आगे अपनायी जा सकती है।

इस विघटन (7 के.सी. का) के अतिरिक्त कॉलेस्ट्रोल ऑक्सीडेंज का औद्योगिक व बायोमेडिकल के क्षेत्रों में भी अत्यधिक उपयोग है। इसमें आगे शोध की आवश्यकता है। पी. एरूजिनोसा PseA की क्लोनिंग व ऑवर एक्सप्रेशन से इसके कम उत्पादन को भी बढाया जा सकता है। जीन की क्लोनिंग pGEM-TEasy वेक्टर द्वारा ई. कोलाइ XL1 Blue-MRF' स्ट्रेन में किया गया। ये काफी कन्जरर्वड स्वभाव का 1794 bp बेस पेयर का जीन है व अन्य स्यूडोमोनास स्ट्रेन से समानता रखता है। इसका सिक्वेंस नम्बर KU315227.1 GI: 1031987706 जीन बैंक में जमा कर दिया गया है। 'इन सिलिको' प्रयासे काफी संरचनायों की तरफ इशारा करती है। Phyre^2 व I-Tasser की समानता सोलवेंट टॉलरेंट कॉलेस्ट्रोल ऑक्सीडेंज क्रोमोबैक्टीरियम sp. DS-1 (PDBID-3js 8A) व B. sterolicum (PDB ID- 1i 19A) से समान पायी गयी। रेस्ट्रिक्शन क्लोनिंग हेतु pET22b(+) वेक्टर द्वारा, ई. कोलाई रोज़ेटा (DE3) स्ट्रेन में किया गया। इसकी कार्यशीलता सोल्यूशन में नहीं किंतु प्लेट में पायी गयी। अतः अतिरिक्त शोध की आवश्यकता कॉलेस्ट्रोल ऑक्सीडेंज़ की कार्यशीलता के लिए जरूरी है।

आर. इरिथ्रोपोलिस MTCC 3951 को कॉलेस्ट्रोल ऑक्सीडेंज उत्पादक होने के बाद भी क्लोनिंग हेतु चयन नहीं किया गया, इसकी वजह कम कन्जर्वड जीन के कारण है। प्रोसेस ऑप्टीमाईजेशन के द्वारा इस एन्जाइम का उत्पादन बढ़ाया जा सकता है। इस प्रोटीन को तीन पदों में संशोधित किया जा सकता है। प्रथम 10 kDa मॉलिक्यूलर वेट कट–ऑफ, द्वितीय क्यू–सेफेराज़ एनाइन–एक्सचेंज क्रोमेटोग्राफी व तीसरा पद फिनाइल सेफेरोज हाइड्रोफोबिक इन्टरेक्शन क्रोमेटोग्राफी। एक बिलकुल नवीन मोनामेरिक 35 kDa प्रोटीन पाया गया जो कि रोडोकोकस स्ट्रेन में सामान्यतः नहीं पाया जाता है। SDS-PAGE पुष्टी की गयी, जिसमें Native-PAGE की स्टेनिंग भी की गयी। इस एन्जाइम को संरचनात्मक व जैव रासायनिक कार्यशीलता द्वारा पहचान बनायी गयी। इस एन्जाइम के लिए PH 7.5, तापमान 30^0 C सबसे बेहतर देखी गयी। PH (4.0-9.0), तापमान (30-50^0C) में यह एन्जाइम उपयुक्त पाया गया। (KM 22.75 mM, V_{max} 9.21mM/min) संरचनात्मक शोध में फ्लोरोसेन्स, Far-UV, CD-स्पेक्ट्रोस्कोपी में 36.3% Helix, 0.9% एण्टीपेरेलल व 6% पेरेलल β-Sheets, 8.5% Turns व 18.3% अन्य संरचनाएं (3_{10}-Helix) प्रोटीन में विद्यमान थीं।

इन सभी की उपयोगिता बढ़ाने के लिए, कोलेस्ट्रोल ऑक्सीडेंज़ (ChOx) एन्जाइम जो पी. एरूजिनोसा PseA (ChOxP) व आर. इरिथ्रोपोलिस MTCC 3951 (ChOxR) स्ट्रेन द्वारा प्राप्त किया गया उसे स्ट्रेप्टोमाइसिस कॉमर्शियल वेरियन्ट के साथ आयरन ऑक्साइड (ii, iii) (MNP) व सिलिका नैनोपाट्रिकल (SNP) द्वारा इममोबेलाइज़्ड किया गया। ChOxP

ChOxR व ChOxS (71, 91 व 86% क्रमशः) MNP- नैनोबायोकेटेलिस्ट द्वारा कॉलेस्ट्रोल ऑक्सीडेंज का उत्पादन देखा गया। SNPs के इमोबलाइजेशन द्वारा ChOxP ChOxR व ChOxS (68, 86 व 83% क्रमशः) की कार्यशीलता देखी गयी। दो गुणी कार्यशक्ति का बढ़ना, स्टेबिलिटी का भी बढ़ना, 30-50^{0}C तापमान में सुरक्षित, PH (4.0-9.0), इनके लिए सही पाया गया है। परन्तु सर्वाधित उचित तापमान 30^{0} व PH 7.5 पायी गयी है। नैनोबायोकोन्जुगेट्स का दस चक्र तक पुनः उपयोग किया जा सकता है। इस एन्जाइम का इमोबेलाइजेशन FTIR, SEM व TEM द्वारा सत्यापित भी किया गया। नैनोबायोकॉन्जूगेट के इस्तेमाल से 7 के.सी. व कोलेस्ट्रोल का बायोट्रॉस्फोरमेशन द्वारा 4–कॉलेस्टीन–3– ऑन, 4–कॉलेस्टीन–3, 7, डाइऑन जैसे फॉरमोस्यूटीकल रूप से उपयोगी पदार्थों का निर्माण किया गया है। नेनोपार्टिकल इममोबिलाइजेशन की मदद से कोलेस्ट्रोल ऑक्सीडेंज की उपयोगिता में इज़ाफा हुआ है।

इस थीसिस के अंतिम पाठ में प्रोटीन कणों का नेनोपार्टिकल की सहायता से रूपान्तरण किया गया है। प्रोटीन को तापमान, PH द्वारा डीनेचर किया जा सकता है। इनकी पुनः रिफोल्डिंग से नेटिव फंक्शनल स्तर पर आ जाना व नेनोपार्टिकल का अप्राकृतिक रूप से 'शेपेरॉन' की भॉति व्यवहार करना, प्रोटीन के एग्रिगेशन को रोकता है। इसमें तीनों जीवाणु पी. एरूजिनोसा PseA, आर. इरिथ्रोपोलिस MTCC 3951 व स्ट्रेप्टोमाइसिस sp. के कॉलेस्ट्रोल ऑक्सीडेंज एन्जाइम के डीनेचुरेशन को 50–70^{0} C तापमान पर देखा गया है। ये तापमान द्वारा

डीनेचुरेटेड प्रोटीन का क्रियाशील MNPs से रिफोल्डिंग भी देखी गयी। इनकी क्रियाशीलता ChOxP ChOxR and ChOxS (47, 79 व 29.4% क्रमशः) पायी गयी। जिनका पुष्टीकरण FTIR, DLS, Zeta Potential measurement, Fluorescence व Far-UV-CD स्पेक्ट्रोस्कोपी द्वारा किया गया है। इससे यह सिद्ध हो जाता है कि चुम्बकीय नेनोपाट्रिकल पुनः रिफोल्डींग में ChOx जो कि डिनेचर हो चुका था की न सिर्फ संरचनात्मक अपितु क्रियात्मक क्षमता को कई गुणा बढ़ा कर लौटाता है।

अंत में यह शोध कार्य भोज्य साइटोटोक्सीसिटी, बायोडिग्रेडेशन, मोलिक्यूलर विज्ञान, एन्जाइम विज्ञान, नेनो टेक्नोलोजी व संरचनात्मक विज्ञान में आपसी तारतम्य व नये अनगिनत आयाम स्थापित करता है।

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7KC	7-Ketocholesterol
3,4-DHSA	3,4-dihydroxy-9,10-seconandrost-1,3,5-triene-9,17-dione
3,4,7-THSA	3,4,7-trihydroxy-9,10-seconandrost-1,3,5(10)-triene-9,17-dione
3,4,7-THSAP	3,4,7-trihydroxy-9,10-seconandrost-1,3,5(10)-triene-9,17-dione propionic acid
3,4-DHSAP	3,4-dihydroxy-9,10-seconandrost-1,3,5(10)-triene-9,17-dione propionic acid
3D	3-Dimensional
8-OHdG	8-hydroxy-2' -deoxyguanosine
AAc	Negatively charged acrylic acid
AAPH	2,2'-Azobis(2-amidinopropane) dihydrochloride
ACAT	Acyl-CoA cholesterol acyltransferase
Amp	Ampicillin
APTES	(3-Aminopropyl)triethoxysilane
ATP	Adenosine Triphosphate
Au(NPs)	Gold Nanoparticles
AuDA	2-(10-mercaptodecyl)malonic acid functionalized gold nanoparticles
Aβ-40	Amyloid Beta-40
Aβ-42	Amyloid Beta-42
BAPTA-AM	1,2-Bis(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid tetrakis(acetoxymethyl ester)
BeStSel	Beta Structure Selection
BLAST	Basic Local Alignment Search Tool
BLASTn	Basic Local Alignment Search Tool for Nucleotide Databases
BSA	Bovine Serum Albumin
CAD	Caspase-activated DNase
cAMP	Cyclic Adenosine Monophosphate
CD	Circular Dichroism
CH25H	Cholesterol 25-Hydroxylase
ChEt	Cholesterol Esterase
ChOx	Cholesterol Oxidase

ChOxP	Cholesterol Oxidase from *Pseudomonas aeruginosa* PseA
ChOxR	Cholesterol Oxidase from *Rhodococcus erythropolis* MTCC 3951
ChOxS	Cholesterol Oxidase from *Streptomyces* sp.
CHP	Cholesteryl group bearing pullulan
CLEA	Cross-Linked Enzyme Aggregate
CLEC	Cross-Linked Enzyme Crystals
COP	Cholesterol Oxidation Products
DEAE	Diethylaminoethyl
DHA	Docosahexaenoic Acid
DISOPRED	Disorder Prediction Server
DLS	Dynamic Light Scattering
DMAPA	N-[3-(dimethylamino) propyl] acrylamide
DNA	Deoxyribonucleic Acid
DSC	Differential Scanning Calorimetry
EDTA	Ethylenediaminetetraacetic Acid
ESI-MS	Electrospray Ionization-Mass spectrometry
FAD	Flavin Adenine Dinucleotide
FPLC	Fast Protein Liquid Chromatography
FTIR	Fourier-Transform Infrared Spectroscopy
GA	Glycyrrhetinic acid
GC-MS	Gas Chromatography–Mass Spectrometry
GFP	Green Fluorescent Protein
GL	Glycyrrhizin
GSH	Glutathione
HDL	High-Density Lipoprotein
HDMPPA	3-(4'-hydroxyl-3',5'-dimethoxyphenyl)propionic acid
HMG-CoA	3-hydroxy-3-methylglutaryl-CoA
HPLC	High-Performance Liquid Chromatography
HRMS	High-Resolution Mass Spectrometry
HRP	Horseradish Peroxidase
ICAD/DFF	Inactive Caspase-activated DNAse/DNA fragmentation factor

IEC	Ion-Exchange Chromatography
IMTech	Institute of Microbial Technology
IPTG	Isopropyl β- d-1-thiogalactopyranoside
I-TASSER	Iterative Threading ASSEmbly Refinement
kDa	Kilo Dalton
LB	Luria-Bertani medium
LC	Liquid Chromatography
LC-MS	Liquid Chromatography-Mass Spectrometry
LXR	Liver X receptor
MNP	Magnetic Nanoparticles
Mn-TBAP	Mn(III)tetrakis(4-benzoic acid) porphyrin
MS	Mass-Spectrometry
MSM	Minimal Salt Medium
MTCC	Microbial Type Culture Collection and Gene Bank
MW	Molecular Weight
MWCO	Molecular Weight Cut-Off
NAD	Nicotinamide Adenine Dinucleotide
NADPH	Nicotinamide Adenine Dinucleotide Phosphate (reduced)
NB	Nutrient Broth
NCBI	National Center for Biotechnology Information
NIST	National Institute of Standards and Technology
NP	Nanoparticles
NRMSD	Normalized Root-Mean-Square Deviation
OD	Optical Density
ORF	Open Reading Frame
OVA	Ovalbumin
OVAT	One Variable at A Time
PAGE	Polyacrylamide Gel Electrophoresis
PANi	Polyaniline
PCL	Poly(ε-caprolactone)
PCR	Polymerase Chain Reaction

PDB	Protein Data Bank
PEG	poly(ethylene glycol)
Phyre2	Protein Homology/AnalogY Recognition Engine 2.0
PI	Isoelectric Point
PNIPAm	poly(N-isopropylacrylamide)
PS	Polystyrene
PSI-BLAST	Position-Specific Iterative Basic Local Alignment Search Tool
PSIPRED	PSI-blast based secondary structure PREDiction
PTIO	2-phenyl-4, 4, 5, 5,-tetramethylimidazoline-1-oxyl 3-oxide
PUFA	Polyunsaturated Fatty Acids
PVDF	Polyvinylidene Fluoride
QuEChERS	Quick Easy Cheap Effective Rugged Safe
RNAse A	Ribonuclease A
ROS	Reactive Oxygen Species
RP-HPLC	Reversed Phase High Performance Liquid Chromatography
MWD	Multi-Wavelength Detector
RPM	Revolutions Per Minute
rRNA	Ribosomal Ribonucleic Acid
SDS-PAGE	Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis
SEM	Scanning Electron Microscopy
SNP	Silica Nanoparticles
SPE	Solid Phase Extraction
TBAm	N-tert-butylacrylamide
TEM	Transmission Electron Microscopy
TEOS	Tetraethyl orthosilicate
Tet	Tetracycline
UHT	Ultra-high temperature
UV	Ultra-Violet
X-Gal	5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside
β-CD	Beta Cyclodextrin