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Review Article

THERMOSTABLE RESTRICTION ENDONUCLEASES FROM THERMOPHILIC BACTERIA

Gupta Dipali[1] and Sharma Nevadita[2]*
[1]Associate Professor in Department of Biotechnology and Microbiology, Tanveer Malawat College of Biosciences, Bikaner (Rajasthan), India
[2]PhD Scholar in Department of Biotechnology and Microbiology, Tanveer Malawat College of Biosciences, Bikaner (Rajasthan), India
*Corresponding Author Email: nevadita@gmail.com

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ABSTRACT
Restriction endonucleases are not only important for bacteria and archaea as their defense mechanism but they are considered as foundation of recombinant DNA technology. The whole empire of biotechnology depends upon type II restriction endonucleases isolated from the diversified array of bacteria. There are 4,096 known restriction enzymes, including 106 Type I, 3, 950 Type II, 21 Type III and 19 Type IV restriction enzymes. Among which 646 type II enzymes have been cloned and 611 have been sequenced. A number of restriction endonucleases have been isolated from thermophilic bacteria also, having higher thermal stability and tolerance to most of the chemical denaturants. In Restriction Enzyme Database, about 600 type II restriction enzymes have been listed showing growth temperature above 40^0C. Nowadays these enzymes have found their importance in isolating mutant strains and for production of antisense oligonucleotides. Thermostable restrictions enzymes are also applied as important tool for molecular biology, industrial works and in cellular processes.
Keywords: Restriction Endonucleases, Thermostable, Thermophilic

INTRODUCTION

The main function of Restriction endonucleases is to defend their host against foreign DNA. This is achieved by cleaving incoming DNA that is recognized as foreign by the absence of a characteristic modification (N4 or C5 methylation at cytosine or N6 methylation at adenine) at defined sites within the recognition sequence. The host DNA is resistant to cleavage as these sites are modified. Restriction endonucleases have traditionally been placed into two groups based on their cofactor requirements, catalytic properties, and subunit structures. Since the discovery of the first type II restriction endonuclease[1], these enzymes have played important roles in creating recombinant DNA molecules[2,3]. Over 600 type II restriction- modification (R-M) systems have been cloned so far.[4] Among the cloned restriction endonucleases, some enzymes with the same or related DNA recognition sequences have similar amino acid sequences (30 to 100 % identity)[5]. Weak amino acid sequence similarities (16 to 20 % identity) have been reported among some non-isoschizomers[6,7]. The methylases of different R-M systems are more conserved. Nine conserved sequence motifs were found among the aminomethylases (N4-cytosine and N6-adenine methylases)[8] and 10 were found among the cytosine-5 methylases[9]. The aminomethylases are separated into three groups, a, b, and g, based on the circular permutation of conserved motifs[5,8]. The majority of the restriction endonucleases have been isolated from mesophilic bacteria, so whilst the enzymes are stable at temperatures below 45°C, they usually denature rapidly at higher temperatures. Restriction endonucleases that will withstand higher temperatures represent an extremely useful addition to the molecular biologist's tool-kit. In recent years the search for novel and thermally stable restriction endonucleases has been extended to include organisms that normally thrive at high temperatures[10]. The thermophilic genus Thermus and its type species *aquaticus* were first described by Brock and Freeze[11] as aerobic, non-sporulating heterotrophic rods with optimum growth temperatures in the region of 70^0C. Strains of Thermus have been isolated from neutral and alkaline hot water environments in the U.S.A[12], Japan[13], Iceland[14], Belgium[15], Britain[16], New Zealand[17], Portugal[18], the Czech Republic[19] and Thailand[20]. Twenty-two restriction endonucleases, each with a different DNA recognition site, have been discovered within the species and strains of the genus Thermus[21] and four of these enzymes have no known mesophilic isoschizomers.

Thermophilic Microorganisms

Ecological studies have shown that both aerobic and anaerobic species and many morphological and physiological types of microorganisms can exist in thermophilic environments[22,23]. Extreme thermophiles are mostly distributed among the genera of *Bacillus*, *Clostridium*, *Thermoanaerobacter*, *Thermus*, *Thermotoga*, *Aquifex*. Most hyperthermophiles, on the other hand, include the two kingdoms of *Archaea*, *Crenarchacota* (*Sulfolobus*, *Pyrodictium*, *Pyrolobus*.), *Euryarchaeaota* (*Thermococcus*, *Pyrococcus*), methanogenes (*Methanococcus*, *Methanobacterium*), sulfate reducers and halophiles[24]. Archaea bacteria have been first mentioned as a separate group by Woese[25], after his 16S rRNA based three-kingdom system proposal[26]. According to the universal tree, a tripartite division of living world consists of phylogenetic domains Bacteria, Archaea and Eukarya. The root of the universal tree represents a point in evolutionary time that all life on earth shared a common ancestor. It clearly indicates that initial evolution from a universal ancestor was first branched in two directions. These were the bacteria line and the Archaea-eukarya line. Archaea and Eucarya are therefore phylogenetically more related to each other than bacteria [27]. The universal tree shows that the Archaea has branched off the tree at a point closest to the root. This supports the idea

that Archaea include the most primitive organism among the three domains. Placement of the Archaea nearest to the universal ancestor is also supported by the fact that many Archaea tolerate extreme environmental conditions such as high temperature, high salinity, low pH under which the earliest life forms have been thought to be originated. Thus, members of Archaea may be the earth's earliest life forms[27].

Structural Adaptations of Thermostable Proteins and Enzymes

Taxonomically thermophilic microorganisms are related to the mesophilic species. The thermophile's anatomy, ultrastructure, respiration, and metabolic processes are very similar when compared with those of mesophilic organisms. These similarities indicate that thermophiles and mesophiles have evolved from common ancestors. It has been concluded that an enzyme from a thermophilic organism, in general, is remarkably similar to that of a mesophilic organism in physical properties[28]. Thermophiles, on the other hand have been reported to produce proteins called chaperonins that help to refold to their native form after denaturation. The cell membrane of thermophiles is composed of saturated fatty acids that provide hydrophobic environment for the cell. Archaea, which compose most of the hyperthermophiles, have lipids linked with ether on the cell wall. The DNA of thermophiles has also been reported to have a reverse DNA gyrase producing positive supercoils in the DNA. Since this difference increases the melting point of DNA, the stability at high temperatures is achieved[29]. No general strategy of stabilization has yet been established[30]. Thermostability appears to be achieved by proteins using the same forces involved in folding acquired by small structural modifications[31]. Several parameters may be effective for the stability of thermophilic proteins like; Increased number of ion pairs and hydrogen bonds, extended hydrophobic subunit interactions and improved packing of hydrophobic core, shortened chain termini, decreased hydrophobic area, increased helicity, less cavity volume, large number of additional salt bridges, stabilization of α-helices, replacement of conformationally strained residues by glycines, strong docking of N-terminal methionine etc[30]. Alterations in the amino acid composition of proteins bring about additional electrostatic interactions, formation of hydrogen and disulfide bonds, and enhancement of hydrophobic interactions or compaction of the structure[32]. There are only a few cysteine residues in thermophilic enzymes or they are completely absent. Since the fact that inactivation is often caused by oxidation of SH- groups, lower cysteine content could enable the protein protected against the oxidation type of inactivation. In some cases localization of cystein residues is also important[32] as alcohol dehydrogenase from *Bacillus sterarothermophilus* has the same number of cysteine residues as its mesophilic analogue, but its SH-groups are localized inside the protein globule[31,33]. Many significant substitutions in thermophilic enzymes as Lys to Arg, Ser to Ala, Ser to Thr and Val to Ile have been reported[31]. These substitutions cause an increase in the internal hydrophobicity. Thermophilic proteins generally show a decreased flexibility and increased hydrophobicity within the α-helical regions. The amino acids responsible for decreased flexibility are located in the helices at non-buried or surface positions so that they are tightly packaged by means of increased hydrophobic contacts[31]. Furthermore a helix-favoring residue, arginine, occurs more frequently whereas helix-dis favoring residues cysteine, histidine and proline have lower frequencies in thermophilic proteins[34]. Some thermophilic enzymes having proteolytic or amylolytic action are stabilized by Ca, Mg, Zn and other ions. The mechanism of stabilization is through the binding of cations to the labile parts of the globule. It is also known that some mesophilic enzymes are also stabilized by metal ions[33]. Hydrophobic interactions are considered the main driving forces in protein folding. In thermophilic proteins, polar surface areas are larger than in mesophilic proteins[31]. However it has been suggested that hydrophobicity shows little quantitative differences between thermophiles and mesophiles[34]. Differ in the numbers of hydrogen bonds and salt bridges may also be another factor for stability, as about 19 additional hydrogen bonds have been detected in thermophilic proteases which are not present in its mesophilic counterpart[33]. Changes in the number of hydrogen bonds change the secondary structure of a protein. It has been observed that the main players of thermal stability are salt bridges and hydrogen bonds. The salt bridges around the active site may help to keep the active site region together by opposing disorder due to greater atomic mobility at high temperatures[34]. It has also been suggested that deletion or shortening of loops may increase the thermal stability and that oligomerisation can be another contributing factor[34].

Thermostable Restriction Endonucleases

The presence of thermostable restriction endonucleases was established by Thomas Brock through his extensive and pioneering studies in Yellowstone National Park (Wyoming, USA) from 1968 to 1978. It is now more than 30 years since he discovered *Thermus aquaticus*, the first isolated organisms shown to grow above 70°C[35]. Brock also isolated *Sulfolobus acidocaldarius* and *Thermoplasma acidophilum*, the first representatives of the thermoacidophilic archaea. Over 10,000 eubacteria and archae bacteria from culture collections, hospitals, soil, and water samples from around the world have been screened for restriction enzymes until now. Among the 4,096 known restriction enzymes, there are about 600 enzymes which grow at above 40^0C temperature[36]. The majority of the restriction endonucleases have been isolated from mesophilic bacteria[37]. The search for novel and thermally stable restriction endonucleases has been extended to include microorganisms that normally thrive at high temperatures[38,39]. Most thermostable type II restriction endonucleases recognizing different DNA sequences have been discovered among species of the genus *Thermus* and strains of *Bacillus stearothermophilus*[37]. Restriction endonucleases with 26 different specificities have been discovered within the species of the genus *Thermus*[37] including 10 prototypes: Taq I[40], Taq II[41], Tfi I, Tse I, Tsp4CI[10], TspEI[42], Tsp45I[43], TspRI, Tth111I[44] and Tth111II[45]. A restriction endonuclease, Bst PI, was purified from a strain of *B. stearothermophilus*, and its cleavage specificity was determined[46]. In 1981 Stetter and Zillig[47] isolated *Thermophile*, the first anaerobic, extreme thermoacidophile, from Icelandic hot springs. Since then, several new species and genera have been isolated, including the landmark discoveries by Karl Stetter and his colleagues[48], *Pyrodictium,* the first organism to grow optimally at 105°C and *Pyrolobus*, which has a maximum growth temperature of 113°C, the highest growth temperature yet recorded for any living organism. Some isolates belong to *Thermus scotoductus* because the small subunit (SSU) rRNA gene sequence analysis showed 98.6 % sequence similarity and 84 % DNA: DNA re association to *Thermus scotoductus* NMX2

A.1[49]. Based on phylogenetic analysis of the small-subunit rRNA (16S rRNA) gene sequences, the order *Aquificales* is the deepest lineage within the domain Bacteria[50,51]. A Thermostable, Co^{++}-Requiring restriction enzyme BflI, an Isoschizomer of BsiYI was isolated from a thermophile, *Anoxybacillus flavithermus*, isolated from geothermal areas in the northern Himalayan region of India. The isolate produced BflI, a Type II restriction endonuclease, which recognized the sequence 5′-CCNNNNN/NNGG-3′ and was the isoschizomer of BsiYI. The purified enzyme (MW 36 kDa) worked best at 60°C. The enzyme showed high specific activity and worked in the presence of high concentrations of β-mercaptoethanol (200 mM), Triton-X-100 (25 %), urea (30 %), formamide (6 %) and guanidine (40 mM) and showed no star activity in the presence of 40 % glycerol[52]. Five hydrogen-oxidizing, thermophilic, strictly chemolithoautotrophic, micro aerophilic strains, with similar (99–100 %) 16S rRNA gene sequences were isolated from terrestrial hot springs at Furnas, Sao Miguel Island, and Azores, Portugal. The strain, designated Az-Fu1T, was characterized and it is proposed that Az-Fu1T belongs to the recently described genus *Sulfurihydrogenibium*. It is further proposed that Az-Fu1T represents a new species, *Sulfurihydrogenibium azorense*[53]. *Caldicellulosiruptor bescii* genome sequence revealed the presence of both a HaeIII-like restriction endonuclease (Athe 2438) and DNA methyltransferase (Athe 2437). Preliminary analysis of other *Caldicellulosiruptor* species suggested that this restriction/modification activity is widespread in this genus. A novel thermostable restriction enzyme CbeI was isolated from *Caldicellulosiruptor bescii*. CbeI is distinct from other members of this group and classified as a member of a novel subfamily of HaeIII-like enzymes[54]. Two thermostable restriction enzymes, MspNI and MspNII, isoschizomers of prototype Type II restriction endonucleases AvaII and BstYI, were extracted from an extreme thermophile bacterium belonging to the genus Meiothermus, isolated from the hot sulphur springs in north Himalayan region of India where temperature and pH ranged from 60 to 80°C and 7.5 to 8.5, respectively. They recognized and cleaved 5′-G/GWCC-3′ and 5′-R/GATCY-3′ sites, respectively. MspNI and MspNII worked optimally on 6 and 5 mM $MgCl_2$, respectively and showed no star activity in organic solvents. Both were resistant to sequence methylation and were stable up to 25 PCR cycles[55].

Biotechnological and Industrial Applications of Thermophiles

One of the most attractive attributes of thermophiles is that they produce enzymes capable of catalyzing biochemical reactions at temperatures higher than those of mesophilic organisms[56]. The property of higher thermal stability and tolerance to most of the chemical denaturants or organic solvents enables them to resist harsh process conditions. They also show high catalytic activity at high temperatures and longer shelf-life as commercial products[57,58]. The increase of temperature in biotechnological processes has an influence on the bioavailability and solubility of organic compounds such as poly-aromatic, aliphatic hydrocarbons and polymeric substances. The elevation of temperature is accompanied by a decrease in viscosity and an increase in the diffusion coefficient of organic compounds. Consequently, higher reaction rates due to smaller boundary layers are expected[59]. Biological processes where high operational temperatures above 60ºC employed, the risk of contamination by other organisms is also become substantially reduced[60]. Furthermore, in large scale fermentations with heat sensitive microorganisms, extensive efforts must be given for cooling the fermentation process and as much as ten percent of the energy cost of a microbial fermentation may be for heat transfer. Thermophilic fermentations, on the other hand, need not to be cooled[59]. A restriction site mutation assay has been successfully developed as a mutation assay by using thermostable restriction enzymes. It has been applied to detect mutations in a variety of genes and organisms. The RSM assay is capable of answering questions on the mutability of DNA sequences which are contained within restriction enzyme sites. The RSM assay represents a powerful technique for mutation analysis, producing quantitative mutation data rapidly and allowing the screening of a large number of mutagen-treated samples[61]. Another novel application of thermostable restriction enzyme is the development of a PCR based technique, polymerase-endonuclease amplification reaction (PEAR), for production of antisense oligonucleotides drugs.Antisense oligonucleotides targeting microRNAs or their mRNA targets prove to be powerful tools for molecular biology research and may eventually emerge as new therapeutic agents[62].

CONCLUSION

Nowadays, interests in thermostable restriction endonucleases are expanding into evermore diverse fields such as molecular biology, whole genome sequencing, bioinformatics etc. Due to their uncountable properties and advantages in a number of processes, thermostable restriction endonucleases have become a hot spot in current studies. Therefore, this review article has emphasized on adaptations found in thermostable enzymes at molecular level, research going on isolation and characterization of thermostable restriction enzymes and their possible role in advanced areas of molecular biology, like screening of mutants and oligonucleotide drug synthesis.

REFERENCES

1. Smith HO, Wilcox KW. A restriction enzyme from *Hemophilus* influenzae. I. Purification and general properties. Journal of Molecular Biology 1970; 51: 379–391. http://dx.doi.org/10.1016/0022-2836(70)90149-X
2. Heitman J. On the origins, structures and functions of restriction-modification enzymes. In: Setlow JK, editor. Genetic Engineering. Plenum Press: New York; 1993. p. 57–108. http://dx.doi.org/10.1007/978-1-4899-1666-2_4
3. Roberts RJ, Halford SE. Type II restriction enzymes. In: Linn SM, Lloyd RS, Roberts RJ, editors. Nucleases. 2nd edition. New York: Cold Spring Harbor Laboratory Press: Cold Spring Harbor; 1993. p. 35–88.
4. Roberts RJ, Macelis D. Rebase-restriction enzymes and methylases. Nucleic Acids Research 1998; 26: 338–350. http://dx.doi.org/10.1093/nar/26.1.338
5. Wilson GG, Murray NE. Restriction and modification systems. Annual Review of Genetics 1991; 25: 585–627. http://dx.doi.org/10.1146/annurev.ge.25.120191.003101
6. Morgan RD, Camp RR, Wilson GG, Xu SY. Molecular cloning and expression of NlaIII restriction-modification system in *E. coli*. Gene 1996; 183: 215–218. http://dx.doi.org/10.1016/S0378-1119(96)00561-6
7. Siksnys V, Timinskas A, Klimasauskas S, Butkus V, Janulaitis A. Sequence similarity among type-II restriction endonucleases, related by their recognized 6-bp target and tetranucleotide-overhang cleavage. Gene 1995; 157: 311–314. http://dx.doi.org/10.1016/0378-1119(94)00632-3
8. Malone T, Blumenthal RM, Cheng X. Structure-guided analysis reveals nine sequence motifs conserved among DNA amino-methyltransferases, and suggest a catalytic mechanism for these enzymes. Journal of Molecular Biology 1995; 253: 618–632. http://dx.doi.org/10.1006/jmbi.1995.0577
9. Posfai J, Bhagwat AS, Posfai G, Roberts RJ. Predictive motifs derived from cytosine methyltransferases. Nucleic Acids Research 1989; 17: 2421–2435. http://dx.doi.org/10.1093/nar/17.7.2421

10. Welch SG, Williams RAD. Two thermostable Type 11 restriction endonucleases from Icelandic strains of the genus Thermus: Tsp4C I (ACN/GT), a novel Type 11 restriction endonuclease, and Tsp8E 1, an isoschizomer of the mesophilic enzyme BgI I (GCCNNNN/NGGC). Biochemical Journal 1995; 309: 595-599.
11. Brock TD, Freeze H. *Thermus aquaticus* gen. n. and sp.n., a Non-sporulating Extreme Thermophile. Journal of Bacteriology 1969; 98: 289-297.
12. Munster MJ, Munster AP, Woodrow JR, Sharp RJ. Isolation and preliminary taxonomic studies of Thermus strains isolated from Yellowstone National Park, USA. Journal of General Microbiology 1986; 132(6): 1677–1683.
13. Oshima T, Imahori K. Description of Thermus Thermophilus (Yoshida and Oshima) comb Nov a Non-sporulating *Thermophilic* Bacterium from a Japanese Thermal Spa. International Journal of Systematic Bacteriology 1974; 24: 102-112. http://dx.doi.org/10.1099/00207713-24-1-102
14. Pask Hughes R, Williams RAD. Yellow-pigmented strains of *Thermus* spp. from Icelandic hot springs. Journal of Genetic Microbiology 1977; 102: 375-383. http://dx.doi.org/10.1099/00221287-102-2-375
15. Degryse E, Glansdorff N, Pierard A. A comparative analysis of extreme thermophilics bacteria belonging to the genus *Thermus*. Archives of Microbiology 1978; 117: 189-196. http://dx.doi.org/10.1007/BF00402307
16. Pask Hughes R, Williams RAD. Extremely thermophilic gram- negative bacteria from tap hot water. Journal of General Microbiology 1975; 88: 321-328. http://dx.doi.org/10.1099/00221287-88-2-321
17. Hudson JA, Morgan HW, Daniel RM. A numerical classification of some Thermus isolates. Journal of General Microbiology 1986; 132: 531-540.
18. Santos MA, Williams RAD, Costa MSD. Numerical taxonomy of Thermus isolates from hot springs in Portugal. Systematic and Applied Microbiology 1990; 12: 310-315. http://dx.doi.org/10.1016/S0723-2020(89)80079-7
19. Peckova M. Properties of a *Hyperthermophilic* Bacterium (*Thermus* sp.) Isolated from Carlsbad Spring. Folia Microbiologica 1991; 36(6): 515-521. http://dx.doi.org/10.1007/BF02884029
20. Kanasawud P, Teeyapan S, Lumyong S, Hoist O Mattiasson B. Thermus 2S from Thai hot springs: isolation and immobilization. World Journal Microbiology and Biotechnology 1992; 8: 137-140. http://dx.doi.org/10.1007/BF01195833
21. Robert RJ, Macelis D. Restriction endonucleases and their iso-shizomers. Nucleic Acids Research 1993; 21: 3125-3137.
22. Brock DT. The Origins of Research on Thermophiles. In: Reysenbach AL, editor. Thermophiles Biodiversity Ecology and Evolution. Kluwer Academic/Plenum Publishers New York; 2001. p. 1-22.
23. Madigan MT, Martinco JM, Parker J. Ecology and Biotechnological Applications of Thermophiles and Hyperthermophiles. In: Brock Biology of Microorganisms. Prentice-Hall Inc; 1997a. p. 167.
24. Bertoldo C, Antranikian G. Starch-Hydrolyzing Enzymes from Thermophilic Archea and Bacteria. Current Opinion in Chemical Biology 2002; 6: 151-160. http://dx.doi.org/10.1016/S1367-5931(02)00311-3
25. Woese CR, Magrum LJ, Fox GE. Archaebacteria. Journal of Molecular Evolution 1978; 11(3): 245–252. http://dx.doi.org/10.1007/BF01734485
26. Madigan MT, Martinco JM, Parker J. Microbial Evolution Systematics and Taxonomy. In: Brock Biology of Microorganisms. Prentice-Hall Inc; 1997 b. p. 621-633.
27. Madigan MT, Martinco JM, Parker J. Microbial Growth. In: Brock Biology of Microorganisms. Prentice-Hall Inc; 1997 c. p. 165- 168.
28. Ljungdahl LG, Sherod D. Proteins from Thermophilic Microorganisms. In: Edwards C editor. Extreme Environments, Adaptation of Microorganisms. Manchester: Open University Press; 1960. p. 147-181.
29. Haki GD, Rakshit SK. Developments in industrially important thermostable enzymes: a review. Bioresource Technology 2003; 89: 17-34. http://dx.doi.org/10.1016/S0960-8524(03)00033-6
30. Jaenicke R, Bohm G. The Stability of Proteins in Extreme Environments. Current Opinion in Structural Biology 1998; 8: 738-748. http://dx.doi.org/10.1016/S0959-440X(98)80094-8
31. Scandurra R, Consalvi V, Chiaraluce R, Politi L, Engel PC. Protein Thermostability In: Extremophiles. Societe Française de Biochimeet Biologie Moleculaire 1998; 80: 933-941.
32. Yavuz E. Genotypic Characterization of Extracellular Enzyme Producing Thermophilic Bacteria in Balçova Geothermal Region. A Dissertation Submitted to the Graduate School in Partial Fulfillment of the Requirements for the Degree of Master of Science; 2003. p. 1-20.
33. Mozhaev VV, Martinek K. Structure- Stability Relationships in Proteins: New Approaches to Stabilizing Enzymes. Enzyme Microbial Technology 1984; 6: 50-57. http://dx.doi.org/10.1016/0141-0229(84)90034-6
34. Kumar S, Tsai C, Nussinov R. Factors Enhancing Protein Thermostability. Protein Engineering 2000; 13: 179-191. http://dx.doi.org /10.1093/protein/13.3.179
35. Hreggvidsson GO, Kristjansson JK. Thermophily. Extremophiles; 2000. p. 1.
36. Roberts RJ, Vincze T, Posfai J, Macelis D. Rebase: restriction enzymes and methyltransferases. Nucleic Acids Research 2003; 31: 418–420. http://dx.doi.org/10.1093/nar/gkg069
37. Roberts RJ, Macelis D. Rebase - restriction enzymes and methylases. Nucleic Acids Research 2001; 29: 268-269. http://dx.doi.org/10.1093/nar/29.1.268
38. Hjorleifsdottir S, Petursdottir SK, Korpela J, Torsti AM, Mattila P, Kristjansson JK. Screening for restriction endonukleazes in aerobic, thermophilic eubacteria. Biotechnology Techniques 1996; 10: 13-18. http://dx.doi.org/10.1007/BF00161077
39. Repin VE, Lebedev LR, Puchkova L, Serov GD, Tereschenko T, Chizikov VE, Andreeva I. New restriction endonucleases from thermophilic soil bacteria. Gene 1995; 157: 321-322. http://dx.doi.org/10.1016/0378-1119(94)00781-M
40. Sato S, Hutchison III CA, Harris JI. A thermostable sequence-specific endonuclease from *Thermus aquaticus*. Proceedings of the National Academy of Sciences USA 1977; 74: 542- 546. http://dx.doi.org/10.1073/pnas.74.2.542
41. Barker D, Hoff M, Oliphant A, White R. A second type II restriction endonuclease from *Thermus aquaticus* with unusual sequence specificity. Nucleic Acids Res 1984; 12: 5567- 5581. http://dx.doi.org/10.1093/nar/12.14.5567
42. Raven NDH, Kelly CD, Carter ND, Eastlake P, Brown C, Williams RAD. A new restriction endonuclease, T*sp*EI, from the genus *Thermus* that generates cohesive termini compatible with those of EcoRI. Gene 1993a; 131: 83-86. http://dx.doi.org/10.1016/0378-1119(93)90672-P
43. Raven NDH, Williams RAD, Smith KE, Kelly CD, Carter ND. T*sp*45I, a new thermostable site-specific endonuclease that cleaves the recognition sequence 5′↓GTSAC-3′. Nucleic Acids Research 1993b; 21: 4397. http://dx.doi.org/10.1093/nar/21.18.4397
44. Shinomiya T, Sato S. A site-specific endonuclease from *Thermus thermophilus* 111, *Tth*111I. Nucleic Acids Research 1980a; 8: 43-56. http://dx.doi.org/10.1093/nar/8.15.3275
45. Shinomiya T, Kobayashi M, Sato S. A second site specific endonuclease from *Thermus thermophilus* 111, *Tth*111II. Nucleic Acids Research 1980b; 8: 3275-3285. http://dx.doi.org/10.1093/nar/8.15.3275
46. Pugatsch T, Weber H. A thermostable, sequence-specific restriction endonuclase from *Bacillus stearothennophilus*: BstPI. Nucleic Acids Research 1979; 7(6): 1429-1444. http://dx.doi.org/10.1093/nar/7.6.1429
47. Zillig W, Stetter KO, Schäfer W, Janekovic D, Wunderl S, Holz I, Palm P. *Thermoproteales*: a novel type of extremely thermoacidophilic anaerobic archaebacteria isolated from Icelandic solfataras. Zbl. Bakt. Hyg, I. Abt. Orig 1981; C2: 205-227.
48. Stetter K, Segerer A, Zillig W, Huber G, Fiala G, Huber R, Konig H. Extremely Thermophilic Sulfur-Metabolizing Archaebacteria, System. Journal of Applied Microbiology 1986; 7: 393-397.
49. Skirnisdottir S, Hreggvidsson GO, Holst O, Kristjansoon JK. Isolation and characterization of a mixotrophic sulfur-oxidizing *Thermus scotoductus*. Extremophiles 2001; 5: 45-51. http://dx.doi.org/10.1007/s007920000172
50. Burggraf S, Olsen GJ, Stetter KO, Woese CR. A phylogenetic analysis of *Aquifex pyrophilus*. Systematic and Applied Microbiology 1992; 15: 352–356. http://dx.doi.org/10.1016/S0723-2020(11)80207-9
51. Pitulle C, Yang Y, Marchiani M, Moore ERB, Siefert JL, Aragno M, Jurtshuk P, Fox GE. Phylogenetic position of the genus *Hydrogeno bacter*. International Journal of Systematic Bacteriology 1944; 44: 620–626. http://dx.doi.org/10.1099/00207713-44-4-620
52. D Souza DR, Morgan RD, Parashar V, Capalash N, Sharma P. Characterization of BfII – A Thermostable, Co^{++}-Requiring Isoschizomer of BsiYI from *Anoxybacillus flavithermus*. World Journal of Microbiology and Biotechnology 2004; 20(6): 593-598. http://dx.doi.org/10.1023/B:WIBI.0000043173.88074.14
53. Aguiar P, Beveridge TJ, Reysenbach AL. Sulfuri hydrogenibiumazorense, sp. nov., a thermophilic hydrogen-oxidizing micro aerophile from terrestrial hot springs in the Azores. International Journal of Systematic and Evolutionary Microbiology 2004; 54: 33–39. http://dx.doi.org/10.1099/ijs.0.02790-0
54. Chung DH, Huddleston JR, Farkas J, Westpheling J. Identification and characterization of CbeI, a novel thermostable restriction enzyme from *Caldicellulosiruptor bescii* DSM 6725and a member of a new subfamily of HaeIII-like enzymes. Journal of Industrial Microbiology and Biotechnology 2011; 38(11): 1867-77. http://dx.doi.org/10.1007/s10295-011-0976-x
55. Gupta R, Xu S, Sharma P, Capalash N. Characterization of MspNI (G/GWCC) and MspNII (R/GATCY), novel thermostable Type II restriction endonucleases from *Meiothermus* sp., isoschizomers of AvaII

and BstYI. Molecular Biology Reports 2012; 39(5): 5607. http://dx.doi.org/10.1007/s11033-011-1365-2
56. Demirjian D, Moris VF, Cassidy CS. Enzymes from Extremophiles. Current Opinion in Chemical Biology 2001; 5: 144-151. http://dx.doi.org/10.1016/S1367-5931(00)00183-6
57. Aguilar A, Ingemansson T, Magnien E. Extremophile Microorganisms as Cell Factories: Support from the European Union. Extremophiles 1998; 2: 367-373. http://dx.doi.org/10.1007/s007920050080
58. Kristjansson JK. Thermophilic Organisms as Sources of Thermostable Enzymes. TIB tech 1989; 7: 349-353. http://dx.doi.org/10.1016/0167-7799(89)90035-8
59. Niehaus F, Bertoldo C, Kahler M, Antranikian G. Exteromophiles as a Source of Novel Enzymes For Industrial Application. Applied Microbial Biotechnology 1999; 51: 711-729. http://dx.doi.org/10.1007/s002530051456
60. Adams MWW, Kelly RM. Finding and Using Hyperthermophilic Enzymes. TIB tech 1998; 16: 329-332. http://dx.doi.org/10.1016/S0167-7799(98)01193-7
61. Jenkins GJS, Suzen HS, Sueiro RA, Parry JM. The restriction site mutation assay: a review of the methodology development and the current status of the technique. Mutagenesis 1999; 14(5): 439-448. http://dx.doi.org/10.1093/mutage/14.5.439
62. Wang X, Gou D, Xu SY. Polymerase-Endonuclease Amplification Reaction (PEAR) for Large-Scale Enzymatic Production of Antisense Oligonucleotides. PLoS One 2010; 5(1): e8430. http://dx.doi.org/10.1371/journal.pone.0008430

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