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Production and Process Optimization of Protease using Various Bacterial Species – A Review

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Abstract: This review constrains mostly to protease production using various bacterial strains. Haloalkaliphilic bacterial species have found to be more profoundly used in recent days of modern enzyme production technology. The extremophiles have been known to be involved in enzyme technology for over a decade. However, their ability to survive harsh climatic changes and extreme conditions has made them more popular and useful, compared to the other bacterial species. Therefore this review would provide a clear idea on the optimization and production process of the various proteases.
Keywords: Haloalkaliphilic, extremophiles, proteases, optimization.

Introduction

Protease is an enzyme which is known to breakdown complex protein molecules into simpler forms. Microbial world serves as an excellent source for the production of these enzymes [1] because of their high chemical diversity, feasibility of mass culture and ease of genetic manipulation 2.The enzymes produced by these microbes play a major role in the advanced biotechnology. The exploration of this microbial world to some extent revealed that a majority of them did not suit for the extreme harsh conditions of industrial and environmental processes because of their subtle and sensitive nature.

Considering these situations scientists have moved on to concentrate more on microbes that thrive even at extreme conditions (pH, temperature, salinity, etc.)[1,2]. Proteases are one among the important groups of biocatalysts which comprise a wide range of enzymes based on their source, mechanism of action and specificity [3, 4, 5, 6.]Protease production accounts for nearly one fourth of the total global enzyme production[7,8.]

These extracellular enzymes are synthesised from various common as well as remote groups of microbes like fungi, bacteria, yeasts[9.]Among bacterial species Bacillus strains produce more most of the commercial proteases[10,11].

Alkaline proteases and Halo alkaline proteases are a few among the wide variety of extremophiles studied in recent years. Microbial alkaline protease have gained significance in commercial production due to inherent advantages of the microbial system like short doubling time, less space requirement and ease to genetic manipulation.

Alkaline proteases isolated from several microbial species have been studied extensively to figure out

their industrial relevance. An in detail study of their properties like optimal ph., temperature, effect of solubilising agents and inhibiting factors gives a good insight of its exact industrial application. They are known to grow and withstand high alkaline ph. and even highly saline conditions [1].

Alkaline proteases being one of the most important groups of industrial enzymes, account for nearly 60% oftotal worldwide enzyme sales [12].Its applications includes detergent industry, recovery of silver from used X-ray films, as bleaching agent , dehairing agents in leather industries and for deproteinizing shrimp waste to produce chitinetc [13,14,1,2].

The growth substrates involved in the production of these enzymes hold the major cost in the overall process, which approximates to 30~40% of total production cost [15].Thus this cause demands the need for a cost effective growth medium which can drastically bring down the costs involved in these enzyme production.

The substrates involved in the production of these enzymes including various agricultural residues (like soybeanmeal, rice bran and wheat flour), dairy products (like cheese whey) and marine by products are been read from literature (bl rp1).The industrial fermentation methodology Involved in the large scale production of these enzymes have also changed from traditional, large empirical operations to controlled and knowledge based processes. The need to study about growth kinetics of the organisms involved in the production of these bio products has increased to meet the industrial needs [16].

Microbial Source

The microbial source varies from a very nutrient environment to an extremely salty lake. In this review we restrict the protease producing microbes to only Bacterial species wherein protease enzymes and activity has also been found in many species of yeast, fungi, algae and other microbes.

As with time the various studies conducted brought to light the high potential of the haloalkalibacteria and the other bacterial strains of the extremophile category .In this review we could concentrate more on it than any other source known.

There has been no firm evidence which suggest the idea of thermostable enzymes being derived from thermophilic microbes in particular, but it has been believed so in order to explore more about these microbes.[17]

The proteases being essential component in all life forms on this earth, it is present in varying amounts among all the species starting from prokaryotes, fungi, plants and animals[18.]

Thus the microbes involved in protease activity to any feeble extent can be isolated from any place on earth. In this review we analyse the bacterial strains isolated from polluted waters SFAX city (Tunisia) [19,] oil sewage station: fishing port SFAX City (Tunisia) [20,] alkaline soda lake (Ethiopia)[21,] saline alkali soils[22,] alkaline soils Himalayas [23,]sea water coastal Gujarat (India) [1,]and many other environments.

Selection of Organism and Maintenance

The various bacterial strains known to be involved in protease production were first isolated from their natural habitats and then sub cultured invitro using appropriate media components [1, 2, 13, 14, 23, 24.] The potential strains were then identified from the formed colonies and their enzyme activity displayed, when added on to the proteinaceous substrates[2.]The identified strains were then isolated for study of their characteristics –growth kinetics, optimum conditions for the maximum growth and activity [25, 26, 27.]

Protease Assay

The protease activity was determined quantitatively by using various proteinaceous substrates. The enzyme activity was measured on the basis of the amount of protease that could breakdown a unit measure of the suitable substrate supplied to the system. Mostly the substrates used include either azocasein [27] and casein [19,28,29,30,31,32,33]

The protease assay was carried out using the method of Kembhavi et al (1993) using casein as a substrate. [20,33.] During the assay the measure of substrate break down was noted keeping either time or volume of the species as a constant or both in very few cases.

The assay was carried out many times individually to confirm the results and then an ANOVA table was formed. (Table- 1)

Table 1: Quantitative determination of proteinaceous substrates (ANOVA table)

Protease origin, Activity test	Molecular weight (kDa)	pH optimum and stability	Temp. optimum and stability (^oC)	Detergents, inhibitors	Metal ions	Reference
Bacillus cereus - BG-1 Casein solubilisation	34	8.0	60	EDTA	Ca^{2+}, Mg^{2+}, Cu^{2+}, Mn^{2+}, Zn^{2+}	33,34
Bacillus cereus - MCM B-326 Caseinolytic assay	36 - 45	9.0	55	SDS (1%), Sodium tripolyphosphate(1%), Sodium tetra-borate(1%) PMSF(1mM), EDTA(2 & 5 mM), DTT(2 & 5 mM), iodoacetamide(2mM), Trypsin(100µg)	1 & 5mM Hg^{+}, Na^{+}, Fe^{2+}, Cu^{2+}, Ca^{2+}, Mg^{2+}, Mn^{2+}, 5mM Zn^{2+}	14
Bacillus cereus - SV-1 Casein solubilisation	42	7.0	55	EDTA(10Mm),SDS,Tween 80, Tween 20, and Triton X-100	Stimulate: Fe^{2+}, Mg^{2+} Inhibit: Mn^{2+}, Zn^{2+}, Co^{2+}, Cu^{2+}, Hg^{2+},Na^{+},	20,32
Bacillus clausii - Casein solubilisation	40	10.5	35	Inhibit: organic nitrogen sources, excessive amino acid and ammonium ions	K^{+},Mg^{2+}, Na^{+} , Fe^{2+}, Cu^{2+},Zn^{2+}	35
Bacillus clausii GMBAE-42 Casein solubilisation	26.5	11.3	30-40	DEPC,TLCK,TPCK, PMSF,SDS(0.2% w/v)	Ca^{2+}, Ba^{2+}, Zn^{2+}, Mg^{2+} ,Cu^{2+}	36
Bacillus clausii I-52 Biorad protein assay	28	10.6	37	Stable to laundry detergents, stable to CMC 20%(w/v), AOS 20% (v/v), Zeolite, LAS 20%, EDTA	K^{+},Na^{+}	28
Bacillus orikoshii Casein digestion	30	9.0	45-50	PMSF, APMSF, aprotinin, LBTI ,SBTI	K^{+},Na^{+}	37
Bacillus licheniformis B-36 Casein solubilisation	42	7.5	55	EDTA(10Mm),SDS,Tween 80, Tween 20, and Triton X-100	Ca^{2+},K^{+}	38
Bacillus licheniformis RP-1 Casein	38	10.0 – 11.0	70	bleach-based detergent formulations	Stimulate: Ca^{2+} (0.07%) Inhibit:	39

solubilisation					Cu^{2+}, Zn^{2+}, Mn^{2+}	
Bacillus mojavensis Casein solubilisation	30	7.0	50	Inhibit:PMSF(1Mm), iodoacetatic acid Increased:TLCK,TPCK	Cu^{2+},Mn^{2+} Co^{2+},Zn^{2+}	25,29
Bacillus mojavensis A21	-	7.0-9.0	30-37	-	Ca^{2+},Na^{+}, Mg^{2+},K^{+}	32
Bacillus pantotheneticus -Casein solubilisation	-	8.4 7.0 – 10.7	30 – 60	-	Stimulate: Fe^{2+}, Ca^{2+}, Mg^{2+} Inhibit: Ni^{2+}, Mn^{2+}, Hg^{2+}	13
Bacillus pseudofirmus - AL-89 Casein solubilisation	24	11.0 8.0 – 12.5	60 - 70	PMSF(1 & 10 mM), EDTA(10mM) 3,4-DCI(0.1mM), Pepstatin(0.1mM), IAA(5mM), PcMBA(5mM), 1,10-Phenanthroline	Ca^{2+}	40
Bacillus sp. Casein digestion	26-29	8.0 – 11.5	35 - 50	EDTA (5mM), PMSF (1mM)	Hg^{2+},Mg^{2+} ,Cu^{2+} (all 1mM)	41
Bacillus sp. I-312 Casein solubilisation	-	11.0	60	Stable: Triton X-100, Tween 20, SDS Stable:H_2O_2, Sodium perborate, TLCK Inhibit: PMSF (0.5 – 1.0 mM)	-	42
Bacillus sp. K-30 Casein solubilisation	-	9.0	55	-	-	3
Bacillus sp. Po-2 Casein digestion	-	8.0 7.0 – 9.0	37	Inhibit: 1% glucose, inorganic nitrogen	Stimulate: Na^{+}	2
Bacillus sp. SB5	-	10.0	60-70	SDS, Surf		43
Bacillus sp. SMIA-2 Azocasein solubilisation	-	8.0	60	EDTA,PMSF	Inhibit: K^{+}, Hg^{2+}, Cu^{2+} Stimulate: Mn^{2+}, Ca^{2+}	24
Bacillus sp. Ve-1 Casein digestion	-	7.0 – 9.0	65	EDTA,PMSF		44
Bacillus sphaericus Casein digestion	-	10.5 8.5-11.5	50-55	EDTA,PMSF	Mg^{2+},K^{+}, Na^{+},	7,23
Bacillus sphaericus MTCC-B-0014	-	11.0 10.5 – 11	50 50 – 55	Stable towards laundry detergents, stable as detergent additive	Stimulate: Ca^{2+}	13

Casein digestion						
Bacillus subtilis DM-04 Casein digestion	-	8.0 – 9.0	35	Stable to laundry detergents	-	45
Bacillus subtilis EAG-2 Kunitz method	27	8.5 6.5 – 9.0	65	APMSF, PMSF, pepstatin, leupeptin, EGTA, EDTA, Alcalase, Esperase	Stimulate: Ca^{2+}, Co^{2+}, Fe^{2+}, Na^{+}, Zn^{2+} Inhibit: Cu^{2+}, Mn^{2+}, Ni^{2+}, Mg^{2+}	9
Bacillus subtilis IQQDB-32 Casein digestion	-	7.0	50 – 55	EDTA,PMSF, *o*-PHEN ($P < 0.01$), *p*-CMB ($P < 0.05$)	-	46
Bacillus subtilis PE-11	-	7.5	45	-	Ca^{2+}	47
Bacillus pumilus c172	-	8.5-9.0	40	PMSF(1mM)	Ca^{2+},Mg^{2+} ,Na^{+},K^{+}	48
Haloalkaliphillic bacterium S 20-9 Casein digestion	-	9.0 7.0 – 9.0	37	-	Ca^{2+}, Mg^{2+}, Mn^{2+}, Hg^{2+}, K^{2+}	1
Microbacterium sp. Casein digestion	-	9.5 – 11.5	65	EDTA,PMSF	Ca^{2+}	2
Nesternkonia sp. - AL-20 Casein	23	7.5 – 11.5	70	PMSF(1 & 10 mM), EDTA(10mM) 3,4-DCI(0.1mM), Pepstatin(0.1mM), IAA(5mM), PcMBA(5mM), 1,10-Phenanthroline	Ca^{2+}	40

Optimization of Production Parameters

Most of the Bacillus sp. had their pH range for optimum activity between 6.0-11.5. Bacillus cereusBG-1 had the optimum relative enzyme activity at a slightly alkaline pH of 8.0 [34,] while Bacillusclausii GMBAE-42 had itsoptimum at a highly alkaline pHof 11.3 [36.]Bacillus sp. are active and have their optimum temperatures from a broad range of 35^{o}C-70^{O}C. Sandeepkaur et.al reported that Bacillus sp.P-2 is highly thermostable in nature exhibiting the maximum protease activity at 90^{o} [17.] On the other end, SeyedehFaranakGhaemiOskouie et.al reported that Bacillus clausii had its optimum temperature as 35^{o}C [35.]Alya Sellami-Kamoun et.al reported that sardinelle and viscera substrate can be used to provide carbon, nitrogen and salt needed by the strain with the a production (8,473 U/ml) with medium supplemented with 20g/l CHVSP and 1g/l $CaCl_2$[33].R.H.Joshi et.al examined for Optimum alkaline protease production from S-20-9 in presence of various mono and di-valentcations which resulted in maximum production in KCl at 0.5 %w/v (350U/ml)[1.]Bacillus cereus BG1 had maximum protease induction at $CaCl_2$ at 2g/L (4,036U/ml)[34, 49-55.]

Conclusion

The protease activity of various bacterial species depends upon physical factors like temperature, pH, method of isolation and maintenance. However the appropriate conditions can be provided with proper knowledge about the bacterial species to be used in order to attain maximum activity. This review provides information about 40 bacterial strains of various origins. The current protease study revolves around the various genetic modifications that can be applied in order to enhance the protease production and activity. Assuring the

increasing need of proteasesfor various small scale and industrial application the research for protease production continues.

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