STUDY ON CAPABILITY OF PRODUCING ENZYMES AND ANTIMICROBIAL SUBSTANCES OF A BACTERIAL STRAIN ISOLATED FROM SOIL SAMPLES COLLECTED IN CATBA NATIONAL PARK

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Abstract. TL53, a bacterial strain, isolated from soil samples in CatBa National Park of HaiPhong city, was assigned to the Genus *Bacillus* based on morphological and physiological characteristics. Based on sequence analyses of 16S rDNA, TL53 strain was phylogenetically closely related to *Bacillus subtilis*. This strain showed high activity of antimicrobial substances and enzymes degrading starch, protein, chitine, lipid and cellullose. This strain may be chosen for production of probiotic used in aquaculture.

1. Introduction

There are a lot of reports about the flora of animals and plants in CatBa National Park, but is only few about microbial flora. The aim of our research is to find new isolates of microorganisms which are capable of producing bioactive substances such as exoenzymes and antimicrobial substances which promote animal and plant growth and screen of new microorganisms controlling the environment.

2. Material and Methods

Baterial strain. The bacterial strain TL53 in this study was isolated from soil samples collected in CatBa National Park, VietNam.

Test microorganism: Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus, Sarcina lutea, Klebsiella sp, Fusarium oxysporum, Bacillus cereus, Shigella flexneri, Pseudomonas mirabilis, Bacillus pumilus.

Morphological and physiological characteristics. Methods in this study used for examination of morphological and physiological characteristics were described by John, G.H. et al. The morphological properties of strain TL53 were examined by the scanning electron micrograph. The enzymatic activities were tested by using agar diffusion assay.

Taxonomy. The characterization and identification of the TL53 strain were carried out according to John, G.H and the molecular methods were described by Ausubel, F.M.

The 16S rDNA sequence of strain TL53 was determined after DNA amplified by using PCR. Then both strands of 16S rDNA obtained was sequenced directly by ABI 3100 Avant sequencing machine of Perkin-Elmer (USA). Generated sequences were aligned with related species by using Clustal W ver.1.83 software. Reference sequences used for phylogenetic study was obtained from the database of gene bank (http: //www.ncbi.nlm.nih.gov). The phylogenetic tree was constructed from the evolutionary distance data arcoding to Kimura (1990) using the neighbor-joining method (Saitou and Nei, 1987). Sites where gaps exsisted in any sequence were excluded. Bootstrap analysis (Felsenstein) were performed from 1000 random resampling. All of phylogenetic analyses were carried out using the PHYLIP package (Felsenstein, 1993).

3. Results and discussion

Among 153 baterial strains with hydrolytic activity of organic compounds isolated from soil samples in CatBa National Park, TL53 strain had highest activity of enzymes hydrolyzing starch, casein, CMC, Tween 80 and chitine.

Cultural characteristics

Mophorlogical, physiological characteristics of strain TL53:

The colonies of strain TL53 are round. The streak culture is pale yellow, smooth and shining. Its vegetative cells are rod, produce endospores and are able to motile.

The results were shown at table 1:

Table1. Some of cultural and physiological properties of strain TL53

Characters		Characters	
Cultural properties		Physiological properties	
Aerial mass color	Yellow	Sunfate reduction	-
Reverse side color	Gray	Catalase test	+
Diffuse pigment	-	Oxidase test	+
Endospore	round	Nitrate reduction	-
Flagella	-	Fermentation product	lactate
Gram stain	+	Respiration type	aerobic
Motility	+		
Salt tolerance (NaCl)	7%		

Determination of optimal media

The strain TL53 was cultivated at 50^{0}C on a rotary shaker at 200 rpm for 48h in various media: Hutchison-Clayton, Fish extract, Fish extract-CMC, bean sprout extract, modified medium. After 48h, the cultural broth obtained was tested for activities of CMCase, lipase, kitinase, amylase by using the agar diffusion assay. The results were shown at Table 1.

Table 2. Enzymatic activities of TL53 in different culture media

Media	Final pH	OD	Enzyme activities (D -d , mm)				
			Amilase	Chitinase	Protease	Lipase	CMC-ase
1	8.3	0.80	25	20	27	17	27
2	7.5	0.82	27	24	30	15	29
3	8.0	0.84	24	24	32	24	27
4	7.7	0.85	22	26	30	20	26
5	8.5	0.87	28	26	33	25	31

Determination of optimal pH

The strain TL53 was incubated at at 50°C on a shaker at 200 rpm for 48h in modified medium of nutrients broth. The pH is changed from 4 to 9. After 48h, the cultural broth obtained was tested for activities of CMC-ase, lipase, kitinase, amylase and protease by using the agar diffusion assay. The results were shown at Table 3.

Table 3. The influence of pH on enzymatic activities of TL53 strain

Initial pH	Final pH	OD	Enzymes activities (D -d , mm)				
			Amilase	Chitinase	Protease	Lipase	CMC-ase
4	6.32	0.626	10	12	15	18	18
5	8.13	0.742	25	22	26	18	29
6	8.24	0.843	27	25	27	21	30
7	8.30	0.950	29	29	32	25	33
8	8.40	0.903	27	27	24	22	31
9	8.00	0.800	24	25	20	22	28

The results showed that pH range for the growth was relative large from 5 to 8.

The pH of fermentation after culturing is relative constant. In suitable pH, the enzymatic activities and cell growth increased. The optimal pH was 7.

Determination of optimal temperature

The strain TL53 was incubated on a rotary shaker at 200 rpm for 48h at 20°C, 25°C, 30°C, 35°C, 40°C, 50°C, 60°C in modified medium. After 48h, the cultural broth obtained was tested for activities of CMC-ase, lipase, chitinase, amylase and protease by using agar diffusion assay and measured for pH and OD.The results were shown at Table 4

Table 4. The influence of temperature on enzymatic activities and growth of TL53

Temperatu - re	Final pH	OD	Enzymes activities (D -d , mm)				
			Amilase	Chitinase	Protease	Lipase	CMC-ase
25	7.71	0.800	24	23	24	18	25

30	8.04	0.850	26	24	24	18	25
35	8.15	0.890	26	27	31	23	28
40	8.21	0.930	30	30	34	27	34
45	8.2	0.860	28	27	30	25	31
50	8.41	0.840	28	25	31	25	29
55	7.36	0.780	20	19	15	20	20
60	7.17	0.560	10	-	12	10	13

The results showed that strain TL53 had a relative large temperature range. The enzymatic activities increased in directly propotion to temperature.

Determination of optimal time

The strain TL53 was incubated at 40°C on a rotary shaker at 200 rpm for 72h in modified medium. Every 12h, the fermentation broth obtained was tested for CMC-ase, lipase, chitinase, amylase and protease by using agar diffusion assay. The results were shows at Table 5

Table 5. The influence of time on enzymatic activities and growth of TL53.

Time (h)	finalpH	OD	Enzymes activities (D -d , mm)				
			Amylase	Chitinase	Protease	Lipase	CMC-ase
12	7.71	0.550	25	22	22	16	26
24	8.08	0.870	28	28	30	20	30
36	8.20	0.910	28	29	31	22	31
48	8.31	0.940	32	29	34	29	33
60	8.43	0.910	33	29	32	29	32
72	8.03	0.860	29	27	28	23	28

The results showed that the enzymatic activities and growth of TL53 increased relatively stably from 36 to 72h. However, after 48h of culture, the enzymatic activities increased only slightly or even not at all.

Antimicrobial activity: To determine the capability of producing antimicrobial substances, strain TL53 was cultivated on a rotary shaker (180 rpm) for 72h at 28°C in modified medium of nutrients broth. After 48h, the cultural fluid was centrifuged 9000prm then the supernatant obtained was added to the wells on the agar plates which had been spread the test microorganism. The Petri dishes were placed in a refrigerator from 4-8h then were kept in a warm cup board. After 24h, the petri dishes were taken out to observe the diameter of antimicrobial-zone. The results were shown at Table 5.

Table 6. Antimicrobial activities against test-microorganism

Test Microorganism	Antimicrobial activities (D-d, mm)
Pseudomonas mirabilis	17
Sarcina lutea	34
Staphylococcus aureus	17
Salmonella typhi	–
Klebsiella sp	–
Fusarium oxysporum	32
Pseudomonas aeruginosa	–
Bacillus cereus	–
Bacillus pumilus	–
Candida albicans	–
Escherichia coli	3

Sequencing and phylogenetic analysis

The 16S rDNA sequence of TL53 strain was determined after DNA amplified by using PCR. Then both strand of 16S rDNA obtained was sequenced directly by ABI 3100 Avant sequencing machine of Perkin-Elmer (USA). Generated sequences were aligned with related species by using Clustal W ver. 1.74 program. Reference sequences used for phylogenetic study was obtained from the database of genebank (http://www.ncbi.nlm.nih.gov)

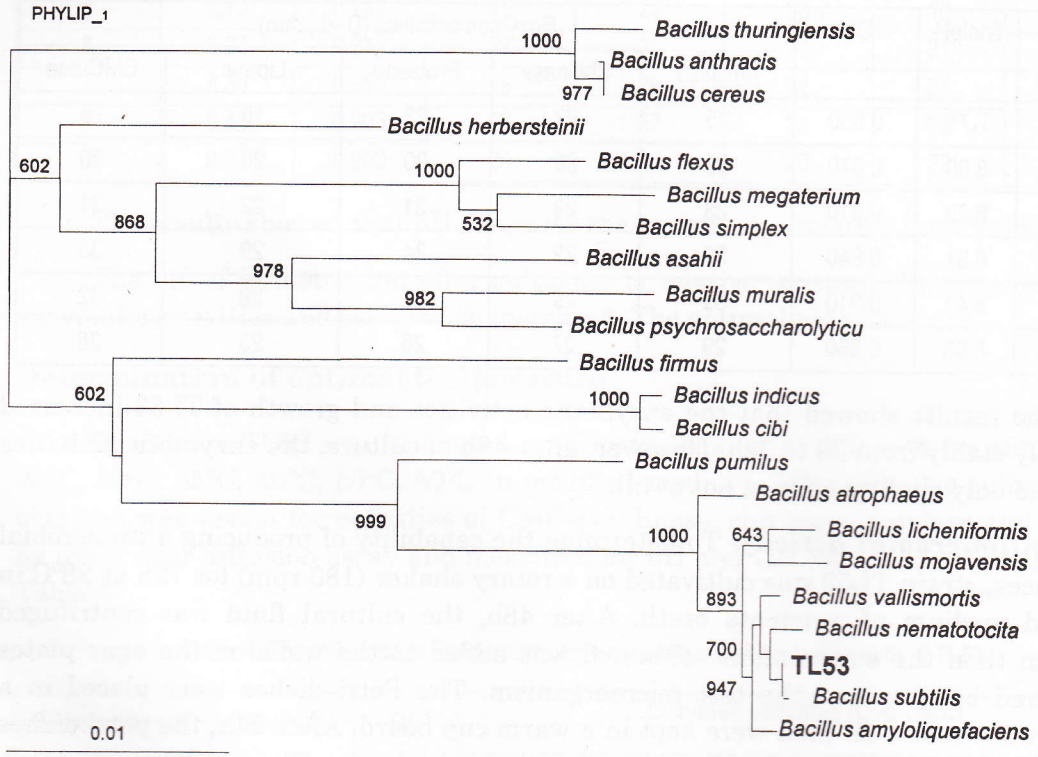

Figure 1. The Phylogenetic tree of TL53 strain

After comparing with 16S rDNAs which have published in gene bank we realized that the similarity between TL53 and *Bacillus subtilis* is 99,7%. The phylogenetic tree was constructed based on 16Sr DNA of TL53 strain and related species of genera *Bacillus*.

The results showed that the TL53 strain was located at the *Bacillus subtilis* clade.

After comparing the morphological, physiological characteristics of TL53 with *Bacillus subtilis* we can confirm that TL53 strain is *Bacillus subtilis*.

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NGHIÊN CỨU KHẢ NĂNG SINH ENZYM VÀ CÁC CHẤT KHÁNG SINH CỦA CHỦNG VI KHUẨN TL53 PHÂN LẬP TỪ CÁC MẪU ĐẤT THU ĐƯỢC TỪ VƯỜN QUỐC GIA CÁT BÀ

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TL53 là chủng vi khuẩn được phân lập từ các mẫu đất ở Vườn Quốc gia Cát Bà, thành phố Hải Phòng. Chủng này có hoạt tính enzym mạnh phân giải các hợp chất hữu cơ như tinh bột, protein, lipit, kitin, celluloz và có hoạt tính mạnh kháng các chủng vi sinh vật kiểm định. Chủng này được xác định thuộc chi Bacillus dựa trên các đặc điểm hình thái, sinh lý và dựa trên việc phân tích trình tự gen mã hóa ARNr 16S thì chủng này có mối quan hệ chủng loại phát sinh gần gũi nhất đối với loài *Bacillus subtilis*. Do có nhiều đặc điểm ưu việt chủng này có thể được xem xét để sử dụng trong chế tạo chế phẩm probiotic dùng trong nuôi trồng thủy sản.