Clone and Sequence Analysis of Trehalose Synthesis from Pseudomonas Stutzeri

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Abstract: We have cloned Trehalose Synthesis gene using PCR from Pseudomonas Stutzeri. We linked this gene with pGEM-T-Easy Vector , analysis the whole gene sequence and transformation the recombinant gene into *Escherichia coli* JM109 .The result of sequence of this gene showed :cloned gene whole length is 2070 bp, has 96.66% homology compared with recorded sequence AF113617in GenBank, encoded 689 amino acids , has 99.71% homology compared with AF113617 . This cloned gene has been recorded by GenBank, accession number is DQ452614. [Nature and Science. 2006;4(4):26-31].

Keywords: Trehalose Synthesis; TreS gene; PCR clone; sequence analysis

Introduction

Trehalose is a deoxidizing disaccharide containing two glucoses which are combined with α，α-1，1 glucosidic linkage. It is abundant in animals, plants and microbe. Trehalose has an important function in protecting plant stress tolerance. It usually produced in the condition of intimidating. The content of the trehalose varys as the condition changes. Trehalose is a irritability metabolite. The reason that why some species performance stress tolerance in inclemency environment is trehalose can have the protection effect on biological giant molecule, such as biomembrane, protein and nucleic acid etc. So according to that, the species which are rich in trehalose can show the peculiar biological speciality. The function of the biological protection of trehalose is that it strongly binds up the water molecules, owning the bound water together with membrane lipid. Or it can instead the function of combining water with the membrane. Consequently preventing the denaturalization of biomembrane and membrane protein. Because of the potential applications of the trehalose on food, cosmetic, medicament and the biological production, the clone and expression of Trehalose synthesis gene become a hotspot of biological researching. In Pseudomonas Stutzeri, maltose is used as a substrate. With the help of Trehalose Synthesis, Trehalose changes the maltose which is combined with α，α-1，4 glucosidic linkage into Trehalose which is combined with α, α-1, 1 glucosidic linkage.

1. Materials

1. 1 Bacteria and Plasmid

Pseudomonas stutzeri1.1803, from the Conservation of microorganism bacteria; E. coli JM109, from Bao Bioengneering Co. of Da Lian, China; pGEM-T-Easy, from Promega Co., USA

1. 2 Reagent

Enzymes and IPTG, X-gal, dNTP, from Bao Bioengneering Co.of Da Lian ,China and Promega Co; T4DNA ligase from GIBCO Co.; UNTQ-10 Kit from Shang Hai Bioengineering Ltd. Co. Primer was synthesized by Bao Bioengneering Co. of Da Lian ,China; Gene sequence analysis was done by Shang Hai Boya Bioengneering Ltd. Co., USA.

2. Methods

2.1 Isolation plasmid

Inoculating the E. coli containing pGEM-T-Easy plasmid into LB liquid culture medium which has specific concentrations overnight. And then isolation plasmid with the method of alkaline lysis.

2. 2. PCR Amplification of TreS gene

Based on the sequence of TreS gene on Genebank, the accession number is AF113617, we designed two primers for PCR reaction. And inserted the restriction enzyme sites of the BamH Ⅰ and Sac Ⅰ on the 5′ end and 3′ end.

P_1 (5' primer) 5'GGGATCCATGAGCATCCCAGA CAACAC 3', BamH Ⅰ;
P_2 (3' primer) 5'GGAGCTCTCAGATCACCGGCGGCGCGG 3', Sac Ⅰ.

Isolating the whole DNA of *Pseudomonas stutzeri* as the template for PCR amplification. Conditions: 94 ℃ 5 min, 94℃ 30 s, 54℃ 30 s, 72℃ 1 min, 72℃ 7 min, 4℃ hold, 40 cycles. The PCR product was tested through 0.8% agarose gel electrophoresis, and purified and reclaimed by 0.8% agarose in order to use in next step.

2. 3 TreS gene clone

Using BamH Ⅰ and Sac Ⅰ sites on pGEM-T-Easy, T_4ligase link PCR product with pGEM-T-Easy vector. Transformating *Escherichia coli* JM109, screen positive clone on LB plate with Amp, IPTG and X-gal. Identifying the recombinant by digestion, and analyze the gene sequence.

2. 4 Sequence Analysis

Commission Shang Hai Boya Bioengneering Ltd., Co. sequence the target whole gene clone. Using BLAST and GeneBank data to analysis the homology of the target sequence.

3. Result

3. 1 Amplification of TreS gene

We use the whole DNA of *Pseudomonas stutzeri* as the template and the designed oligonucleotide as the primers to amplify the TreS gene. PCR product is 2.07 Kb, consistent with result of anticipate (Chart 1). And reclaimed by 0.8% agarose in support.

3. 2 Amplification product clone and analysis

Link the PCR amplification product purified by low melting point agarose with pGEM-T-Easy and then transform into *E. coli* JM109, then cut the recombinant plasmid with the restriction enzymes of BamH Ⅰ and Sac, and test it use PCR. (Chart 2.3) The result shows that the vector has been inserted the 2.07 kb DNA fragment.

3. 3 The result of sequence analysis of TreS gene and the homologous searches with the reported gene

After determining one sequence of the recombinant, the result shows that this fragment whole length is 2070 bp, encoding 331 amino acid. Comparing with the sequence on GeneBank AF113617 (Chart 4). It have 18 differences on 52、225、231、249、831、843、972、1038、1213、1299、1561、1563、1564、1569、1809、1824、1848、2031sites. The results show homologous rate of correspond region are almost 96.66% compared with AF113617.

3.4 Putative Amino Acid Sequence and the homologous analysis

Translating the ORF of TreS gene into amino acid, putative molecular weight 75.7KD, isoelectric point 5.14. Homologous rate of correspond region are almost 99.71% copare with AF113617. The differences between them only on 521 and 522 sites. Send TreS amino acids sequence to the server of NCBI, use BLASTP tool to homologous searches, the result shows the sequences which have high homologous sequence with TreS amino acids sequence (Chart 5). They are the amino acids sequence of glycosidase, putative and hypothetical protein. By using DNAMAN4.0 to compare homologous sequence of TreS gene with 3 items of TreS genes which were published in GeneBank, the result shows that the homologous rate of all of them are above 86.07% (Chart 6).

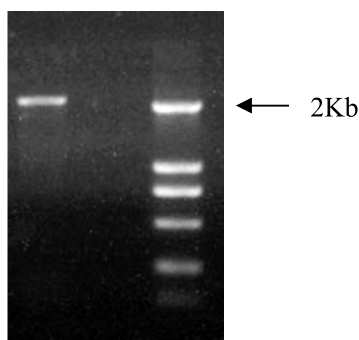

Chart 1. cDNA PCR amplification

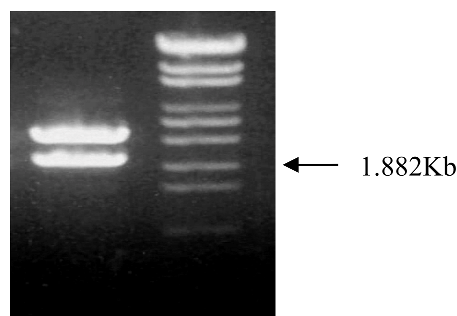

Chart 2. Recombinant of TreS gene plasmid was cutted by BamH Ⅰ and Sac Ⅰ

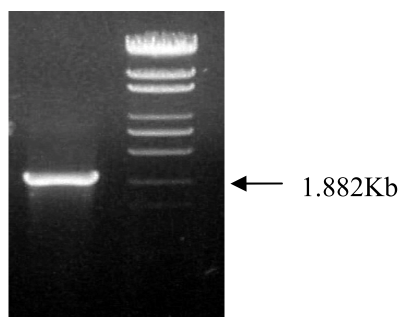

Chart 3. PCR identification of recombinant plasmid

1 ATG…ATGTTG…GGT…GAT…CTG…GCA…CAT….CTT…ATA…
2 ATG…ATGCTG…GGC…GAC…CTC…GCG…CAC…CTC…ATC…
1 …CTG…CTA…CCCGCCGAA…CCC…CCA…GAA…CTC
2… TTG…CTG…GCGCCCGAG…CCG…CCG…GAG…CTT

Chart 4. TreS gene sequence
1. DQ452614 bacteria 2. AF113617 bacteria

Sequences producing significant alignments:		(Bits)	Value
gi|6724082|gb|AAF26837.1|	trehalose synthase [Pseudomonas stutze	1387	0.0
gi|66045972|ref|YP_235813.1|	glycosidase, putative [Pseudomon...	1144	0.0
gi|71557609|gb|AAZ36820.1|	trehalose synthase [Pseudomonas sy...	1141	0.0
gi|28870130|ref|NP_792749.1|	glycosidase, putative [Pseudomon...	1139	0.0
gi|82737280|ref|ZP_00900131.1|	Trehalose synthase [Pseudomonas p	1056	0.0
gi|26989637|ref|NP_745062.1|	trehalose synthase, putative [Ps...	1055	0.0
gi|67987823|gb|EAM75610.1|	hypothetical protein KradDRAFT_254...	776	0.0

Chart 5. The result of the searches on GeneBank of TreS amino acids sequence

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ZP_00900131.pro    MTQPDPSYVKWLEDRAMLKASQARASLYSGQSRLWQQPYA    40
AAF26837.pro       MSIPDNTYIEWLVSQSMLHAARERSRHYAGQARLWQRPYA    40
DQ452614.pro       MSIPDNTYIEWLVSQSMLHAARERSRHYAGQARLWQRPYA    40
NP_745062.pro      MTQPDPSYVKWLEDRAMLKASQDRASLYSGQSRLWQQPYA    40
Consensus          msipdnsyiewledqamlhaaqerarhyagqarlwqqpya

ZP_00900131.pro    EAQPRRATEIASVWLTVYPDAIIAPEGCSVLGALAHEALW    80
AAF26837.pro       QARPRDASAIASVWFTAYP.AIITPEGGTVLEALGDDRLW    79
DQ452614.pro       QARPRDASAIASVWFTAYPAAIITPEGGTVLEALGDDRLW    80
NP_745062.pro      EAQPRRATEIASVWLTVYPDAIIAPEGCSVLGALAHEALW    80
Consensus          eaqprdasaiasvwftaypdaiiapegcsvlealaddalw

ZP_00900131.pro    KRLSEIGVQGLHTGPIKLSGGIRGRELTPSVDGNFDRISF   120
AAF26837.pro       SALSELGVQGIHNGPMKRSGGLRGREFTPTIDGNFDRISF   119
DQ452614.pro       SALSELGVQGIHNGPMKRSGGLRGREFTPTIDGNFDRISF   120
NP_745062.pro      KRLSEIGVQGLHTGPIKLSGGIRGRELTPSVDGNFDRISF   120
Consensus          kalseigvqgihngpiklsggirgreftpsidgnfdrisf

ZP_00900131.pro    DIDPLYGSEQELIQMSRMAAAHNAVTIDDLIPSHTGKGAD   160
AAF26837.pro       DIDPSLGTEEQMLQLSRVAAAHNAIVIDDIVPAHTGKGAD   159
DQ452614.pro       DIDPSLGTEEQMLQLSRVAAAHNAIVIDDIVPAHTGKGAD   160
NP_745062.pro      DIDPLYGSEQELIQMSRMAAAHNAVTIDDLIPSHTGKGAD   160
Consensus          didpllgseeeliqlsrmaaahnaitiddiipahtgkgad

ZP_00900131.pro    FRLAEIAHGPYPGLYHMVEIREEDWTLLPEVPAGRDAVNL   200
AAF26837.pro       FRLAEMAYGDYPGLYHMVEIREEDWELLPEVPAGRDSVNL   199
DQ452614.pro       FRLAEMAYGDYPGLYHMVEIREEDWELLPEVPAGRDSVNL   200
NP_745062.pro      FRLAELAHGPYPGLYHMVEIREEDWALLPEVPAGRDAVNL   200
Consensus          frlaemahgdypglyhmveireedwellpevpagrdavnl

ZP_00900131.pro    LPAQCDELKARHYIVGQLQRVIFFEPGVKETDWSATPPIT   240
AAF26837.pro       LPPVVDRLKEKHYIVGQLQRVIFFEPGIKDTDWSVTGEVT   239
DQ452614.pro       LPPVVDRLKEKHYIVGQLQRVIFFEPGIKDTDWSVTGEVT   240
NP_745062.pro      LPAQCDELKARHYIVGQLQRVIFFEPGVKETDWSATPPIT   240
Consensus          lpaqcdelkakhyivgqlqrviffepgikdtdwsatgeit

ZP_00900131.pro    GVDGKTRRWVYLHYFKEGQPSLNWLDPTFAAQQMIIGDAL   280
AAF26837.pro       GVDGKVRRWVYLHYFKEGQPSLNWLDPTFAAQQLIIGDAL   279
DQ452614.pro       GVDGKVRRWVYLHYFKEGQPSLNWLDPTFAAQQLIIGDAL   280
NP_745062.pro      GVDGKTRRWVYLHYFKEGQPSLNWLDPTFAAQQMIIGDAL   280
Consensus          gvdgktrrwvylhyfkegqpslnwldptfaaqqliigdal

ZP_00900131.pro    HALDCLGARGLRLDANGFLGVETRASGTAWSESHPLSLVG   320
AAF26837.pro       HAIDVTGARVLRLDANGFLGVERRAEGTAWSEGHPLSVTG   319
DQ452614.pro       HAIDVTGARVLRLDANGFLGVERRAEGTAWSEGHPLSVTG   320
NP_745062.pro      HALDCLGARGLRLDANGFLGVETRASGTAWSESHPLSLVG   320
Consensus          haidclgarglrldangflgverraegtawseghplsltg

ZP_00900131.pro    NQLIGGMIRKAGGFSFQELNLTLDDIAQMSRGGADLSYDF   360
AAF26837.pro       NQLLAGAIRKAGGFSFQELNLTIDDIAAMSHGGADLSYDF   359
DQ452614.pro       NQLLAGAIRKAGGFSFQELNLTIDDIAAMSHGGADLSYDF   360
NP_745062.pro      NQLIGGMIRKAGGFSFQELNLTLDDIAQMSKGGADLSYDF   360
Consensus          nqliagairkaggfsfqelnltiddiaamshggadlsydf
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ZP_00900131.pro    ITRPAYQHALLTGDTEFLRLMLKEMHAFGIDPASLIHALQ    400
AAF26837.pro       ITRPAYHHALLTGDTEFLRMMLREVHAFGIDPASLIHALQ    399
DQ452614.pro       ITRPAYHHALLTGDTEFLRMMLREVHAFGIDPASLIHALQ    400
NP_745062.pro      ITRPAYQHALLTGDTEFLRLMLKEMHAFGIDPASLIHALQ    400
Consensus          itrpayhhalltgdteflrlmlkemhafgidpaslihalq

ZP_00900131.pro    NHDELTVELVHFWTLHAHDMYLYKGQTLPGSILREHIREE    440
AAF26837.pro       NHDELTLELVHFWTLHAYDHYHYKGQTLPGGHLREHIREE    439
DQ452614.pro       NHDELTLELVHFWTLHAYDHYHYKGQTLPGGHLREHIREE    440
NP_745062.pro      NHDELTVELVHFWTLHAHDMYLYKGQTLPGSILREHIREE    440
Consensus          nhdeltlelvhfwtlhahdhyhykgqtlpgghlrehiree

ZP_00900131.pro    IYERLSGEHAPYNLRFVTNGIACTTASLIAAALGIRDLEQ    480
AAF26837.pro       MYERLTGEHAPYNLKFVTNGVSCTTASVIAAALNIRDLDA    479
DQ452614.pro       MYERLTGEHAPYNLKFVTNGVSCTTASVIAAALNIRDLDA    480
NP_745062.pro      IYERLSGEHAPYNLRFVTNGIACTTASLIAAALGIRDLEQ    480
Consensus          iyerlsgehapynlkfvtngiacttasliaaalgirdlda

ZP_00900131.pro    IGATDIELIKKVHLLLVMYNAMQPGVVALSGWDLVGALPL    520
AAF26837.pro       IGPAEVEQIQRLHILLVMFNAMQPGVFALSGWDLVGALPL    519
DQ452614.pro       IGPAEVEQIQRLHILLVMFNAMQPGVFALSGWDLVGALPL    520
NP_745062.pro      IGVADIELIKKVHLLLVMYNAMQPGVVALSGWDLVGALPL    520
Consensus          igpadielikklhillvmfnamqpgvfalsgwdlvgalpl

ZP_00900131.pro    PAEAVAERMLDGDTRWIHRGGYDLAGLDPQAVASVRGMPR    560
AAF26837.pro       APEQVEHLMGDGDTRWINRGGYDLADLAPEASVSAEGLPK    559
DQ452614.pro       PAEQVEHLMGDGDTRWINRGGYDLADLAPEASVSAEGLPK    560
NP_745062.pro      PAEAVAERMLDGDTRWIHRGGYDLAGLDPQAEASVRGMPR    560
Consensus          paeavaelmgdgdtrwihrggydladlapeasasaeglpk

ZP_00900131.pro    ARSLYGSLDSQLDEGDSFACQVKKLLAVRQAYGIATSRQV    600
AAF26837.pro       ARSLYGSLAEQLQRPGSFACQLKRILSVRQAYDIAASKQI    599
DQ452614.pro       ARSLYGSLAEQLQRPGSFACQLKRILSVRQAYDIAASKQI    600
NP_745062.pro      ARALYGSLDRQLDESDSFACKVKKLLAVRQAYGIATSRQV    600
Consensus          arslygslaeqldepdsfacqlkkilavrqaydiaaskqi

ZP_00900131.pro    LVPEVRSPGLLVMVHELPAGRGIQITALNFGQEAIAEELL    640
AAF26837.pro       LIPDVQAPGLLVMVHELPAGKGVQLTALNFSAEPVSETIC    639
DQ452614.pro       LIPDVQAPGLLVMVHELPAGKGVQLTALNFSAEPVSETIC    640
NP_745062.pro      LVPEVSSPGLLVMVHELPAGRGIQITALNFGQDAIAEELL    640
Consensus          lipdvqapgllvmvhelpagkgiqitalnfgaeaiaeeic

ZP_00900131.pro    LTGFTPGPVVDMINETVEGDLTEDGRLMVNLDPYEALCLR    680
AAF26837.pro       LPGVAPGPVVDIIHESVEGDLTDNCELQINLDPYEGLALR    679
DQ452614.pro       LPGVAPGPVVDIIHESVEGDLTDNCELQINLDPYEGLALR    680
NP_745062.pro      LTGFTPGPVVDMINETVEGDLTEDGRLMVNLDPYEALCLR    680
Consensus          lpgfapgpvvdiihesvegdltddcelminldpyealalr

ZP_00900131.pro    IVNSSGHV.    688
AAF26837.pro       VVSAAPPVI    688
DQ452614.pro       VVSAAPPVI    689
NP_745062.pro      IVNSSGHV.    688
Consensus          ivnaaghvi
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Chart 6. Compare Deduce Amino Acid Sequence of TreS Gene with published Amino Acid Sequence of TreS Gene. The Accession Number of Genbank of Comparing Sequence: ZP_00900131、AAF26837、NP_745062

4. Discussion

Through the experiment analysis, the expression product of ORF sequence which is from *Pseudomonas stutzeri* is Trehalose Synthesis gene. The homologous rate of the DNA sequence and reported TreS geneAF113617 is very high. We only searches one TreS gene on GenBank through BLAST. We compared the deduce amino acid sequence with the published amino acid sequence, it has the almost same region with the unknown function amino acid sequence, indicant enzyme and TreS gene sequence. It is reported that these regions correlate to the catalyze of amylum enzyme family and the substrate bind sites. So it has the similar structure domain. It is shows that the differences between amino acid sequences are not the reason of the different functions. So it has the deeply significance to research the third dimensional structure of Trehalose Synthesis protein, and further knows the mechanism of Trehalose Synthesis gene recognizing the substrate and the energy coupling in transferring between two indicans in the process of catalyzing.

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References

1 Crowe J H et al. Science, 1984, 223:701-703.
2 Elbein A D. et al. Carbohydr Chem. Biochen. 1974, 30:227-256.
3 linghong Nie, et al. The Biology Protection Function of Trehalose [J] Chemistry of Life, 2001，21 (3) : 206-208.
4 Suilou Wang. Food and Biotechnology [M]. BeiJing : The Ocean Press，1998.
5 Colaco C. Sen S. et al. Bio/technol. 1992, 10(9):1007-1011.
6 De Smet K A, Weston A, Brown I N, et al. Three pathways for Trehalose biosynthesis in mycobacteria. Microbiology, 2002, 146:199-208.
7 Yongming Li, Yuqi Zhao. Practicality approach of Molecular Biology Manual. BeiJing：Science Press，1998.
8 Grba S, E Oura, H Suomalainen. On the formation of glycogen and trehalose in baker’s yeast. Eur. J. Appl. Microbiol, 1975, 2:29-37.
9 Attfield P V. Trehalose accumulates in Saccharomyces cerevisiae during exposure to agents that induce heat shock response. FEBS Lett. 1987, 225:259-263.
10 Van Laere A. FEMS Microbiol Rev, 1989. 63:201-210.
11 Caffrey M, Victoria F, Carl L A et al. Plant Physiol. 1988, 86:754-758.
12 Zhiyang Dong, et al. The Researching Advance of Nucleus Agriculture BeiJing : Agriculture Science and Technology Press of China， 1996，115-120.
13 Xiuyu Dai, et al. Microbiology Bulletin，1996，22 (2) : 102-104.
14 Koichi Yoshinaga, Hiroe Yoshioka, Hiromu Kurosaki et al. Biosci Biotech Biochem, 1997, 61(1):160-161.
15 Alan D. Elbein, Y.T. Pan, Irena Pastuszak, and David Carroll. New insights on trehalose: a multifunctional molecule. Glycobiology. 2003 ,13: 17 - 27.
16 Kato M, Miura Y, KettokuM, Shindo K, IwamatsuA , Kobayashi K. Purification and characterization of new trehaloseproducing enzymes isolated from the hyperthermophilic archae, Sulfolobus solfataricus KM1. Biosci Biotechnol Biochem. 1996, 60:546 - 50
17 Hongman Chen. Clone and Construction of Plant Express Vector of Vintage Yeast Trehalose-6-Phosphoric Acid Synthesis Gene. Microbiology Journal, 2001, 41(1): 54～ 58.
18 Hongman Chen. Cloning of Trehalose-6-phosphate synthase gene from S.cerevisiae and its plant expression vector construction. Institute of Microbiology, The Chinese Academy of Sciences , Beijing, 2001, 41(1): 54～ 58.
19 Sambrook J, Fritsch EF, Maniatis T. Molecular Cloning A Laboratory Manual 2nd ed BeiJing : Science Press, 1992. 19～ 22