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Full Length Article

DNA Methylation Changes in *Pleurotus eryngii* Subsp. *tuoliensis* (Bailinggu) in Response to Low Temperature Stress

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Abstract

The methylation sensitive amplified polymorphism (MSAP) was used to induce DNA methylation transformation in mushroom mycelia. DNA obtained from the mycelial stages of *P. eryngii* subsp. *tuoliensis* was digested with isoschizomers *Msp I* or *Hpa II* (mixture of *EcoR I*), the ability to digest the sequence CpCpGpG as influenced by their methylation state. The data analysis demonstrated that full-methylated and unmethylation modifications were primary and the hemi-methylated ratio was significantly lower. These results indicated that the pattern of CG hypermethylation is abundant in *P. eryngii* subsp. *tuoliensis*. All fragments that were differentially amplified upon low temperature induction illustrated the feasibility of MSAP in edible mushrooms. Moreover, this study confirmed that genetic and epigenetic changes in *P. eryngii* subsp. *tuoliensis* were induced under low temperature. © 2017 Friends Science Publishers

Keywords: DNA methylation; *Pleurotus eryngii* subsp. *tuoliensis* (Bailinggu); Low temperature induction; MSAP

Introduction

DNA methylation in eukaryotes is a significant crucial epigenetic modification and is the most well-studied phenomenon at present (Gavery and Roberts, 2010). *Pleurotus eryngii* subsp. *tuoliensis* (Bailinggu) is one of the most diffusely cultivated edible mushrooms in China. Together with the white mushroom *Ganoderma lucidum* and *P. eryngii* subsp. *tuoliensis* constitutes a delicious and nutritious food and has antihypertensive, antiviral, enhancement-immunity and antitumor properties (Choi *et al.*, 2006; Miyazawa *et al.*, 2008; Lv *et al.*, 2009). The optimal temperature for *P. eryngii* subsp. *tuoliensis* mycelium growth in 25℃, while induction and formation of fruit within 13–18ºC Moreover, induction stress during environmental temperature of 0–4℃ is a significant factor for primordium growth and development. Epigenetic processes were necessary for growth and variation and the environment may affect the epigenetic phenomenon to cause phenotypic changes. Therefore, the epigenome is tightly in connection to environmental inducement and developmental variation (Yaish *et al.*, 2011).

Many studies have highlighted the relevance of DNA methylation in developmental processes (Fang and Chao, 2007). Moreover, mounting evidence from previous studies indicates environmental stresses, such as light (Omidvar and Fellner, 2015), temperature (Steward *et al*., 2002; Zemach *et al.*, 2010; Naydenov *et al.*, 2015), salt stress (Tan, 2010), Nitric Monoxide (Wang *et al*., 2015) and drought (Wang *et al.*, 2011; Zeng *et al.*, 2015), diversity of phenotypes were detected through the DNA methylation. Thus, there is an epigenetic relation between environmental stress and plants developmental processes (Boyko and Kovalchuk, 2008).

Many studies demonstrated that DNA methylation of fungi genomes shows change at low levels (Antequera *et al*., 1984; Magill and Magill, 1989; Zemach *et al*., 2010; Foulongne-Oriol *et al*., 2013). The genome of *Neurospora* showed approximately 1.5% methylcytosines and insufficient 0.1% methylcytosines in *Schizosaccharomyces pombe* and *Anacystis nidulans* (Antequera *et al.*, 1984; Selker and Stevens, 1987; Foss *et al.*, 1993). DNA methylation is speculated to occur transiently during the sexual stage (Liu *et al*., 2012), DNA methylation may be not exist in *Aspergillus flavus*. DNA methylation prevents extension, transcription in *Neurospora crassa* and *Candida albicans* (Rountree and Selker, 1997; Mishra *et al.*, 2011). In *Magnaporthe oryzae*, methylcytosines content (0.4%) and level (0.39%) of DNA methylation are similar during asexual development (Jeon *et al*., 2015). However, we need to know with respect to the methylation connection between the environmental and developmental influences of edible mushrooms. We applied MSAP to survey DNA methylation in *P. eryngii* subsp. *tuoliensis* to address the concern on the methylation levels and patterns of edible mushroom under low temperature induction.

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Materials and Methods

Strain Culture

P. eryngii subsp. *tuoliensis* was provided by Hengdaxing. Strains were maintained at 4°C on culture slants of potato dextrose agar (PDA) medium. Mycelial block of inoculation (diameter, 5 mm) was sliced from PDA that had grown on PDA medium for 12 days at 25°C in a petri dish (diameter, 9 cm). The medium was autoclaved at 121°C for 15 min.

Temperature Treatments

P. eryngii subsp. *tuoliensis* mycelia were grown at 25°C for 7d (control). After 25°C growth for 7 d, the mycelia were subjected to 4°C low temperature stimulation treatments for 3 d, and then incubated at 13°C under a 12/12 h dark/light cycle for primordium.

Isolation of DNA

Genomic DNA was extracted from mycelia tissues given different treatment using the Cetyl trimethylammonium bromide (CTAB) method (Kidwell *et al.*, 1992). The quality and quantity of genomic DNA was detected by 1.0% agarose gel and spectrophotometer.

Methylation Sensitive Amplified Polymorphism (MSAP) Analysis

MSAP is a improved method of the AFLP fingerprinting technique (Vos *et al.*, 1995). Highly quality genomic DNA is extracted, *EcoR I* /*Hpa II* and *EcoR I* /*Msp I* enzyme combination were used to digest DNA, and linked to adapters, then design pre-amplification primers and PCR amplification. (Salmon *et al*., 2008; Herrera and Bazaga, 2010; Liramedeiros *et al.*, 2010; Paun *et al.*, 2010; Richards *et al.*, 2012).

Each sample mycelium genomic DNA 300 ng was cut off using *EcoR I* (4 units, NEB, USA), *Msp I* / *Hpa II* (4 units, NEB, USA) and cut smart buffer in a total of 10 μl, at 37°C for 5.0 h. Then 3 pM *EcoR I*, 30 pM *Msp I* /*Hpa II* specific adopters (Table 1), 4 units T4 DNA ligase (NEB, USA) were added to the digested reaction at 16°C for overnight. The pre-selective amplification DNA (25 μL) was acquired with the template (digestion), *EcoR I* + X and *Msp I* /*Hpa II* + X (X is one of A, T, C and G nucleotide) primers (Table 1), 5 unit of Taq DNA polymerase (TaKaRa, China), 2.5 mM dNTP, 10×PCR Buffer(Mg^{2+} plus). The PCR reaction profile were considered at 94°C for 5 min, 30 cycles of 94°C for 30 s, 56°C for 30s, at 72°C for 60s, 10 min extension 72°C. There were 96 primer combinations add *Hpa II* /*Msp I* +XYZ (X, Y, Z is one of A,T,C and G nucleotide) for selective amplification. The PCR product (first PCR) was diluted 30 times in TE buffer (pH 8.0). The second PCR reaction profile were considered at 94°C for 5 min, 10 cycles of 94°C for 30s，65°C for 30s (from 65°C to 56°C was reduced by 1°C) and at 72°C for 80s, 30 cycles of 94°C for 5 min, 55°C for 30s and at 72°C for 80s, 10 min extension 72°C. The gel method and silver staining were similar to already described (Bassam *et al*., 1991; Zhang *et al*., 2009). The electrophoretic results were noted by sequencing system (Junyi, Beijing, China).

The methylation status were dividied into four classes under MSAP dates analysis: Type I showed that *EcoR I* /*Hpa II* and *EcoR I* /*Msp I* have bands(E-H/E-M, +/+), Type II showed that *EcoR I* /*Hpa II* has band, but absent in *EcoR I* /*Msp I* (E-H/E-M, +/-), Type III showed that *EcoR I* / *Msp I* has band, but absent in *EcoR I* / *Hpa II* (E-H/E-M, -/+), Type IV showed that *EcoR I* /*Hpa II* and *EcoR I* /*Msp I* bands were absent (Keyte *et al.*, 2006; Zhang *et al.*, 2009).

The specific bands were cut from gel. In BLAST (NCBI, http://www.ncbi.nlm.nih.gov/), the gene fragments were describled by find the homology.

Results

DNA Methylation of *P. eryngii* Subsp. *tuoliensis* by MSAP Analysis

Bailinggu (China) is widely considered as one of the most popular edible mushroom in Asia. Environmental factors that affect mycelium grown require, special low temperature situations to germinate primordia and fruiting bodies. Low temperature (4°C 3 d) stimulation of mycelium growth is the key stage for primordia initiation in edible mushroom. Statistical analysis of MSAP markers bands was used to assess changes in methylated DNA of *P. eryngii* subsp. *tuoliensis*. Nucleotide specificity MSAP primers combinations *EcoR I* and enzymes *Msp I*/ *Hpa II*+ XY (XYZ) are used to generate the MSAP fingerprints of *P. eryngii* subsp. *tuoliensis*. We used 96 primer combinations (three technical replicates) to amplify 2005 to 2022 clear and reproducible bands from *P. eryngii* subsp. *tuoliensis* cultivars. The total methylation of cytosine averaged 28.31%, and 11.85% under low temperature induction (4°C 3 days), which was more than that induced by 25°C (control). Moreover, the full-methylated level was significantly higher than the hemi-methylated level at all times. The number of full-methylated bands (from 12.62 to 19.10%) and hemi-methylated lines (from 3.84 to 9.21%) were significantly greater compared with low temperature induction (Fig. 3).

DNA Methylation Patterns under Low Temperature Stress

DNA fragments are known as more than one CG cover end point from the same recognized site (CCGG) and cut by one or both restriction enzymes (Schulz *et al.*, 2013). To survey the DNA methylation hyper- or hypo-diversity under low temperature induction and control, we defined all

Table 1: Sequences of adapters and pre-selective and selective primers used for MSAP analysis

Primers/adapters	Sequence (5′-3′)
EcoR I adapter	5'-CTCGTAGACTGCGTACC-3' 3'-CATCTGACGCATGGTTAA-5'
EcoR I+1 primer	5'-GACTGCGTACCAATTCA- 3'
Msp I-Hpa II adapter	5'-GACGATGAGTCTAGAA-3' 3'-CGTTCTAGACTCATC-5'
Msp I-Hpa II+1 primer	5'-GATGAGTCTAGAACGGT-3'

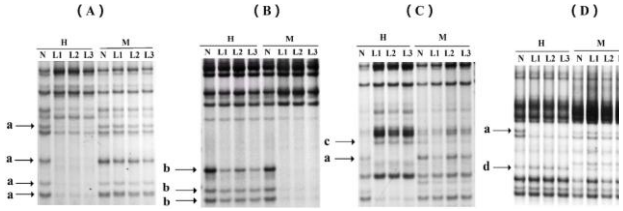

Fig. 1: MSAP profiles showing the various types of locus-specific DNA methylation alterations in Control and three independent low temperature induction *P. eryngii* subsp. *tuoliensis*. a, CG hyper-; b, CHG hyper-; c, CG hypo-; d, CHG hypo-. N, Control; L1, L2 and L3, Low temperature induction. H, *EcoR I + Hpa II*; M, *EcoR I +Msp I*

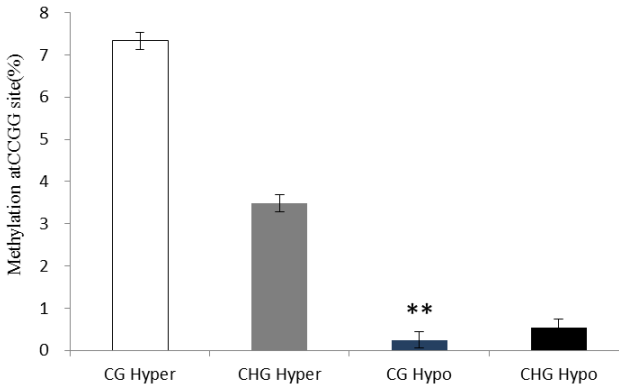

Fig. 2: Levels of the four major patterns of methylation CG hyper-, CHG hyper-, CG hypo- and CHG hypo- calculated based on the MSAP in Control and three independent Low temperature induction of *P. eryngii* subsp. *tuoliensis*. **, $P<0.01$

differential lines as four patterns (CG hypo-, CHG hypo-, CG hyper- and CHG hyper-) (Zhang *et al.*, 2009; Qi *et al.*, 2010) (Fig. 1). We determined the methylation changes analyzed from four methylation patterns compared with the genetics. Low temperature induction caused a more abundant change in CG hyper- and least abundant in CG hypo- compared with the control (Fig. 2). Fig. 2 shows that different methylation patterns changed the relative consistency for three individuals. CG hyper- showed a dramatic difference in the three changed methylation patterns, while CHG hypo-, CG hypo- and CHG hyper- showed similar methylation patterns. These results suggest that low temperature induces a diversity in methylation patterns.

DNA methylation patterns in control and low temperature induction were analyzed as described by Karan (Karan *et al.*, 2012). We count all bands in the gel and compare them for surveying DNA methylation patterns. We observed fifteen different banding patterns in the statistical data. Table 2 showed that from A to C patterns had no change in methylation in response to both control and low temperature induction. From D to I described cytosine demethylation and J-O represented DNA methylation induction of low temperature (Table 2).

All bands averaged 84.97% of the 5′-CCGG-3′ sites that showed unchanged methylation under low temperature induction (Table 2). The percentage of demethylation bands averaged 5.36% under low temperature induction while the methylated bands averaged 9.68% in *P. eryngii* subsp. *tuoliensis* under low temperature induction.

BLAST Result of Poplymorphic Fragment Sequences

Twenty two differential bands were collected and sequenced from different primers (Table 3). BLAST was used to search DNA sequences that are significantly homologous to known-function genes. Most of the DNA fragments showed "No significant similarity found". L6 was 74% homologous to the *Agaricus bisporus* var. *bisporus* H97 chromosome 10 sequence. The sequence of L8 was related to *Trametes versicolor* FP-101664 SS1 PR-1-like protein mRNA. The sequence of L20 was associated with *Mesocestoides corti* genome assembly M_ corti_ Specht_ Voge.

Discussion

Over 100 publications described the use of MSAP primarily to study developmental biology (Portis *et al*., 2004; Hanai *et al*., 2010; Moran and Perezfigueroa, 2011; Meng *et al*., 2012). DNA methylation plays critical roles in various aspects of biological processes (Jullien *et al*., 2012; Diez *et al*., 2014; Heard and Martienssen, 2014; Matzke and Mosher, 2014). Environmental stresses can induce varying patterns of DNA methylation (Steward *et al*., 2002; Wada *et al*., 2004; Verhoeven *et al*., 2010), the regulation of gene expression may be speculated. In plant development, MSAP (Xiong *et al.*, 1999) was put into use to study genome methylation under stress (Portis *et al.*, 2004; Salmon *et al*., 2008; Xuelin *et al*., 2009; Zhong *et al.*, 2009; Yi *et al*., 2010; Wang *et al.*, 2011; Karan *et al*., 2012; Albertini and Marconi, 2013; Ou *et al.*, 2015). DNA demethylation often has been related to temperature induction or stress, such as *Arabidopsis*, maize, Antirrhinum majus and wheat (Steward *et al*., 2002; Hashida *et al.*, 2003; Sherman and Talbert, 2011). However, DNA methylation in edible mushrooms development is not well-studied, and there is a dearth of

Table 2: Variation in the major methylation patterns, including No change, Demethylation and Methylation in Control and Low temperature induction of *P. eryngii* subsp. *Tuoliensis*

Patterns	Class	Control		Stress-induced		Low temperature induction
		H	M	H	M	
No change	A	1	0	1	0	24
	B	0	1	0	1	46
	C	1	1	1	1	1640
	Total					1710 (84.57%)
Demethylation	D	1	0	1	1	19
	E	0	1	1	1	8
	F	0	0	1	1	6
	G	0	1	1	0	7
	H	0	0	1	0	71
	I	0	0	0	1	1
	Total					112 (5.54%)
Methylation	J	1	1	1	0	23
	K	1	1	0	1	151
	L	1	1	0	0	12
	M	1	0	0	1	1
	N	1	0	0	0	1
	O	0	1	0	0	12
	Total					200 (9.89%)

Table 3: Results of the BLAST analysis of the methylated DNA polymorphic sequences

Code	Length (bp)	Identity (%)	BLAST results
H3	90	-	Ns
H5	99	-	Ns
H7	100	-	Ns
H8	113	-	Ns
H10	99	-	Ns
H17	242	-	Ns
H18	136	-	Ns
H22	100	-	Ns
H24	117	-	Ns
H29	141	-	Ns
L1	101	-	Ns
L3	90	-	Ns
L5	110	-	Ns
L6	389	74	*Agaricus bisporus* var. *bisporus* H97 chromosome 10 sequence, CP015466.1
L8	437	74	*Trametes versicolor* FP-101664 SS1 PR-1-like protein mRNA, XM_008034883.1
L9	157	-	Ns
L15	92	-	Ns
L20	86	85	*Mesocestoides corti* genome assembly M_ corti_ Specht_ Voge, scaffold MCOS_scaffold0001241, LM533338.1
L24	98	-	Ns
L21	105	-	Ns
L22	133	-	Ns
01102	134	-	Ns

Ns, No significant similarity found

information that could be potentially useful for mushroom breeders.

In our study, the MSAP method was used to determine whether low temperature induction caused changes DNA methylation of *P. eryngii* subsp. *tuoliensis*. Our results showed the level of DNA methylation rise

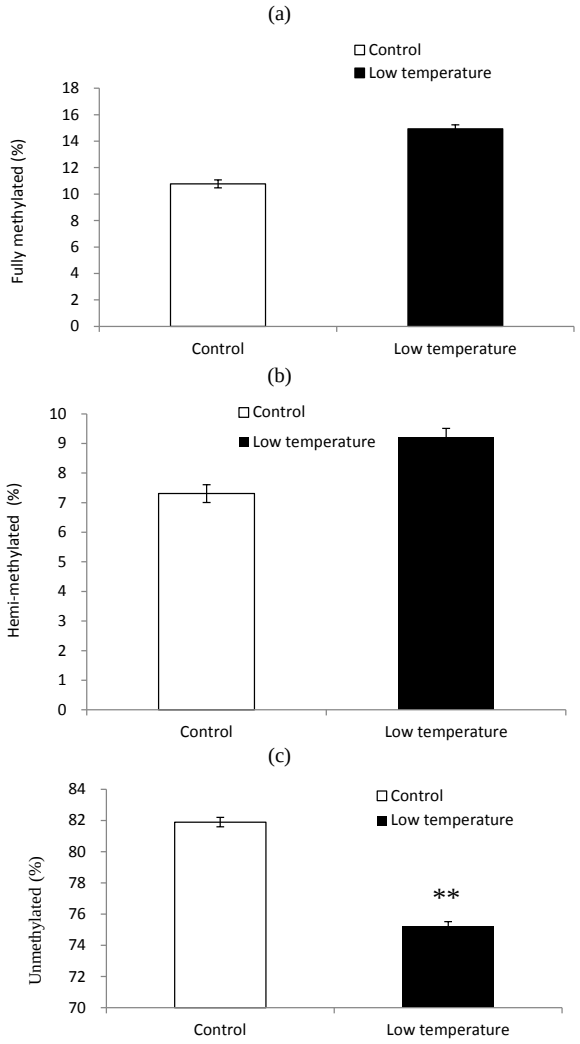

Fig. 3: Full-methylated, Hemi-methylated and Unmethylated in MSAP fingerprints of *P. eryngii* subsp. *tuoliensis* Normal and subjected to low temperature induction. (a) Full-methylated, (b) Hemi-methylated, (c) Unmethylated. **, $P<0.01$. MSAP (%) = [(II + III+ IV) / (I +II + III+IV)] ×100, unmethylated(%)=1-MSAP(%). Full-methylated ratio (%) = [(III+ IV) / (I+II +III +IV)] × 100. Hemi-methylated ratio (%) = [(II) / (I +II + III + IV)] ×100

during mycelium growth in Control and Low temperature induction. Variable frequencies for MSAP bands, different changes in demonstrated genotypes and epigenetic changes, and loss and gain indicated function indicated hypo- and hyper-methylated, which are possibly the primary function of DNA methylation. This is accordingly reflected relation in the relation of various stress-induction environment and re-patterning. Thus, our study showed that low temperature induced genetic and epigenetic changes are relevant for each other. CG hyper- and loss of bands are significantly correlated in stress-induction.

Table S1: List of 96 selective primers combinations used in the MSAP markers

EcoR I+3 (a-h)	*H/M*+3 (1-12)
a GACTGCGTACCAATTCAAC	1 GATGAGTCTAGAACGGTAC
b GACTGCGTACCAATTCAAG	2 GATGAGTCTAGAACGGTAG
c GACTGCGTACCAATTCACA	3 GATGAGTCTAGAACGGTCT
d GACTGCGTACCAATTCACT	4 GATGAGTCTAGAACGGTCG
e GACTGCGTACCAATTCACG	5 GATGAGTCTAGAACGGTA
f GACTGCGTACCAATTCAGC	6 GATGAGTCTAGAACGGTT
g GACTGCGTACCAATTCAGG	7 GATGAGTCTAGAACGGTC
h GACTGCGTACCAATTCAGA	8 GATGAGTCTAGAACGGTG
	9 GATGAGTCTAGAACGGAAC
	10 GATGAGTCTAGAACGGAAG
	11 GATGAGTCTAGAACGGAAA
	12 GATGAGTCTAGAACGGAGA

Table S2: BLAST sequence of twenty-two

Code	BLAST sequence
H3	GCAAAAAAATATGATAATTTCGGTAGATCGACTTGGAATCACGTCAGCTCTAGAAGTGAGCATGAACGAGAGAGCGTACCGTTCTAGACA
H5	GAATCATCGTGCTGGAGACTGCATAGCAAGGTAGCGCCTGGTAGCGTCTAGCGAAAAAAATATTACCTTTATATCCCCCAGACCGTTCTAGAC TCATCA
H7	CCCCCATCGCCAAGGTACTATTCCCTGGATGCAGCAGGCCGCCGTGAGTCCAGCGGTACGGGGCATGCTGCGCATCCAAGGACCGTTCTAG ACTCATCAA
H8	CCAACGTTAGGTTATATGTCATTCAACGGGACGTTGTTCATCCGAGCGGCGGGGTTGACAGCCAAGTGGATGGGTAGGATTAGGGAAGGAG AGAGACCGTTCTAGACTCATCA
H10	GGAAGGTTCCTAATAAGATATTTCGGTAGAACGACTTGAAAACACGTCAGCTCAAAGAAGTGAGCATGAACGAGAGAGCGTACCGTTCTAG ACTCATCA
H17	GGTCCATGCAGCTCAGCCGTTGGGCTTGCGGAGTTCGATATCGGTGGCCATGCAATTAATAGAACAGTGTCTGATCGGTGTGAACCTGGAAG TTCCGAATGATGTCGACCTGACACTTGCATGTTTCTATTCGCTTCAGTGTTCGCTAAGCAAGCAACTTCGCAAGCAACCAACGGAAATTACT CCGTGACAGTGCATGCCAACGTCTTCAGATCTTCAAAATGACCGTTCTAGACTCATCA
H18	ACCTATGGATTGACGGAGTGGCAGGTGATGAACGTCTGTGCGCTTGACATAGACTTAGTTCCGCGAATGGCCAAGGAGCGAGAGGCAGCAG CCACGAGCCTGAGACCGCTATGACATGACCGTTCTAGACTCATCA
H22	GTCCCTAAGAAGAGCGTCTCTGCGGTGGGTGGCGTGATAACTGGTGAGAGGAGCGGGAATAAATCCCACGGTCACTACTGCCACCGTTCTA GACTCATCA
H24	TGATGAGTCTAGAACGGTGGCAGTAGTGACCGTGGGATTTATTCCCGCCCTCTCACCAGTTATCACGCCACACGCGTCTTTCTTGCCATTCCT CAGCTCTGAATTGGTACGCAGTCA
H29	TGACTGCGTACCAATTCAGACGCTGCGTCCTGGATCATACACGCGAAACAAGTACGTCCATCTCGTCTCGTCGAGCTGAGCTGCCCTCACCT ATTGACGTTTCCTCAGTTCTACTGTATATCCACCGTTCTAGACTCATCA
L1	CAATTGCTGACTGAATGCGTGTGAGGCGTGTGATGGGTGGCTTACATTGGCAAATATCTCGAGCAAATTAAGAATTGCTGTATACCGTTCTAG ACTCATCA
L3	GGGACTATAAGCTCCGAGTTCTTGATTGTCACGGCACCTGTGGTGTTCGGCGGGTATACTAAGATTCTATGGACCGTTCTAGACTCATCA
L5	ACCCCTCACTTTAAACTTAAAAACTCTGCAGAGGCGTGTGATAAGGTGGCTTACATTGGCAAATATCTCGAGCAAATTAAGAATTGCTGTAG ACCGTTCTAGACTCATCA
L6	TGATGAGTCTAGAACGGTAGACTGAATACAACGTTAGTTACCGATTTCATCTGGCATCGAGGCTGGCCGTTCAAGATGATGGCATTGGGTATA GACTTGAAACAAAAGTGTCGTGGTAATTACCTGTGCTATATTGATGAAACCTTCTCCGTGCTCATCCATTTCGACCTCACCCTCAACGGCCTC CGTCCCCTACCTCACTTTGGCTTTCTTATCCCGTTCTTCATCGCTGTGTGTCGGTGGCGGTTGTTCGCGAACCTCAAATATACGAAGCAGGTTT GTACGCGCGACTACCACATTGCAAAGGACCTTGGTTCCATCGTCTATCCCCGAATTTTGCGTCGTTGAAGGAGTCAATTTCAAACTGACGAT GAATTGGTACGCAGTCA
L8	TGACTGCGTACCAATTCATCGCATAAGCTCTGAGAATTGAACGGGGATTCATGGGAGAAGACGTACGCAAATTGGCCAATAATATTTCCCTGG GCAGAGTATTCACAAACGAAATATTTCGCCTTCTGCGGCACAACAGAACGCGCGGTCAGTTAATTATTACGGACGAGCCGACAAGACGTGT ACTCACCCCAAACTTAGCGTCGAAAATGCCGTCGCAAAGCGCTTCAGCACATCCAACTTGGCTGCTCCCTTTCCAGACCACTTGGGTGAAA TGGGATGGAACAGGGTTGTTAGGGTTGTAGTCCTCTACGTATTGAGGTGAGCACGTTCCCAGGTAGTTGGAGCGACAACCACTACTCACTG ACTTCGTCCGTCCACGACTTGATGGCAGACTGAATGTCATAAGCGCTCCCAGTACCGTTCTAGACTCATCA
L9	GCCGTAGGTATGCAGATGCGTGCCTTTGGAGCGTGATTCCAGAATGGTGAGGTCGTCTTCACCAGTCGGGGAAATTTCTCCTTCCAGGGCGG GGCCGAGGTGGTTCGCCTCGACATCCCGCTCCTCGATCCTGTCCACCGTTCTAGACTCATCAATG
L15	CCCCTCCATCACGGTCACACCAAGATAGCATTAGGGTCTTGGCTGTGTTGCCAGGAAGATGAGGATGAGCCGTAACCGTTCTAGACTCATCA
L20	GTCCTGACAGGATTTCCGCTCACGTCCCACCGTCACCACCACCACTGTCTCGCCCTCCTCCTCGTCGTACCGTTCTAGACTCATCA
L24	CCCCTGAATCACGGTCACAACCAAGATAGCATTAGGGTCTTGGCTGTGTTGCCAGGAAGATGAGGATGAGCCGTGACCGTTCTAGACTCATC ACAGTG
L21	CCTTCATGACTTAGAGCCAGTAGAGGATGACTGAATGAGTTTCACTCACTCACAACGAGTCTGATTAGGGCATATCGGGGTAGCCTGACCGT TCTAGACTCATCA
L22	TGATGAGTCTAGAACGGTCAGGCTACCCCGATATGCCCTAATCAGACTCGTTGTGAGTGAGTGAAACTCATTCAGTCATCCTCTACTTGGCAT CTTCACCAGGACATGTTTGATCTGAATTGGTACGCAGTCA
01102	CCGGACCTCAGCGTCGCGCTCTTTTCTGGGGCAGGTATATCCCCGCTTGGATACAATCTTCAATAAGGCCATACTTGAGGTTAAACTTGTGAA ACACCTGGTCGGGGAGTTTGTGAACCGTTCTAGACTCATCA

A dramatic change in methylation occurs when there is low temperature induction. However, it is evident that *P. eryngii* subsp. *tuoliensis* responds to low temperature induction by increasing the level of DNA methylation. Our results indicate that low temperature induce DNA methylation (16.47% to avg. 28.31%). Moreover, hemi-methylated bands raised under low temperature induction. However, it was not clear

whether this increase was caused by segmental methylation of unmethylated DNA or incomplete CG or CHG hypomethylated of fully methylated bands.

Conclusion

The role of environmental factors that contribute to the methylation process is well-known. *P. eryngii* subsp. *tuoliensis* is the earliest variety from Xinjiang in China; it needs low temperatures for bud stage and temperature less than 4ºC for the differentiation of mushroom primordia, which are the bottleneck of *P. eryngii* subsp. *tuoliensis* industry development. Therefore, genomic changes are a result of the environment and genotypes, as shown by their markedly variable frequencies and different patterns of methylation changes. These investigations may enhance our comprehension of stress inducted epigenetic transformation in edible mushroom.

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