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Validation of reference genes for quantitative gene expression analysis in *Auricularia cornea*

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ABSTRACT

Auricularia cornea Ehrenb., previously named *A. polytricha* (Mont.) Sacc, has become one of the most widely cultivated mushrooms in China. Considerable research has been conducted on its cultivation, pathogen identification, proteomics, and more. However, to the best of our knowledge, no studies have been performed on reference-gene validation in this species. Formerly, reference genes were selected for their expression levels only relied upon from others species, owing to the fact that the gene stability in this species is unknown. In this study, nine candidate genes, including tubulin alpha-1A chain (*TUBA1A*), β-tubulin (*Btu*), phosphoglucomutase (*Pgm*), actin 1 (*Act*1), protein phosphatase 2A regulatory subunit (*PP2A*), polyubiquitin (*UBQ*), glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*), 18S ribosomal protein (*18S*) and 28S ribosomal protein (*28S*), were evaluated among different strains and developmental stages. Four algorithms (i.e., geNorm, NormFinder, BestKeeper and RefFinder) were used to analyze candidate genes. The results revealed that *UBQ* was the most stable reference gene, while *18S* was the least stable. Despite these results, the candidate genes were largely inadequate and only two were considered suitable. Based on candidate gene stability, *PP2A* and *UBQ* were identified as a set of usable interior control genes for future analyses in this species. This is the first systematic study conducted for selecting reference genes in *A. cornea*, and lays the foundation for identifying genes and quantifying gene expression in this species.

1. Introduction

Auricularia cornea Ehrenb, previously named *A. polytricha* (Mont.) Sacc (Huang et al., 2012; Yang et al., 2014; Zheng et al., 2014), has become the fourth most important cultivated mushroom worldwide (Jia et al., 2017). It has been demonstrated to be a potential agent against diseases, such as tumors, dementia, and hypercholesterolemia, and has been used as an alternative bio-sorbent for detoxification (Huang et al., 2012; Yang et al., 2014; Zheng et al., 2014). Based on this information, more attention is being paid to this interesting species. To date, considerable research efforts have been made to improve its cultivation (Abd Razak et al., 2013), pathogen identification (Sun and Bian, 2012; Peng et al., 2014), and to explore its transcriptomic and proteomic data (Zhou et al., 2014; Jia et al., 2017). However, to our knowledge, an evaluation of reference genes has not been reported, which could be used in real-time reverse transcription quantitative polymerase chain reaction (RT-qPCR) analyses. Thus, we do not know which genes are stably expressed at different development stages or among different strains, and could be used as reference genes to normalize data.

As for reference genes, unfortunately, there is no universal gene that is suitable for every species (Kang et al., 2018; Zhao et al., 2018). For example, actin (*Act*) is unstably expressed in potatoes and cucumbers (Xiang et al., 2018). As another example, hypoxanthine phosphoribosyltransferase (*HPRT*) was suitable as reference gene in placental tissues, but is unsuitable for articular cartilage (Kang et al., 2018). Therefore, it is essential to validate suitable reference genes under different conditions to obtain biologically meaningful expression data. Until now, research has been devoted to the identification and evaluation of reference genes in other mushroom species apart from *A. cornea*, such as *Lentinula edodes* (Xiang et al., 2018), *Ganoderma lucidum* (Xu et al., 2014), *Pleurotus ostreatus* (Zheng et al., 2018a), and *Volvariella volvacea* (Qian et al., 2018).

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Table 1
Descriptions of the nine candidate reference genes and the target gene.

Gene[a]	Gene symbol	Primer sequences	Amplicon length (bp)	Annealing temperature (°C)	Amplification efficiency (%)	R^2
Tubulin alpha-1A chain	*TUBA1A*	F:AAGGAGGACGCCGCCAACAA R:CCGAAGGAGTGGAAGACGAAGAAG	140	60.0	94.9	0.999
Beta-tubulin	*Btu*	F:CACTCGCTTGGTGGTGGAACAG R:GTGTACCGACAGCAGGCAGTTG	162	60.0	90.4	0.999
Phosphoglucomutase	*PGM*	F:CATCGTCCACACCTCGCTTCAC R:GCCAGTTATCGCCGTCCTTCTC	269	60.0	92.0	1.000
Actin 1	*ACT 1*	F:TGCTGTGTCTGTGTTGCGTGTT R:GCGAGCCAAGTCAAGTCAAGTCT	225	60.0	92.4	0.993
Protein phosphatase 2A regulatory subunit	*PP2A*	F:CTGCCGCCGACACCACTTAATC R:GTACAAGTTCTACACCGAGTTCCAGTC	106	60.0	90.7	0.999
Polyubiquitin	*UBQ*	F:CGGATCTAACAGCGTGGACTCTTC R:CCTCCTGAGCGATTGGCACTTG	124	60.0	95.1	0.999
Glyceraldehyde-3-phosphate dehydrogenase	*Gapdh*	F:GCCGTATCGGTCGGATTGTGAC R:TGAGCTTGCCGTCCTTGGTCT	168	58.0	95.6	0.998
18S ribosomal protein	*18S*	F:GGTTAAGGTCTCGTTCGTTATCGGAAT R:GGAGTATGGTCGCAAGGCTGAA	229	58.0	92.2	0.996
28S ribosomal protein	*28S*	F:TTGTGGTGTCTGATGAGCGTGTATT R:CTTGATTGGACCGTCTGGCGAAT	125	58.0	91.4	0.997
Laccase	*Lac*	F:GATACGATGACGGACGGAACCAATC R:ACTTGTGGACGATCTGGAACTTGTG	241	58.0	90.1	0.999

[a] Nine candidate reference genes include tubulin alpha-1A chain (*TUBA1A*), β-tubulin (*Btu*), phosphoglucomutase (*PGM*), actin 1 (*Act1*), protein phosphatase 2A regulatory subunit (*PP2A*), polyubiquitin (*UBQ*), Glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*), 18S ribosomal protein (*18S*), 28S ribosomal protein (*28S*). Laccase (*Lac*) was used as target gene.

Nine genes of tubulin alpha-1A chain (*TUBA1A*) (Li et al., 2011), β-tubulin (*Btu*) (Xiang et al., 2018; Li et al., 2019; Song et al., 2019), phosphoglucomutase (*Pgm*) (Xiang et al., 2018), actin 1 (*Act*1) (Dai et al., 2018; Huang et al., 2018), protein phosphatase 2A regulatory subunit (*PP2A*) (Xu et al., 2015; Lu et al., 2018), polyubiquitin (*UBQ*) (Singh et al., 2019; Song et al., 2019), glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*) (Yang et al., 2019; Zhang et al., 2019), 18S ribosomal protein (*18S*) (Xiang et al., 2018) and 28S ribosomal protein (*28S*) (Singh et al., 2018; Singh et al., 2019) have been commonly used for reference-gene analysis, and were also chosen for candidates in this study, so as to screen out the most stale reference gene. Laccase (*Lac*) demonstrates cresolase and catecholase activities (Yihui et al., 2018), showed different expression levels at various developmental stages (Zhao and Kwan, 1999), and used as a target gene to validate the selected reference genes. These genes were used to study expression stability across different strains of *A. cornea* in samples obtained from different developmental stages, including mycelia (MC), primordia (PM), and fruiting bodies (FB). Four algorithms, geNorm, NormFinder, BestKeeper, and RefFinder, were used to analyze the reference gene stability. This study will hopefully provide a better guide for the gene expression in the mushroom and other similar species.

2. Materials and methods

2.1. Sample preparation

Four *A. cornea* strains, WA24, CBE1, 43,012, and 22, were preserved at the Soil and Fertilizer Institute, Sichuan Academy of Agricultural Sciences, China. The MC, PM, and FB samples were cultivated following the methods outlined by Jia et al. (Jia et al., 2017). Samples were flash-frozen in liquid nitrogen and stored at −80 °C until RNA isolation.

2.2. RNA isolation and cDNA synthesis

Total RNA was extracted from the *A. cornea* MC, PM, and FB samples using a Plant RNA Kit (Omega Biotek Inc., USA) following the manufacturer's instructions. The integrity of RNA was tested by gel electrophoresis. The first strand of cDNA synthesis was performed using a RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific Inc., USA) following the manufacturer's instructions. All cDNA samples were stored at −80 °C until used in further analyses.

2.3. Candidate reference genes and RT-qPCR analysis

Samples were prepared for RT-qPCR analysis by diluting cDNA samples five times with nuclease-free water. Nine candidate reference genes were selected for this study, including *TUBA1A*, *Btu*, *PGM*, *Act1*, *PP2A*, *UBQ*, *Gapdh*, *18S*, and *28S*. *Lac* was selected as a target gene. The original sequences of the candidate reference genes and target gene were derived from the *A. cornea* transcriptome, except *18S* and *28S*, which were derived from the *A. cornea* genome (supplemental dataset). Primers were designed using Primer 5 software (Dai et al., 2018), their detailed information was described in Table 1. The RT-qPCR was performed with the CFX Connect Real-Time PCR Detection System using ChamQ Universal SYBR qPCR Master Mix (Vazyme Biotech Co., Ltd., China). The PCR protocol is as follows: 95 °C for 3 min, 1 cycle; and 95 °C for 5 s, 60/58 °C for 20 s, plate read, and 43 cycles. Primer specificity was verified by heating from 65 to 95 °C with a 0.5 °C w/s and 1.5% agarose gel electrophoresis. Tree replicates were performed for each RT-qPCR reaction. The linear amount of the target gene expression to the calibrator was calculated by $2^{-\Delta\Delta CT}$ (Zhang et al., 2015).

2.4. Stability analysis

The stability of the nine candidate reference genes was analyzed using commonly used algorithms: geNorm (Medrano et al., 2017; Wang

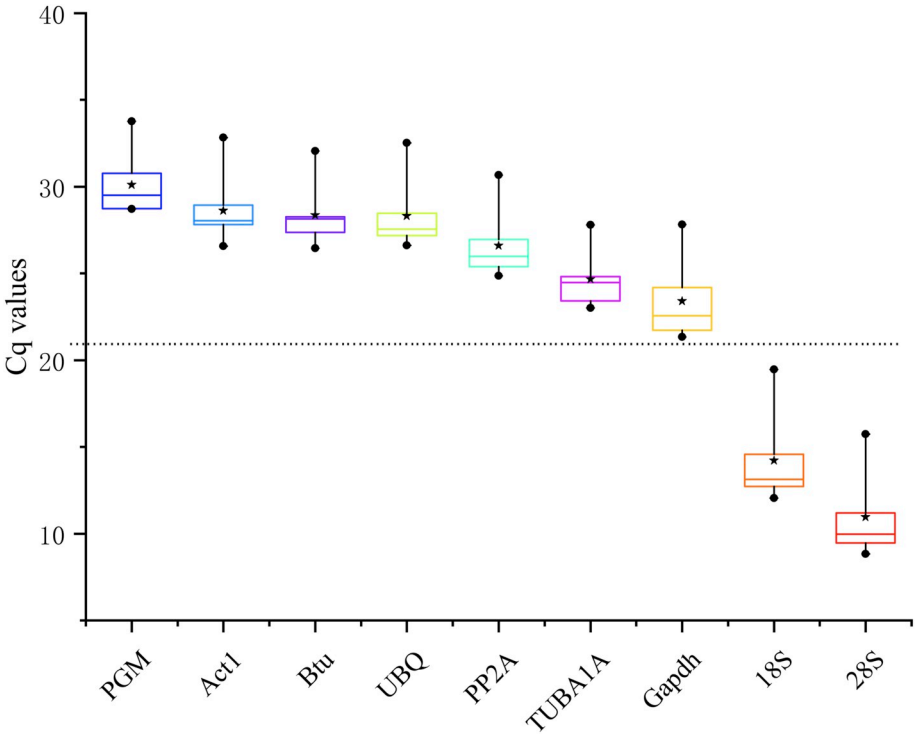

Fig. 1. Expression levels of candidate reference genes across all samples of the four strains at different development stages. The boxes depict the 25/75 percentiles; Lines across the boxes represent the medians; Stars indicate the mean values; Upper and lower dots represent the maximum and minimum values.

et al., 2018; Xiang et al., 2018; Yang et al., 2018; Zheng et al., 2018b), NormFinder (Xu et al., 2018; Yang et al., 2018; Zhao et al., 2018; Zheng et al., 2018b), BestKeeper (Sheshadri et al., 2018; Wang et al., 2018; Xiang et al., 2018; Zheng et al., 2018b) and RefFinder (Sheshadri et al., 2018; Wang et al., 2018; Yang et al., 2018; Zheng et al., 2018b), in order to rank the stability of the tested genes and provide recommendations on the most stable gene. Based on the different candidate reference genes, *Lac* was used as a target gene and its mRNA expression levels were determined.

3. Results

3.1. Selection of candidates

Primer specificity was determined by melting curve and agarose gel electrophoresis. Genes with expected amplified bands and no non-specific amplicons were selected for the study. By 10-fold serial dilutions of cDNA, the PCR efficiency of candidate genes was calculated. Results revealed that the 10 genes' efficiency varied from 91.1% (*Lac*) to 95.6% (*Gapdh*), and their correlation coefficients ranged from 0.993 (*ACT 1*) to 1.000 (*PGM*). These annealing temperatures ranged from 58 to 60 °C (Table. 1, Fig. S1). After primer-specificity assays, the nine candidate reference genes, *TUBA1A*, *Btu*, *PGM*, *Act*1, *PP2A*, *UBQ*, *Gapdh*, *18S*, *28S*, and one target gene, *Lac*, were selected for this study.

3.2. Expression profiles of reference genes

After measuring Cq values, the expression stability of each gene was evaluated. The candidate gene with the lowest Cq value was *28S*, followed by *18S*, *Gapdh*, *TUBA1A*, *PP2A*, *UBQ*, *Btu*, *Act1*, and *PGM*. The 9 reference genes' Cq values ranged from 8.83 (*28S* at the PM stage of strain 43,012) to 33.77 (*PGM* at the MC stage of strain WA24), indicating a wide range of variation. All Cq values of each candidate gene were above 21, except *18S* and *28S*. The lowest gene expression variation was observed in *TUBA1A* (23.02–27.81), while *18S* was the most variable (12.05–19.46) (Fig. 1). These wide ranges indicate that none of the reference genes had a constant expression pattern across the different samples. It is important to evaluate expression stability of the candidate reference genes, so that a stable gene can be selected for normalizing gene expression data.

Table 2
Comprehensive ranking results for the stability assessment of candidate reference genes at different developmental stages of the four strains.

	BestKeeper		geNorm		NormFinder		RefFinder	
	Standard deviation	Ranking	M	Ranking	Stability value	Ranking	Geomean	Ranking
TUBA1A	1.234	1	0.643	8	0.380	8	4.000	5
Btu	1.240	2	0.538	3	0.269	3	2.621	2
PGM	1.374	3	0.580	7	0.297	6	5.013	6
Act1	1.425	4	0.574	4	0.290	4	4.000	4
PP2A	1.479	6	0.432	2	0.051	2	2.884	3
UBQ	1.442	5	0.430	1	0.051	1	1.710	1
Gapdh	1.789	7	0.574	5	0.292	5	5.593	7
18S	1.829	9	0.663	9	0.404	9	9.000	9
28S	1.790	8	0.578	6	0.317	7	6.952	8

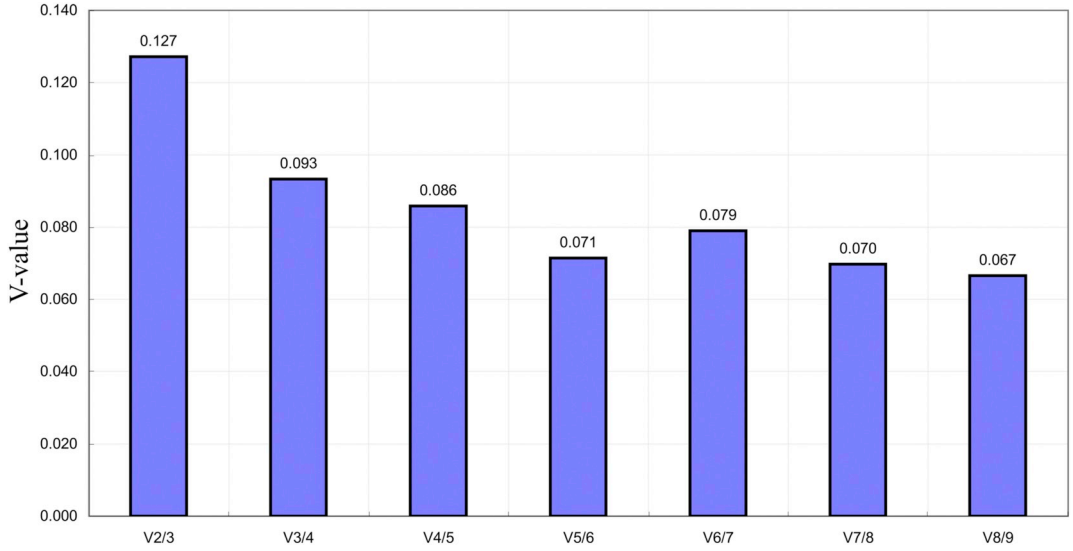

Fig. 2. Determination of the optimal number of control genes for normalization. In which all V-values were below 0.15, indicating that the optimal number of control genes was two and there was no need to include a 3rd gene in the normalization factor.

3.3. Expression stability of the candidate reference genes

Analyzing the stability of nine candidate reference genes with the algorithms of geNorm, NormFinder, BestKeeper and RefFinder, the resulting data was obtained (Tables. 2 and S1). By the BestKeeper, the most stably expressed genes were found resulting from evaluating the coefficient of correlation (r) and its index (Xiang et al., 2018). Lower standard deviation (SD) values represent higher expression stability of a given candidate gene. Results revealed that the most and least stable reference genes were *TUBA1A* and *18S*, respectively.

Using the geNorm software, M-values were calculated. Lower M values represent higher stability (Shu et al., 2018). Results revealed that the reference gene with the lowest M-value (most stable) was *UBQ*, followed by *PP2A*, while the reference gene with the highest M-value (least stable) was *18S*.

Using the NormFinder software, the stability values (SV) were calculated. Lower SV-values represent higher stability (Zhao et al., 2019). Results revealed that the two most stable genes were *UBQ* and *PP2A*, while the least stable gene was *18S*, which matched the geNorm findings.

RefFinder calculates the geomean of all stability weights from the geNorm, NormFinder, and BestKeeper results by assigning an appropriate weight to an individual gene (You et al., 2018), which contributes to a comprehensive ranking of the most stable candidate genes (Lu et al., 2018). Comprehensive ranking revealed that *UBQ* was the most stable gene, while *18S* was least stable, which matched the findings of geNorm and NormFinder.

Table 3
Expression stability determination among various developmental stages and different strains.

Gene/category		Stability value/gene	
		Developmental stage	Strain
Individual gene	*TUBA1A*	0.342	0.209
	Btu	0.262	0.182
	PGM	0.24	0.193
	Act1	0.282	0.155
	PP2A	0.091	0.078
	UBQ	0.093	0.08
	Gapdh	0.265	0.199
	18S	0.358	0.234
	28S	0.3	0.208
	Best gene	*PP2A*	*PP2A*
	Stability value	0.091	0.078
Best combination	Pair	*Btu* and *Gapdh*	*PP2A* and *UBQ*
	Stability value	0.072	0.074

3.4. Determination of the optimal number of reference genes for normalization

The geNorm software calculated a pairwise comparison (Vn/n + 1) to determine the optimal number of reference genes. The V-value cutoff is 0.15; There is no need to include an additional reference gene when the values below this level (Zhao et al., 2019). If the average geNorm M-value was lower than 0.2, the gene was considered stable (Shu et al., 2018). In this study, the lowest M-value was above 0.2 and all V-values were below 0.15, indicating that only one reference gene was inadequate for normalizing gene-expression data, but two were sufficient (Fig. 2). Therefore, *UBQ* and *PP2A* were considered as a suitable combination based on their M-values.

3.5. Expression stability among different strains and various developmental stages

The intra- and intergroup variations were calculated by NormFinder, which combines the two results into a stability value for each candidate gene (Zheng et al., 2018b). A lower SV-value represents higher expression stability (Xiang et al., 2018). Across the different developmental stages, the SV-value of *PP2A* was 0.091, indicating that it was the best gene. The combination of *Btu* and *Gapdh* had the best SV-value of 0.079. Among the four strains, *PP2A* had the best SV-value of 0.078. Additionally, *PP2A* and *UBQ* also had a good SV-value of 0.074 (Table. 3). Combined with the results of the optimized reference gene numbers, *PP2A* and *UBQ* were identified as the best set for expression data normalization.

3.6. Differential target gene mRNA expression profiles based on the selection of internal genes

The target gene, *Lac* (EC 1.10.3.2), is a multicopper oxidase. It belongs to an extracellular enzyme and is able to degrade a variety of substrates, especially phenolic components (Shao et al., 2018). *PP2A* and *UBQ* were used as a reference-gene set, and *18S* was used as a least stable control. When *PP2A* and *UBQ* were used to normalize the

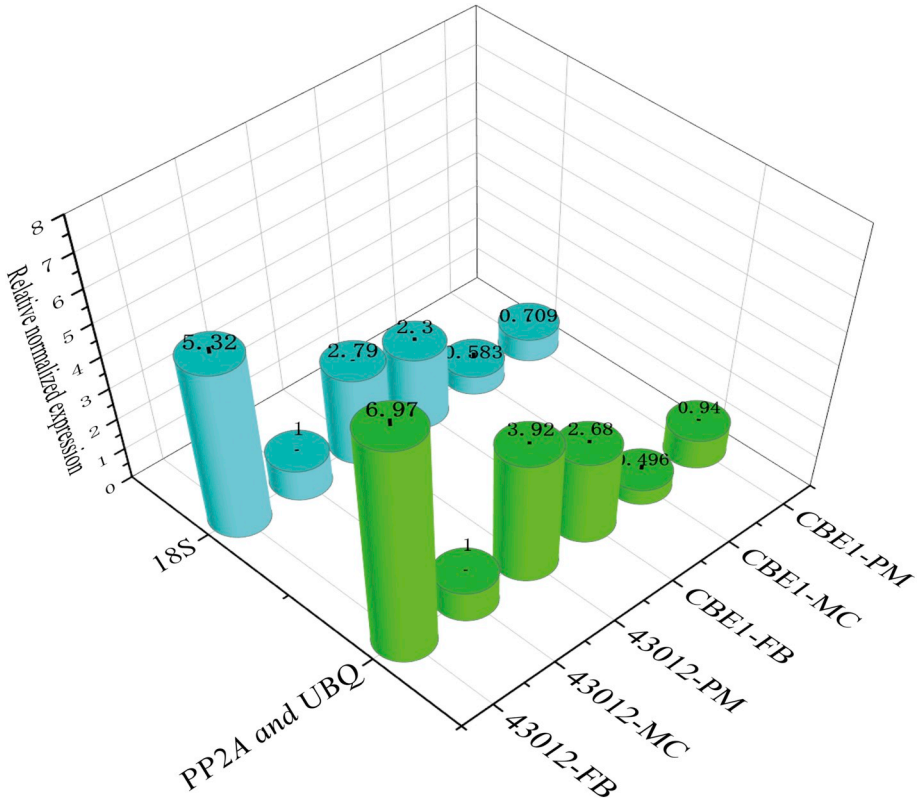

Fig. 3. Expression profile of *Lac* in the tissues of mycelium (MC), primordium (PM) and fruiting bodies (FB) of strain 43,012 and CBE1. The best stable combination of reference genes (*PP2A* and *UBQ*) and the least stable reference gene (*18S*) were used to normalize the expression data. The sample of 43,012-MC was used as a control. The data show the mean expression ± standard deviation calculated from three biological replicates.

expression of *Lac* in sample 43,012-FB, this gene was upregulated 6.97-fold ($p < .01$, Fig. 3). When *18S* was used, *Lac* was upregulated 5.32-fold ($p < .01$). In another sample, *Lac* was upregulated 2.68-fold ($p < .01$) in CBE1-FB when *PP2A* and *UBQ* were used, but upregulated 2.30-fold ($p < .01$) when *18S* was used. The results reveal that there is a marked difference in expression level using different reference genes, indicating that the biologically meaningful expression data has to depend on the stable reference genes. This confirms the importance of validating reference genes before use in experimental applications.

4. Discussion

The experiments were performed in triplicate. Genes with amplification efficiency > 90% and $R^2 > 0.99$ were selected for validation analyses. The most stable genes were obtained through a comprehensive analysis utilizing four algorithms, geNorm, BestKeeper, NormFinder, and RefFinder, which made the experimental data more reliable.

When the geNorm M-value was ≤0.2, the reference gene had high stability. Moreover, when using the BestKeeper algorithm, candidate genes with SD-values > 1 were considered unstable and unsuitable for use as a reference gene (Shu et al., 2018). The results revealed that across the three developmental stages, all M-values were above 0.4 and SD-values were above 1 (Table. 2). However, specifically for the PM and FB stages, M-values were below 0.5 and SD-values were below 0.6 (Table. S2). This indicates that there is a great deal of variation in gene expression from stage MC to stage FB. Although it has smaller variation between stages of PM and FB, all genes were above the cut-off for M- and SD-values. Combined with the determination of the optimal number of reference genes, the results indicate that any one of reference genes is inadequate, but the combination of *PP2A* and *UBQ* is the most stable and sufficient in either the PM and FB stages or all stages.

To date, reference-gene evaluation has been employed in *Lentinula edodes* (Xiang et al., 2018), *Ganoderma lucidum* (Xu et al., 2014), *Pleurotus ostreatus* (Zheng et al., 2018a), *Volvariella volvacea* (Qian et al., 2018), *Cordyceps militaris* (Lian et al., 2014) and *A. cornea*, and their most stable reference genes are ribosomal protein L4(*RPL4*), *RPL4*, secretion associated GTP-binding protein (*Sar1*), SPRY domain protein (*SPRYp*), polymerase II large subunit (*Rpb1*) and *UBQ* respectively (Table. 4). Their M-values, generated by geNorm, were almost above 0.2, indicating that only one reference gene is inadequate for these species. It is of help to the follow-up mushroom researchers.

In this study, *PP2A* and *UBQ* were identified as the most stable reference genes in *A. cornea* samples. Similarly, *PP2A* expressed at the most stable levels in *Sorghum bicolor* (Sudhakar Reddy et al., 2016) and *Ganoderma lucidum* (Xu et al., 2014; Lu et al., 2018). Though expressed stably in *A. cornea*, *UBQ* presented the least stability in *Robinia pseudoacacia* (Wang et al., 2018) and *Hemerocallis citrina* (Hou et al., 2017). Thus, there is clearly no universal reference gene that is suitable for every species. It is important to validate reference gene stability before studying gene expression or use in experimental applications. In conclusion, from the nine candidate reference genes, *PP2A* and *UBQ* were the most sable combination, and *18S* was the least stable. In order to obtain the most reliable results of quantitative gene expression in *A. cornea*, *PP2A* and *UBQ* are recommended as a set of usable interior control genes for RT-qPCR analysis.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mimet.2019.105658.

Table 4
Information on the most stable reference genes and sets within six mushroom species.

Species	Most stable reference gene	The least stable reference gene	The optimal number of reference genes	Most stable reference-gene set	Primer sequences	Annealing temperature (°C)	Amplicon length(bp)
Ganoderma lucidum	RPL4	18S	Two	*RPL4*	F: GTCAACAAGGGCGTTCTCTT R: ACAGCGTCTTGAGGAAGGT	60	151
				BTU	F: CAGTTCACGGCGATGTTCA R: CGACGGTAGCATCCTGGTA	60	142
Lentinula edodes	*Rpl4*	*F-actin*	Four	*Rpl4*	F: AATCGTAGACACCGTCAGCG R: TGACGAAACGGCCAAGATGA	63	160
				BTU	F: CAGTTCACGGCCATGTTCA R: CGACGGTGGCATCCTGGTA	63	152
				RPL2	F: AATCGATCCCTCGGGAAAGC R: CAGGCTTCCTTGTCCTCGTT	63	181
				Tsb	F: CTGGTGTTGGACCTGAGCAT R: CCTTAGGAAGCGTCTTGGCA	63	183
Pleurotus ostreatus	*Sar1*	*Cyc*	Three	*Sar1*	F: GGATAGTCTTCCTCGTCGATAG R: GGGTGCGTCAATCTTGTTAC	63	133
				Pep	F: TGATTCCAGAGGACAAGGACGCAA R: AAATCTTCCGCGATACGGGTCACT	63	148
				Phos	F: CATCGCAAATCATCGATCGCACCA R: GCTCTCCAGCCAATGCACCAATTT	63	125
Volvariella volvacea	*SPRYp*	*18S*	Two	*SPRYp*	F: GCATTCTTCTTGATGTCGGTGGTCG R: AACCCTGAAGTGTTGGATGCTCTGG	60	130
				TUBα	F: GAGCCCAATGTTATCGATGAAGTGC R: GTTCTTTGCCAATTGTGTAGTGCCC	60	130
Cordyceps militaris	*Rpb1*	*Actin*	Five	*Rpb1*	F: CTGTTCCCCCTCCTCCTGTG R: CTGTTCCCCCTCCTCCTGTG	58	/
				Gpd	F: GCAACGCCGTCGAGCACAA R: AAAACACCGTGGGAGGAGTCATAC	58	110
				Try	F: CGATGCTTCCCTTGAACCACT R: GTCTCCCTCATGGCAGAGATCA	58	/
				Ubi	F: ACCGCTGAAGTTGCCATATAACC R: GTCGCACCCTTTGTATTTGTTGT	58	135
				Tef1	F: GTCAAGGAAATCCGTCGTGGTAA R: GCAGGCGATGTGAGCAGTGTG	58	162
Auricularia cornea	*UBQ*	*18S*	Two	*PP2A*	F: CTGCCGCCGACACCACTTAATC R: GTACAAGTTCTACACCGAGTTCCAGTC	60	106
				UBQ	F: CGGATCTAACAGCGTGGACTCTTC R: CCTCCTGAGCGATTGGCACTTG	60	124

Abbreviations: Ribosomal protein L4(*RPL4*), 18S ribosomal protein (*18S*), Beta-tubulin (*BTU*), Ribosomal protein L2 (*RPL2*), tryptophan synthase-β (*Tsb*), Secretion associated GTP-binding protein (*Sar1*), cyclin-like F-box (*Cyc*), SPRY domain protein (*SPRYp*), Alpha-tubulin (*TUBα*), polymerase II large subunit (*Rpb1*), glyceraldehyde-3-phosphate dehydrogenase (*Gpd*), tryptophan synthase (*Try*), polyubiquitin binding protein (*Ubi*), the elongation factor 1-alpha (*Tef1*), protein phosphatase 2A regulatory subunit (*PP2A*), polyubiquitin (*UBQ*).

Authors and contributions

Wei-Hong Peng conceived and designed the experiments. Ding-Hong Jia performed the experiments. Bo Wang, Xiao-Lin Li, Wei Tan and Bing-Cheng Gan analyzed the data. Ding-Hong Jia wrote and revised the paper.

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Declaration of Competing Interests

The authors declare they have no conflict of interest.

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