Bangladesh J. Bot. **54**(3): 783-790, 2025 (September) Special DOI: https://doi.org/10.3329/bjb.v54i30.85128

EFFECTS OF EXOGENOUS MICROBIAL ADDITIVES TO IMPROVE TOBACCO LEAF QUALITY

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Keywords: Tobacco leaf, Fermentation, Microbial agents, Chemical composition, Sensory quality

Abstract

To investigate the effect of adding microbial agents in the fermentation stage on the quality of tobacco leaves prepared by imitating black tea method. Using Yunyan 87 middle leaves as materials, two microbial agents of *Schwanniomyces occidentalis* and *Bacillus subtilis* were sprayed onto the leaves before fermentation, and the effects of different microbial treatments on the main chemical composition, sensory quality, microbial diversity of tobacco leaves were analyzed. The starch content in tobacco leaves treated with *S. occidentalis* and *B. subtilis* decreased by 25.00 and 47.64%, and the total pigment content decreased by 46.15 and 30.77%, respectively. Tobacco leaves treated with two microbial agents can increase the characteristic aroma of burnt sweet and sour sweet respectively. The treatment of the two agents can improve the community richness and species diversity of tobacco leaves, and effectively improve the abundance of dominant bacteria such as *Firmicutes* and *Candida*. Both exogenous microorganisms can effectively reduce the starch and pigment contents in tobacco leaves, improve the sensory quality of tobacco leaves, improve the diversity of microbial community, and then improve the quality of tobacco leaves prepared by imitating black tea method.

Introduction

The intrinsic chemical composition of tobacco leaves directly determines their quality, thereby influencing their usability and grade classification (Chen *et al.* 2020). During tobacco processing, relying solely on the enzymatic activity within the leaves is insufficient to achieve complete transformation of uncoordinated components or significant quality improvement within a short period, failing to meet industrial requirements for tobacco quality (Wang *et al.* 2015). Therefore, appropriate microbial inoculation may optimize the internal chemical composition of tobacco leaves (Lu *et al.* 2023). Microbial metabolic processes produce various enzymes that effectively promote the decomposition and conversion of macromolecular compounds in tobacco leaves, facilitate the transformation and accumulation of aroma constituents, and reduce harmful components, ultimately enhancing smoking quality (Hao *et al.* 2022).

While numerous studies in China have explored microbial applications for improving tobacco quality, most focus on microbial roles during aging and fermentation processes (Chen *et al.* 2019a, Chen *et al.* 2019b, Bie *et al.* 2021, Wu *et al.* 2021), with limited research on microbial enhancement during the tobacco modulation stage. Drawing inspiration from black tea processing techniques (Dai *et al.* 2021), this study introduces microbial inoculants during tobacco modulation to analyze their effects on major chemical components, sensory quality, and surface microbial diversity. The study further evaluates the potential of microbial treatments to enhance the quality of tobacco leaves prepared by imitating black tea method, providing a scientific basis for improving tobacco usability.

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Materials and Methods

The test tobacco variety was Yunyan 87, cultivated in Yong'an Town, Liuyang, China. Mature fresh leaves from the 8th to 9th positions (middle leaves) were selected. The microbial strains used were *Schwanniomyces occidentalis* CGMCC1548 and *Bacillus subtilis* CGMCC7417, obtained from Beijing Bio-Bank Co. Ltd. The culture media included Yeast extract, Peptone, and Dextrose (1% peptone, 1% glucose, 0.5% yeast extract) and Luria-Bertani (1% peptone, 1% NaCl, 0.5% yeast extract), both sterilized at 121°C for 25 min.

The modulation process followed: fresh leaves → withering → rolling → fermentation → drying. Based on optimized parameters established in previous laboratory studies (Dai *et al*. 2021), the withering temperature was set at 38°C for dry bulb temperature and 36°C for wet bulb temperature, withering time: 36 hrs, rolling quantity: 678 u 2 kg, kneading time: 20 min, fermentation temperature: 41°C, fermentation time: 13 hrs, drying mode: 85-105°C two-stage drying.

Schwanniomyces occidentalis was inoculated from solid medium into 50 ml of YPD liquid medium and cultured at 37°C with shaking at 120 r/min for 24 hrs. The resulting seed culture was centrifuged at 4°C and 10,000 r/min for 10 min, and the supernatant was discarded. The pellet was resuspended in sterile deionized water and adjusted to 10^8 cfu/ml using the McFarland turbidity method. *Bacillus subtilis* was inoculated into 50 ml of LB liquid medium and cultured at 37°C with shaking at 160 rpm for 24 hrs. The subsequent steps were identical to those for *S. occidentalis*. 50 ml microbial suspension (10^8 cfu/ml) was evenly sprayed onto 1 kg of tobacco leaves before fermentation. Sterile water was applied as a control (CK), and conventional flue-cured tobacco served as an additional control (CK1), *Schwanniomyces occidentalis* (W1), *Bacillus subtilis* (W2). After 13 hrs of fermentation, samples were collected using a five-point sampling method (50 g leaves per treatment), immediately frozen in liquid nitrogen, and stored in an ultra-low temperature refrigerator at 80°C for microbial amplicon sequencing.

All inoculated fermentation tests were set up in 3 parallel groups. The measured parameters included total sugars, total nitrogen, total alkaloids, reducing sugars, chloride, potassium, starch, protein, and plastid pigments. Analytical methods followed China Tobacco Industry Standards: YC/T 159-2019 (total and reducing sugars), YC/T 468-2021 (total alkaloids), YC/T 162-2011 (chloride), YC/T 217-2007 (potassium), YC/T 161-2002 (total nitrogen), YC/T 216-2013 (starch), YC/T 249-2008 (protein), and 95% ethanol extraction-colorimetric method (plastid pigments). Organized by the Technology Center of China Tobacco Hunan Industrial Corporation, a panel of certified tobacco evaluators assessed the sensory characteristics of the four treatments. Evaluation criteria included aroma quality, aroma intensity, concentration, strength, irritation, Offensive taste, aftertaste, combustibility and so on. Liquid nitrogen samples from the end of fermentation of the three treatments W1, W2 and CK were taken for microbial amplicon sequencing. DNA was extracted and used as a template for PCR amplification of the bacterial 16S rRNA V3-V4 region and the fungal ITS1 region. Purified PCR products were sequenced by Novogene Bioinformatics Technology Co. Ltd. (Beijing). Experimental data were processed using Microsoft Excel 2019, and statistical analysis was performed with SPSS 26.0. Microbial community structure analysis was conducted on the Novogene Cloud Platform (https://magic.novogene.com/customer/main #/homeNew).

Results and Discussion

The chemical composition of tobacco leaves is intrinsically linked to their quality. As presented in Table 1, all microbial treatments resulted in significant reductions in reducing sugar content compared to the control (CK). Notably, treatment W1 exhibited a marked decrease in total sugar content, with the hierarchical order of total sugar content being CK > CK1 >W2 >W1. The observed reduction in reducing sugar content in treated leaves may be attributed to microbial metabolic utilization of sugars as energy sources, as well as sugar-amino acid reactions generating aroma-contributing compounds (Chen *et al.* 2022). Starch content demonstrated differential reductions of 25.00% (W1) and 47.64% (W2) relative to CK. *Bacillus*, are frequently utilized for starch degradation and quality improvement (Hao *et al.* 2022, Duan *et al.* 2023). While protein content showed a decreasing trend across treatments, the differences were not statistically significant. Table 2 reveals that total plastid pigment content was significantly reduced by 46.15% (W1) and 30.77% (W2) compared to CK. Both treatments induced significant decreases in carotenoid and chlorophyll b content, with W1 showing a particularly pronounced 42.86% reduction in chlorophyll a. *S. occidentalis* fermentation broth can degrade carotenoids while increasing aromatic compounds (Liu *et al.* 2016). These findings suggest that microbial inoculation significantly influences pigment metabolism during the tea-style processing of tobacco leaves.

Table 1. Conventional chemical composition of tobacco leaves treated by different microbial inoculum.

Treatment (%)	W1	W2	CK	CK1
Total sugar	25.99 ± 1.40b	27.19 ± 0.63ab	29.15 ± 1.07a	28.25 ± 1.29ab
Total nitrogen	1.59 ± 0.14a	1.57 ± 0.12a	1.67 ± 0.09a	1.65 ± 0.14a
Nicotine	1.58 ± 0.12a	1.49 ± 0.13a	1.51 ± 0.05a	1.47 ± 0.23a
Reducing sugar	24.51 ± 0.82b	26.40 ± 1.09b	28.69 ± 0.63a	26.13 ± 1.56b
Chlorine	1.37 ± 0.11a	1.43 ± 0.08a	1.42 ± 0.13a	1.35 ± 0.06a
Potassium	2.03 ± 0.12a	1.91 ± 0.09a	1.88 ± 0.08a	1.94 ± 0.12a
Starch	5.73 ± 1.53ab	4.00 ± 1.18b	7.64 ± 1.71a	6.37 ± 1.27ab
Protein	8.25 ± 0.82a	8.20 ± 0.63a	8.81 ± 0.80a	8.72 ± 0.98a

The same row data with different lower-case letters indicate the significant difference at the 5% level.

Table 2. Plastid pigment content in tobacco leaves treated by different microbial inoculum.

Treatment	Plastid pigment/(mg.g^{-1})				
	Chlorophyll a	Chlorophyll b	Carotenoid	Total chlorophyll	Gross
W1	0.04b	0.02c	0.08c	0.07b	0.14c
W2	0.05ab	0.02c	0.11b	0.07b	0.18b
CK	0.07a	0.06b	0.14a	0.12ab	0.26a
CK1	0.07a	0.09a	0.10b	0.16a	0.26a

Data with different lower-case letters after the same column indicate the significant difference at the 5% level.

The sensory evaluation results (Tables 3 and 4) revealed that, compared to CK and CK1, W1-treated leaves demonstrated enhanced aroma intensity and improved permeability, while W2-treated leaves exhibited superior aroma quality, reduced irritation, and increased cleanliness. *S. occidentalis* fermentation broth and *Bacillus species* can improve sensory evaluation scores (Zheng *et al.* 2016, Fan *et al.* 2019, Hu *et al.* 2019). The comprehensive sensory scores ranked the treatments as W2 > W1 > CK > CK1, indicating that imitation black tea modulated tobacco leaves exhibited superior sensory characteristics to conventional flue-cured tobacco. W1 treatment introduced a distinct caramel-like sweetness with elevated aromatic notes, whereas W2 treatment developed caramel sweetness, fruity acidity, and enhanced purity. Furthermore, yeast fermentation has been reported to produce floral and fruity aromas, and *Bacillus* fermentation can enhance alcoholic, fruity, and sweet notes (Lu *et al.* 2023). Our treatments specifically developed caramelized and acidic aroma profiles, potentially due to the unique black tea-style processing conditions.

Table 3. Sensory evaluation results of tobacco leaves treated by different microbial inoculum.

Indicator	W1	W2	CK	CK1
Aroma quality	6.3	6.4	6.3	6.3
Aroma quantity	6.6	6.5	6.5	6.5
Permeability	6.6	6.5	6.5	6.5
Offensive taste	6.4	6.4	6.4	6.3
Concentration	6.6	6.6	6.6	6.6
Strength	5.1	5.1	5.1	5.2
Irritation	6.2	6.4	6.3	6.3
Cleanliness	6.3	6.4	6.3	6.3
Dryness	6.2	6.1	6.2	6.2
After taste	6.4	6.4	6.4	6.3
Total score	62.7	62.8	62.6	62.5

Table 4. Assessment of the sensory quality of tobacco leaves treated with different microbial inoculum.

Treatment	Evaluation results
W1	Increased caramelized sweetness, hay, toasted notes, uptick in aroma profile, increased Irritation, slightly green and miscellaneous
W2	Increased sourness, toasted notes, hay, slightly caramelized sweetness and tartness, increased cleanliness, increased dryness, slightly decreased richness
CK	Honey-sweet, toasted, hay, slightly gritty, clean finish
CK1	Toasted, caramelized sweetness predominates, medium texture, medium strength, less wilted caramelized notes

Alpha diversity analysis of bacterial and fungal communities (Table 5) showed complete sequence coverage (Coverage index = 1.000) for all treatments, confirming data reliability. Bacterial community analysis revealed higher Chao1 indices and OTU counts in W1 >W2 >CK, with W2 demonstrating increased Shannon and Simpson indices compared to CK, indicating enhanced bacterial diversity and evenness. Fungal communities in W1 showed greater richness (higher OTU counts and Chao1 index) (Cheng *et al.* 2023), while both W1 and W2 exhibited increased Shannon and Simpson indices, suggesting improved fungal diversity and evenness (Fan *et al.* 2019, Cai *et al.* 2023). OTU analysis (Fig. 1A) identified 77 shared bacterial OTUs among W1, W2, and CK, representing 28, 33.77, and 35.98% of respective OTUs, with unique OTUs numbering 156, 112, and 92 per treatment. Fungal analysis (Fig. 1B) detected 45 shared OTUs (21.43, 33.33 and 30.61% per group), with treatment-specific OTUs numbering 141 (W1), 67 (W2), and 73 (CK).

Table 5. Microbial Alpha diversity index of tobacco leaves with different microbial inoculum.

Microbes	Treatment	Chao1	Coverage	OTUs	Shannon	Simpson
Bacteria	W1	280.526	1.000	275	2.955	0.769
	W2	231.750	1.000	228	4.707	0.933
	CK	222.000	1.000	214	3.472	0.841
Fungus	W1	213.111	1.000	210	2.257	0.640
	W2	135.300	1.000	135	2.342	0.664
	CK	147.000	1.000	147	1.796	0.545

The phylum-level taxonomic profiling of bacterial and fungal communities revealed distinct compositional patterns among treatments (Fig. 1). Proteobacteria and Cyanobacteria emerged as the dominant bacterial phyla shared across all treatments and controls, collectively accounting for 83.59 to 99.69% of relative abundance. Comparative analysis showed that W1 treatment significantly increased the relative abundance of Cyanobacteria and Firmicutes while decreasing Proteobacteria abundance compared to CK. Notably, Firmicutes demonstrated particularly substantial enrichment in W1-treated samples. In contrast, W2 treatment exhibited higher relative abundances of both Proteobacteria and Firmicutes but reduced Cyanobacteria levels relative to the control group. Fungal community analysis (Fig. 1B) identified Ascomycota and Basidiomycota as the predominant phyla present in all experimental groups. Both microbial treatments enhanced Ascomycota abundance compared to CK, with W1 and W2 showing increases of 11.47 and 19.42%, respectively. Divergent effects were observed for Basidiomycota, where W1 treatment increased its relative abundance while W2 treatment resulted in decreased levels relative to the control. At the phylum level, Proteobacteria and Cyanobacteria emerged as shared dominant bacterial phyla across treatments, while Ascomycota and Basidiomycota represented the predominant fungal phyla - findings consistent with previous reports (Bao *et al*. 2018, Chen *et al*. 2019c, Fan *et al*. 2019, Cai *et al*. 2023).

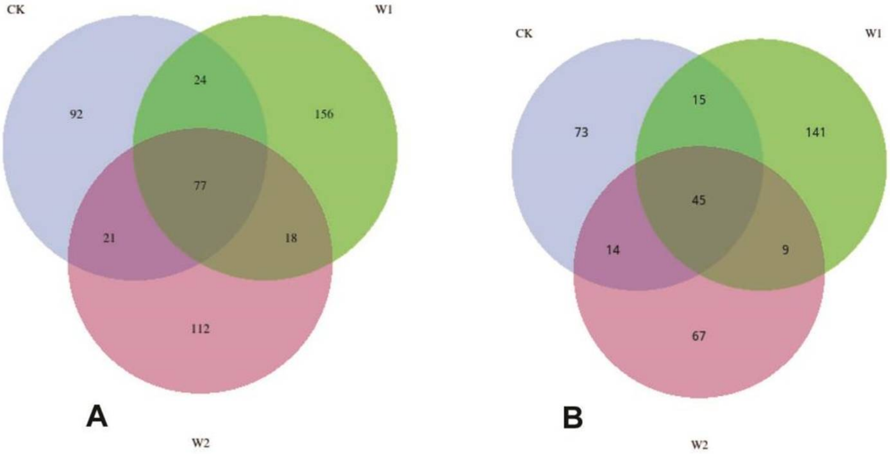

Fig. 1. Venn diagram of bacterial (A) and fungal (B) communities in tobacco leaves treated with different microbial agents.

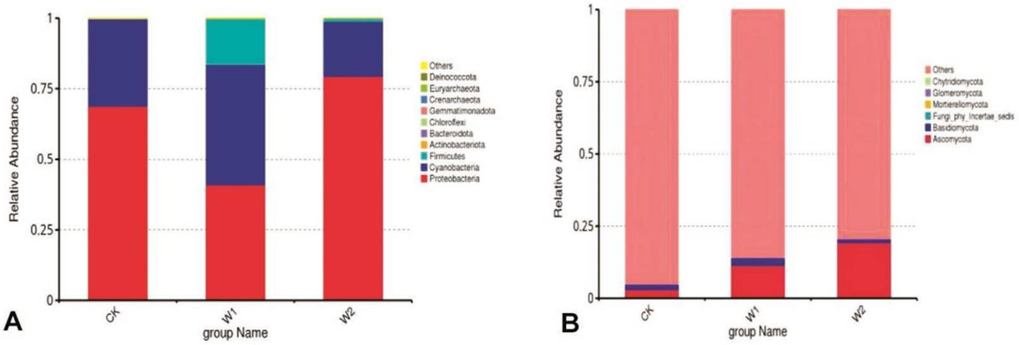

Fig. 2. Relative abundance of bacteria (A) and fungi (B) phyla levels in tobacco leaf samples.

The genus-level microbial composition exhibited significant variations among different treatments (Fig. 2). Analysis of bacterial communities (Fig. 2A) revealed *Pantoea* as the predominant genus across all samples, accounting for 18.24-55.84% of relative abundance. W1 treatment showed substantial enrichment of *Staphylococcus* (26.08%), while W2 treatment demonstrated increased abundances of *Franconibacter* (15.42%) and *Klebsiella* (10.59%) compared to the control.

Fungal community analysis (Fig. 2B) indicated that W1 treatment led to increase in the relative abundances of *Candida*, *Aspergillus*, and *Sampaiozyma* by 4.38, 3.61, and 0.97%, respectively, whereas W2 treatment increased the proportions of *Candida*, *Diutina*, and *Issatchenkia* by 11.37, 4.72, and 1.69%, respectively. Conversely, W2 treatment resulted in decreased abundances of *Aspergillus* and *Sampaiozyma* relative to the control group. This study also found that *Pantoea*, *yeasts*, and *Staphylococcus* are the dominant microorganisms in fermented tobacco leaves (Cai *et al*. 2022, Chen *et al*. 2024).

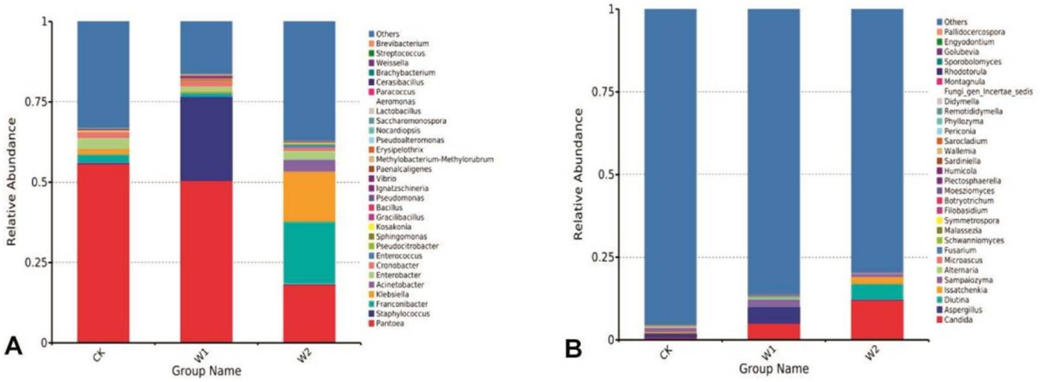

Fig. 3. Relative abundances of bacteria (A) and fungi (B) genus levels in tobacco leaf samples.

Heatmap analysis of the top 30 genera (Fig. 3) revealed distinct treatment-specific patterns in both bacterial and fungal communities. For bacterial populations (Fig. 3A), W1 treatment showed increased abundances of *Staphylococcus* and *Sphingomonas*, while W2 treatment exhibited enrichment of *Enterococcus* and *Acinetobacter* compared to CK. Fungal community analysis (Fig. 3B) demonstrated that W1 treatment enhanced the abundance of *Schwanniomyces*, whereas W2 treatment specifically increased the proportion of *Candida*. These shifts in community structure likely reflect complex microbial interactions and competition induced by exogenous inoculants (Chen *et al*. 2023, Huang *et al*. 2023).

This study demonstrates that both *Schwanniomyces occidentalis* and *Bacillus subtilis* effectively reduce starch and plastid pigment contents in imitation black tea modulated tobacco leaves. *S. occidentalis* exhibited superior performance in degrading total plastid pigments and carotenoids, achieving reductions of 46.15 and 42.86% respectively, while *B. subtilis* showed optimal starch degradation efficiency with a reduction rate of 47.64%. The microbial treatments distinctly enhanced characteristic aroma profiles, with *S. occidentalis* promoting caramel-like sweetness and *B. subtilis* developing acidic notes, both contributing to improved sensory quality. The inoculations significantly altered the indigenous microbial communities, particularly increasing the relative abundance of dominant genera such as *Candida.* This work provides preliminary evidence for the advantages of microbial applications in enhancing black tea-style tobacco quality and establishes a scientific basis for improving industrial applicability. Future research will focus on elucidating the underlying metabolic pathways and enzymatic dynamics to further validate the relationships between microbial activities and tobacco quality enhancement.

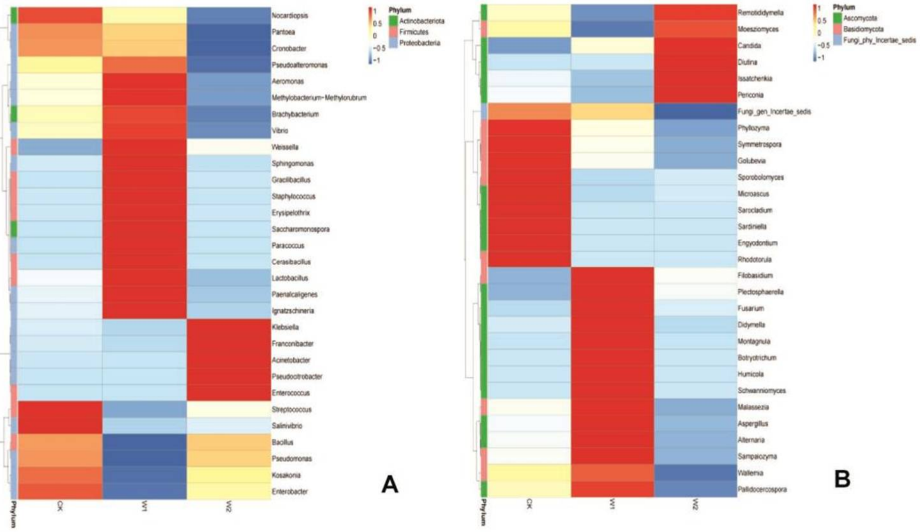

Fig. 4. Cluster heat map of the bacteria (A) and fungi (B) dominant flora at the genus level.

Acknowledgement

The authors are grateful for project support from the Hunan Tobacco Industry Co. Ltd. (202343000834073) for this study.

References

Bao KX, Chen SY, Chen YQ, Chen SB, Fan JQ, He W, Li JJ, Lin J and Zhang RQ 2018. High-throughput sequencing fungal community structures in aging tobacco strips from different growing areas and stalk positions. Tob. Sci. Tech. **51**(04): 12-19.

Bie XM, Hu AT, Kong LY, Lu ZX, Mao SR, Yu ST and Zhu HY 2021. Study on screening of microorganisms for improving tobacco leaf quality and their effects. J. Nj. Agr. Univ. **44**(04): 766-777.

Cai W, Hu WR, Li DL, Liu YF, Luo C, Zhang ZJ and Xue F 2023. Influence of fermentative medium on the chemical compositions and microbial communities of cigar tobacco leaves. J. Light Ind. **38**(01): 90-100.

Cai XH, Chen Z, Chai ZS, Zheng LL, Zhao L and Shi XD 2022. Analysis of bacterial and fungal community diversity during industrial secondary fermentation of cigar core tobacco leaves. Acta Tabacaria Sin. **28**(05): 121-128.

Chen D, Ding SS, Li XN, Li M, Li LL, Shi XD and Wan DJ 2019a. Role of microorganisms in tobacco fermentation and alcoholization: a review. Microbiol. China **46**(06): 1520-1529.

Chen QL, Cai LT, Huang Y, Li Z, Wang HC, Xie HL and Zhou H 2019b. Fungal composition and diversity of tobacco phyllosphere from cured tobacco leaves. Acta Microbiol. Sin. **59**(12): 2401-2409.

Chen W, Guo ZG, Guo B, Jiang YY, Lai HX, Xue L and Zheng ZH 2019c. Screening and application of aroma-enhancing bacteria for tobacco. Chin. Tob. Sci. **40**(05): 60-67.

Chen XN, Duan RM, Deng SJ, Jin YM, Wu LJ, Zhang Ge, Zhang Y and Zhang HB 2023. Changes in chemical composition and bacterial communities during fermentation of Yunnan cigar tobacco leaves and their correlation. Acta Tabacaria Sin. **29**(04): 124-134.

Chen YJ, Chen JH, Chen YP, Gong JSQ, He X, He CL, Li JY, Ren K, Wang C, Xia ZY, Yue C, Zhao GK and Zou CM 2020. Metabolism of macromolecular substances in flue-cured tobacco with different maturity. Southwest China J. Agr. Sci. **33**(09): 1950-1957.

Chen ZF, Feng YJ, Huang XY, Huang S, Liu Q, Mao DB, Zhou LF and Zhang JL 2022. Isolation and identification of a *Bacillus subtilis* strain and its effects on chemical composition and sensory quality of tobacco leaves. Tob. Sci. Tech. **55**(08): 10-19.

Chen ZG, Sun GW, Sun JG, Xiang HB, Yang Y and Zhao W 2024. Screening and effect evaluation of starch degrading bacteria in tobacco leaf. China Tob. Sci. **45**(01): 70-78.

Cheng Y 2023. The effect of *Saccharomyces cerevisiae* on the microbial community and quality of flue-cured upper tobacco leaves. Gz Univ.

Dai LJ, Liu YK, Li Y, Shen CW, Wang YF and Zhou DB 2021. Smoking quality and differential compounds analysis for tobacco leaves prepared imitating black tea modulation process. J. Hn Agr. Univ. (Nat. Sci.) **47**(06): 642-647.

Duan K, Gao LW, Hu WY, Li ZF, Wang LP, Xia YZ, Zhang P and Zhang ZF 2023. Condition optimization of enzyme production from *Bacillus mojavensis* in tobacco leaf and its effect for improving tobacco quality. China Tob. Sci. **44**(03): 69-76.

Fan XS, Hao H, Ma K, Tian XH, Wang L, Mao DB, Fan XS, Ye JB, Yang F, Yan J, Yang XP and Zhang Z 2019. Improving quality of reconstituted tobacco via fermenting extract of its raw material with *Paenibacillus* sp. Acta Tabacaria Sin. **25**(01): 33-38.

Hao J, Ji Q, Li LQ, Sun ZK, Wu N, Wu H and Zheng C 2022. Research progress on improving aroma of tobacco leaf with bio-enzymes and microorganism technology. Curr. Biotechnol. **12**(06): 817-824.

Hu ZZ, Jiang Y, Long ZD, Liu H, Li JG, Sun JS, Su Z and Xu CP 2019. Study on the effect of different inoculation amounts of *Schwanniomyces occidentalis* on flavor enhancement and quality improvement of redried tobacco leaves. J. Yn. Univ. (Nat. Sci. ed.) **41**(04): 797-804.

Hu ZZ, Jiang Y, Long ZD, Liu H, Li JG, Su Z, Sun JS, Xu CP, Yang LY and Zou KX 2018. Study on improvement of tobacco quality by fermentation technology with aroma producing yeast. Food Mach **34**(11): 200-204.

Liu FL, Shi JJ, Mao DB, Xu CP, Zheng JQ and Zhao N 2016. Study on biotransformation of β-carotene to generate flavor components using *Schwanniomyces occidentalis*. China Food Addit. **4**: 133-138.

Lu YS, Liu ZH, Sun FY, Wang L, Yang XP, Yang C, Yang N and Zeng X 2023. Research progress of tobacco aroma enhancing biotechnology. Yn Chem. Tech. **50**(02): 9-14.

Huang S, Liu DY, Liang S, Mao DB, Wei JH, Yang C, Zhang JL and Zhang ZH 2023. Study on improving tobacco quality mechanism of Shandong province by *Enterobacter* and *Acinetobacter* mixed fermentation. J. Light Ind. **38**(01): 45-53.

Wang L, Xu ZC, Zhu JF and Zhang JH 2015. Influence of spraying different enzyme preparations on chemical composition, free and glycosidic neutral aroma components in upper leaves of flue-cured tobacco. Curr. Biotechnol. **5**(06): 455-460.

Wu X, Zhu P and Li D 2021. Bioaugmentation of *Bacillus amyloliquefaciens-Bacillus* kochii co-cultivation to improve sensory quality of flue-cured tobacco. Arch. Microbiol. **203**(9): 5723-5733.

Zheng JQ, Tian S, Tie JX and Xu CP 2016. Experiments on enhancement of the aroma of tobacco from *Schwanniomyces occidentalis* and dioxygenase. Hb Agric. Sci. **55**(21): 5612-5617.

(Manuscript received on 03 September 2025; revised on 11 October 2025)