


Effect of Temperature and Relative Humidity on Growth of *Aspergillus* and *Penicillium* spp. and Biocontrol Activity of *Pseudomonas protegens* AS15 against Aflatoxigenic *Aspergillus flavus* in Stored Rice Grains

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ABSTRACT

In this study, we evaluated the effect of different temperatures (10, 20, 30, and 40 °C) and relative humidities (RHs; 12, 44, 76, and 98%) on populations of predominant grain fungi (*Aspergillus candidus*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Penicillium fellutanum*, and *Penicillium islandicum*) and the biocontrol activity of *Pseudomonas protegens* AS15 against aflatoxigenic *A. flavus* KCCM 60330 in stored rice. Populations of all the tested fungi in inoculated rice grains were significantly enhanced by both increased temperature and RH. Multiple linear regression analysis revealed that one unit increase of temperature resulted in greater effects than that of RH on fungal populations. When rice grains were treated with *P. protegens* AS15 prior to inoculation with *A. flavus* KCCM 60330, fungal populations and aflatoxin production in the inoculated grains were significantly reduced compared with the grains untreated with strain AS15 regardless of temperature and RH (except 12% RH for fungal population). In addition, bacterial populations in grains were significantly enhanced with increasing temperature and RH, regardless of bacterial treatment. Higher bacterial populations were detected in biocontrol strain-treated grains than in untreated control grains. To our knowledge, this is the first report showing consistent biocontrol activity of *P. protegens* against *A. flavus* population and aflatoxin production in stored rice grains under various environmental conditions of temperature and RH.

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Rice (*Oryza sativa* L.) is a primary grain crop that is cultivated worldwide. In Korea, harvested rice in the form of unhulled grains is often stored for extended periods [1]. During storage, fungal contamination is one of the most important problems related to the decreased quality of the rice grains. Fungi can contaminate rice grains before harvest, during harvesting, or even at the storage facility [2]. However, storage conditions, particularly temperature and relative humidity (RH), may determine the type of fungi that develop in the stored grains [3]. Unlike field fungi, the growth and development of xerophilic fungi, such as *Aspergillus* and *Penicillium* spp., are enhanced during storage because these fungi are relatively tolerant to lower RH [4–7].


Aspergillus and *Penicillium* spp. are common contaminants of stored food products, particularly cereal grains, and can deteriorate grain quality. More importantly, these fungi can produce mycotoxins that are hazardous secondary metabolites. Mycotoxins, including aflatoxins B₁, B₂, G₁, and G₂

are mainly produced by *A. flavus* and *A. parasiticus* [8]. Aflatoxin B₁ is one of the most carcinogenic compounds known, with several adverse effects on human and animal health by direct or indirect consumption of contaminated food or feed [9,10]. Several control methods, including chemical measures, have been used to inhibit mycotoxigenic fungi. Increased awareness of the adverse health effects of agricultural chemicals and the possible emergence of fungicide resistance have prompted researches on safer alternatives for the management of fungal contamination of stored grains [11]. The biological control of deleterious fungi using antagonistic microorganisms is a potentially feasible and environment-friendly alternative to chemical control during grain storage [11]. However, previous studies have inadequately considered the influence of temperature and RH on the biocontrol of microorganisms capable of suppressing fungal contamination or aflatoxin production in stored rice grains.

In our previous studies, *A. candidus* AC317, *A. flavus* AF57, *A. fumigatus* AF8, *Penicillium*

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 Supplemental data for this article can be accessed [here](#).

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fellutanum KU53, and *Penicillium islandicum* KU101 were identified as the predominant fungi in stored rice grains [12–14]. Furthermore, a total of 460 bacterial strains were isolated from rice grains and screened for their potential as biocontrol agents for the fungal contaminants. Among the tested strains, *Pseudomonas protegens* AS15, which produces volatile compounds, exhibited the most effective antifungal activity, with inhibition of *A. flavus* growth in unhulled rice grains and biodegradation of aflatoxins produced by the fungus in a liquid medium [15–17]. In this study, therefore, we evaluated the effect of temperature and RH on the growth of the predominant *Aspergillus* and *Penicillium* spp., and the biocontrol activity of *P. protegens* AS15 against *A. flavus* population and aflatoxin production in stored rice grains.

A. candidus AC317, *A. flavus* AF57, *A. fumigatus* AF8, *P. fellutanum* KU53, and *P. islandicum* KU101 isolated from our previous studies [12–14] were used in this study. The fungal isolates were stored on potato dextrose agar (PDA) at 4 °C until use. To prepare fungal inocula, conidia from cultures grown on PDA at 25 °C for 5 days were harvested in sterile distilled water (SDW) containing 0.03% Tween 20 and then adjusted to 10⁷ conidia/ml using a hemocytometer. *P. protegens* AS15 was used in this study as it exhibited significant biocontrol activity against *A. flavus* and aflatoxin production in our previous study [16]. This strain was stored in nutrient broth (NB) containing 20% glycerol at –70 °C until use. To prepare the bacterial suspension, *P. protegens* AS15 was streaked on nutrient agar (NA) and incubated for 48 h at 28 °C. Single colonies appearing on NA were transferred to NB and incubated at 28 °C in a rotary shaker (160 rpm) for 48 h. Bacterial cells were harvested by centrifugation as described by Mannaa et al. [16]. The harvested cells were suspended in MgSO₄ solution and adjusted to 10⁸ cells/ml (OD₆₀₀ = 0.5) using a spectrophotometer.

Four saturated salt solutions (LiCl, K₂CO₃, NaCl, and K₂SO₄) were prepared and adjusted to four different RH levels (12, 44, 76, and 98%, respectively), as described by Greenspan [18]. One hundred milliliters of each solution were aseptically poured into plastic containers (15 cm in width × 11 cm in length × 5 cm in height; Daiso, Higashihiroshima, Japan) disinfected with 99% ethanol [19] to create an internal atmosphere with the designed RH, in which RH (%) = water activity (*a_w*) × 100 at equilibrium. These containers containing each saturated salt solution were stored at different temperatures (10, 20, 30, and 40 °C). HOBO[®] temperature/RH data-logging units (Onset Computer Corporation, Bourne, MA) and HOBOWare for Windows version 3.7.11 (Onset Computer Corporation) were used to

monitor temperatures and RHs in the plastic containers containing the solutions (Supplementary Table S1). At equilibrium, RH in the atmosphere of the containers was maintained, with slight fluctuations (±1% in the target RH adjusted by each saturated salt solution), by temperature changes [20]. The designed RH conditions showed almost the expected water activity when rice grains were measured using an AquaLab water activity meter (Model 4TE; Decagon Devices, Pullman, WA) (Supplementary Table S2).

Two grams of stored unhulled rice (cv. “Ilpum”) grains obtained from Korea University Farm (Namyangju, Korea) were surface-sterilized with 1% sodium hypochlorite for 3 min and 70% ethanol for 5 min [21]. Grains were then washed three times in SDW and blotted on Whatman No. 1 filter paper. The surface-sterilized grains were inoculated with 200 µl of a conidial suspension (10⁷ conidia/ml) of each tested fungus, which was equivalent to 10⁶ conidia/g dry weight of rice grains. Inoculated grains were placed in a Petri plate (60 mm in diameter) and the plates were put in a plastic container (replicate) containing each of the saturated salt solutions prepared as described above. These containers sealed with Uniwrap[®] (Uniwrap Co., Hwaseong, Korea) were tightly closed using lids. They were then incubated in the dark at 10, 20, 30, and 40 °C. After 2 weeks of incubation, fungal populations in inoculated rice grains were determined, as described previously [16]. Briefly, rice grains from a Petri plate from each container were finely ground with an analytical mill (A11 basic; IKA Works, Wilmington, DE). One gram of ground grains was suspended in 10 ml of SDW and incubated in a rotary shaker (160 rpm) at 28 °C for 30 min. After serial dilution, aliquots (200 µl) of the dilutions were spread on dichloran 18% glycerol agar (DG18; Fluka 40587; Sigma-Aldrich, St. Louis, MO) amended with 50 mg/ml chlortetracycline [22]. Smear plates (three plates per replicate) were incubated in the dark at 28 °C for 4 days and the colony-forming units (CFUs) per g dry weight of rice grains was assessed.

The biocontrol activity of *P. protegens* AS15 against *A. flavus* and aflatoxin production in unhulled rice (cv. “Ilpum”) grains was examined at different temperatures (10, 20, 30, and 40 °C) and RHs (12, 44, 76, and 98%). In this experiment, the aflatoxigenic strain, *A. flavus* KCCM 60330, obtained from the Korean Culture Center of Microorganisms (Seoul, Korea) was used because *A. flavus* AF57 produces a low level of aflatoxins [16]. Briefly, two grams of surface-sterilized unhulled rice (cv. “Ilpum”) grains were immersed in 10 mM MgSO₄ solution (untreated control) or a bacterial

suspension of *P. protegens* AS15 for 3 h. The rice grains, bacterial suspensions, and fungal inocula were prepared as described above. Treated rice grains were inoculated with 10^6 conidia of *A. flavus* KCCM 60330/g dry weight of grains. Inoculated grains were then placed in plastic containers prepared as described above and these containers were incubated at different temperatures. Populations of *A. flavus* KCCM 60330 in treated rice grains were evaluated after 2 weeks of incubation as described above. In addition, total bacteria of the same inoculated grains were assessed on NA supplemented with 50 mg/l cycloheximide. Fungal and bacterial CFUs were assessed on smeared plates (three plates per replicate) after 4 and 2 days of incubation at 28 °C, respectively. In addition, total aflatoxins, including aflatoxin B₁ and B₂, produced by *A. flavus* KCCM 60330 were analyzed using a competitive direct enzyme-linked immunosorbent assay with Veratox[®] HS (Neogen Co., Lansing, MI), as described by Manna et al. [16].

The experiments were established with factorial designs to observe the effect of temperature and RH with four different levels on fungal and bacterial populations and mycotoxin production in rice grains. All experiments were performed twice with three replicates per treatment except for RH measurement (10 replicates) in plastic containers. Statistical analyses were performed using the Statistical Analysis Systems software (SAS Institute, Cary, NC). Data from repeated experiments were combined after confirmation of the homogeneity of variances using Levene's test [23]. The fungal and bacterial population data were analyzed after logarithmic transformation. Multiple linear regression analysis was conducted to detect the effect of both temperature and RH on fungal populations. Analysis of variance was conducted using general linear model procedures and means were separated using Tukey's HSD test at $p < .05$.

When growth on unhulled rice grains inoculated with the tested fungi (*A. candidus* AC317, *A. flavus* AF57, *A. fumigatus* AF8, *P. fellutanum* KU53, and *P. islandicum* KU101) was assessed at different temperatures and RHs, growth of all fungi was significantly ($p < .0001$) enhanced by both increased temperature and RH (Figure 1; Supplementary Figure S1 and Supplementary Table S3). There were significant ($p < .0001$) temperature \times RH interactions in all the tested fungal populations (Supplementary Table S3). In general, populations of *A. flavus* AF57 and *A. fumigatus* AF8 were higher than the other tested fungi, regardless of temperature and RH (Figure 1). Multiple linear regression analysis to observe the effect of both temperature and RH on fungal populations revealed significantly ($p < .0001$)

positive relationships in all the tested fungi (Table 1). The increase in all the fungal populations was higher in response to one unit temperature increase than RH according to the regression coefficients (Table 1).

When rice grains treated with or without the biocontrol strain, *P. protegens* AS15, were inoculated with *A. flavus* KCCM 60330, fungal populations in the inoculated grains were significantly ($p < .0001$) enhanced by increasing temperature from 10 to 40 °C and by increasing RH from 12 to 98% (Figure 2(A); Supplementary Figure S2 and Supplementary Table S4). Treatment with *P. protegens* AS15 significantly ($p < .01$) inhibited fungal populations in rice grains compared with untreated control grains, regardless of the temperature and RH (Figure 2(A); Supplementary Table S4). There were significant ($p < .0001$) temperature \times RH interactions in the fungal populations, regardless of the biocontrol strain treatment (Supplementary Table S4). The biocontrol strain, *P. protegens* AS15, significantly ($p < .01$) suppressed *A. flavus* KCCM 60330 populations in the grains from 10 to 40 °C, compared with control grains not treated with strain AS15, at 44–98% RH (Figure 2(A)). However, at 12% RH, no significant differences were detected in fungal populations between rice grains that were untreated or treated with the biocontrol strain at the tested temperatures, except at 30 °C (Figure 2(A)).

In biocontrol strain-treated and untreated rice grains inoculated with the aflatoxigenic strain, *A. flavus* KCCM 60330, total aflatoxin production was significantly ($p < .0001$) enhanced with increased temperature and RH (Figure 2(B); Supplementary Table S4). Significant ($p < .001$) temperature \times RH interactions in aflatoxin production were detected regardless of biocontrol strain treatment (Supplementary Table S4). In general, biocontrol strain, *P. protegens* AS15, consistently suppressed aflatoxin production by *A. flavus* KCCM 60330 in rice grains from 10 to 40 °C, regardless of RH (Figure 2(B)). However, aflatoxin production by *A. flavus* KCCM 60330 was significantly ($p < .01$) higher in the control grains not treated with strain AS15 than in the treated grains at all temperatures and RHs. In addition, the highest levels of aflatoxin production were detected in grains at 30 °C among the tested temperatures, regardless of biocontrol bacterial treatment (Figure 2(B)).

In rice grains treated with or without *P. protegens* AS15 prior to inoculation with *A. flavus* KCCM 60330, total bacterial populations were significantly ($p < .0001$) enhanced by increased temperature and RH, regardless of whether the grains were treated with the biocontrol strain or not (Supplementary Table S4; Table 2). However, bacterial populations

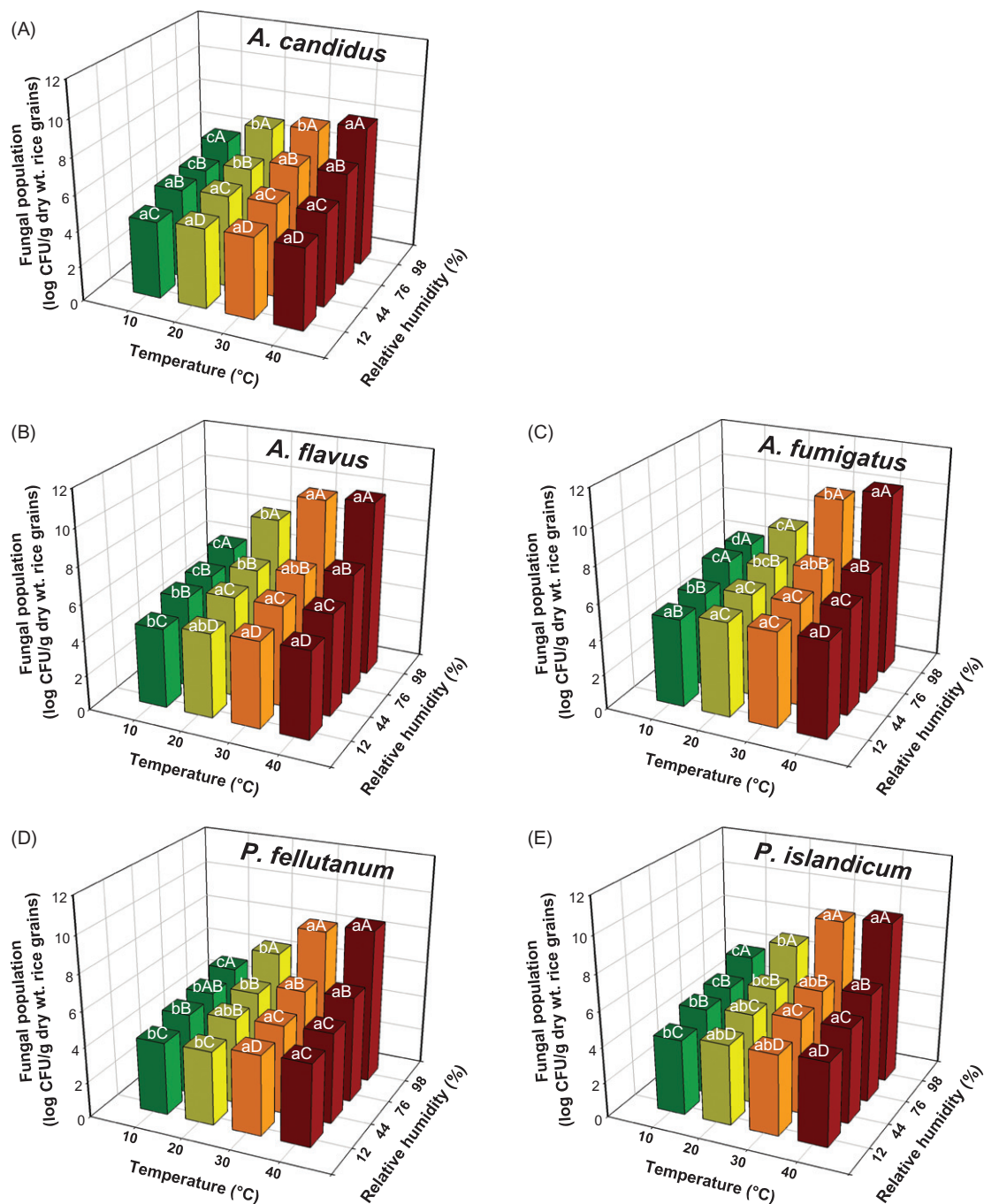


Figure 1. Effect of different temperatures (10, 20, 30, and 40 °C) and relative humidities (RHs; 12, 44, 76, and 98%) at equilibrium on fungal populations 2 weeks after inoculation of rice grains with (A) *Aspergillus candidus* AC317, (B) *Aspergillus flavus* AF57, (C) *Aspergillus fumigatus* AF8, (D) *Penicillium fellutanum* KU53, or (E) *Penicillium islandicum* KU101. Surface-sterilized rice grains were inoculated with 10^6 fungal conidia/g dry weight of rice grains. Fungal populations were evaluated after 4 days of incubation on dichloran 18% glycerol agar amended with 50 mg/ml of chlortetracycline. Different lowercase and uppercase letters on bars ($n = 6$) are significantly different between different temperatures for a given RH and RHs for a given temperature, respectively, according to Tukey's HSD test at $p < .05$. CFU: colony-forming unit.

in grains not treated with the biocontrol strain at 12 and 44% RH could not be assessed, because the natural bacterial populations were below detectable levels (Table 2). Significant ($p < .0001$) temperature \times RH interactions were observed in bacterial populations in rice grains treated with the biocontrol strain, but not in grains that were not treated with the biocontrol strain (Supplementary Table S4). High bacterial populations (5.85–10.06 log CFU/g dry weight of rice grains) were detected

in biocontrol strain-treated grains at 98% RH, regardless of temperature (Table 2). However, bacterial populations in untreated grains remained at low levels (3.30–4.03 log CFU/g dry weight of rice grains) at 76 and 98% RH, regardless of temperature (Table 2).

The growth of storage grain fungi is generally dependent on environmental conditions such as water activity and temperature [6,24]. The xerophilic nature of these fungi allows them to tolerate

Table 1. Multiple linear regression analysis (fungal population^a = intercept + $\beta_1 \times$ temperature + $\beta_2 \times$ relative humidity) relating the effects of both temperature (10, 20, 30, and 40 °C) and relative humidity (RH; 12, 44, 76, and 98%) on the population of *Aspergillus candidus* AC317, *Aspergillus flavus* AF57, *Aspergillus fumigatus* AF8, *Penicillium fellutanum* KU53, and *Penicillium islandicum* KU101.

Fungal isolate	Variable	Regression coefficient	Standard error	t	p	R ²
<i>A. candidus</i> AC317	Intercept	3.317	0.139	23.91	<.0001	0.821
	Temperature	0.032	0.004	7.68	<.0001	
	RH	0.027	0.001	19.47	<.0001	
<i>A. flavus</i> AF57	Intercept	2.769	0.251	11.01	<.0001	0.735
	Temperature	0.053	0.007	7.05	<.0001	
	RH	0.037	0.003	14.70	<.0001	
<i>A. fumigatus</i> AF8	Intercept	3.322	0.252	13.16	<.0001	0.686
	Temperature	0.051	0.008	6.74	<.0001	
	RH	0.033	0.003	12.81	<.0001	
<i>P. fellutanum</i> KU53	Intercept	2.517	0.216	11.63	<.0001	0.716
	Temperature	0.050	0.006	7.77	<.0001	
	RH	0.030	0.002	13.48	<.0001	
<i>P. islandicum</i> KU101	Intercept	2.743	0.232	11.84	<.0001	0.713
	Temperature	0.045	0.007	6.57	<.0001	
	RH	0.033	0.002	13.97	<.0001	

^aFungal population is expressed as log colony-forming unit/g dry weight of rice grains.

relatively low water activity, unlike field fungi, which need higher water activity for spore germination and growth [7]. Although several previous studies have assessed the effect of temperature and water activity on storage fungi, the environmental effect on rice grains has been often examined with a narrow range of temperature and RH [25]. Therefore, in this study, we examined the effect of various temperatures and RHs, ranging from 10 to 40 °C and 12 to 98%, respectively, on populations of predominant storage rice fungi (*A. candidus*, *A. flavus*, *A. fumigatus*, *P. fellutanum*, and *P. islandicum*). We also examined the biocontrol activity of *P. protegens* AS15 against aflatoxigenic *A. flavus* KCCM 60330 in rice grains under these conditions. The growth of all the tested fungi was significantly enhanced in rice grains by increased temperature and RH. *P. protegens* AS15 significantly inhibited fungal population and aflatoxin production in grains inoculated with *A. flavus* KCCM 60330 under the same conditions.

Holmquist et al. [26] reported that water activity had a greater effect than temperature on the growth of *A. flavus* and *A. parasiticus*. Furthermore, they found that fungal growth at low temperatures occurred only at high water activity. In another study, 11 out of 20 fungi, including *A. flavus* and *A. fumigatus*, showed visible growth at 20 and 30 °C and RHs of 60–80% [27]. Similarly, in this study, varying degrees of both temperature and RH significantly affected the populations of all tested fungi. In particular, temperature (i.e., one unit increase) had a greater influence than RH on fungal populations. In addition, significantly positive effects of both temperature and RH on fungal populations were evident in the multiple linear regression analysis. Although low RH is considered a limiting factor for fungal growth in rice grains, the observed populations would be related to conditions within

individual rice grains, which may provide a micro-environment favorable for fungal growth [28].

P. protegens AS15 displayed effective biocontrol activity by reducing fungal growth and aflatoxin production in rice grains inoculated with the aflatoxigenic strain, *A. flavus* KCCM 60330. Strain AS15 significantly suppressed fungal populations in the grains compared with bacterium-untreated control grains regardless of temperature and RH. Interestingly, aflatoxin production in the grains was consistently inhibited to a low level by the biocontrol strain regardless of temperature and RH. These results indicate that the biocontrol strain can suppress aflatoxin production by *A. flavus*, even in unfavorable environmental conditions. Moreover, the consistent inhibitory activity of *P. protegens* AS15 indicates that it can effectively compete with *A. flavus* KCCM 60330 and survive in its original habitat, rice grains. This survival ability is further supported by the observation of bacterial populations in rice grains. In addition, the effective colonization of grains by this biocontrol strain was demonstrated in our previous study [16]. Although the bacterial population was negatively affected by the environmental conditions, *P. protegens* AS15 could exist at high levels in rice grains, regardless of temperature and RH. However, natural bacterial populations in the untreated grains remained undetectable (at 12–44% RH) or at low (at 76–98% RH) levels, regardless of temperature. Recently, Punja et al. [29] observed a similar phenomenon, in which the biocontrol activity of *Bacillus subtilis* persisted at a low temperature. However, even though its population was dramatically affected, *B. subtilis* was still able to minimize postharvest disease development in tomatoes. Previously, Lahlali et al. [30] reported the efficient biocontrol activity of an antagonistic yeast strain against *Penicillium italicum*, the causal agent of blue mold, under controlled

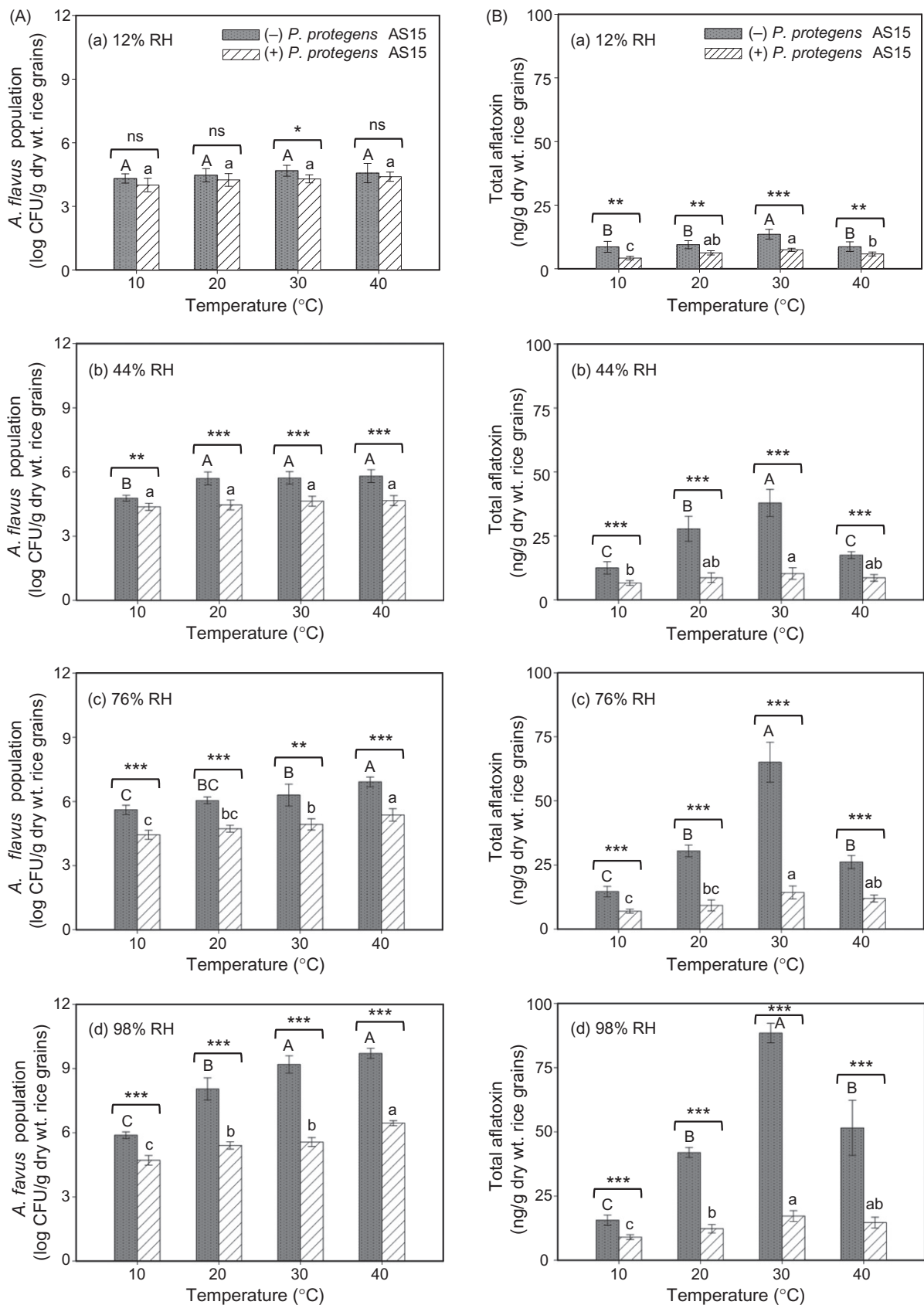


Figure 2. Effect of different temperatures (10, 20, 30, and 40 °C) and relative humidities (RHs; 12, 44, 76, and 98%) at equilibrium on (A) fungal population and (B) aflatoxin production in rice grains treated with (+) or without (-) the biocontrol strain, *Pseudomonas protegens* AS15, followed by inoculation with the aflatoxigenic strain, *Aspergillus flavus* KCCM 60330. Fungal population and total aflatoxin amount were assessed 2 weeks after inoculation with 10^6 conidia/g dry weight of rice grains. Different lowercase and uppercase letters on bars with error bars (standard deviations, $n = 6$) indicate significant differences between different temperatures of rice grains treated with and without *P. protegens* AS15, respectively, according to Tukey's HSD test at $p < .05$. Asterisks (*, **, and ***) on bars at a given temperature indicate significant differences between rice grains treated with or without *P. protegens* AS15 at $p < .05$, .01, and .001, respectively. ns, not significant; CFU, colony-forming unit.

Table 2. Effect of different temperatures (10, 20, 30, and 40 °C) and relative humidities (RHs; 12, 44, 76, and 98%) at equilibrium on total bacterial populations 2 weeks after rice grains treated with (+) or without (–) the biocontrol strain, *Pseudomonas protegens* AS15.

Temperature (°C)	Total bacteria (log CFU/g dry weight of grains) at equilibrium RH (%) ^a							
	12		44		76		98	
	(–) AS15	(+) AS15	(–) AS15	(+) AS15	(–) AS15	(+) AS15	(–) AS15	(+) AS15
10	– ^b	4.07 ± 0.30aD	–	4.69 ± 0.32aC	3.30 ± 0.10 bB	5.40 ± 0.25bB	3.51 ± 0.12cA	5.85 ± 0.14cA
20	–	4.28 ± 0.35aD	–	4.93 ± 0.14aC	3.58 ± 0.10aB	5.66 ± 0.22abB	3.72 ± 0.16bcA	8.80 ± 0.18bA
30	–	4.33 ± 0.32aD	–	4.93 ± 0.33aC	3.59 ± 0.09aB	5.91 ± 0.12aB	3.88 ± 0.10abA	9.97 ± 0.20aA
40	–	4.36 ± 0.36aD	–	5.07 ± 0.13aC	3.69 ± 0.14aB	5.92 ± 0.20aB	4.03 ± 0.09aA	10.06 ± 0.20aA

^aBacterial populations were determined after 2 days of incubation at 28 °C on nutrient agar containing cycloheximide. Means ± standard deviations ($n = 6$) with different lowercase and uppercase letters indicate significant differences between different temperatures for a given RH and RHs [(–) or (+) AS15] for a given temperature, respectively, according to Tukey's HSD test at $p < .05$. In addition, significant ($p < .001$) differences were observed between rice grains treated with or without the biocontrol strain, *P. protegens* AS15, at 76 or 98% RH.

^bNot detected. CFU, colony-forming unit.

environmental conditions, in which the biocontrol activity was not affected by different temperatures and RHs.

In this study, we observed that aflatoxin production in rice grains was highly influenced by temperature and RH. Mislivec et al. [31] previously demonstrated that active fungal growth of mycotoxigenic *Aspergillus* and *Penicillium* spp., which are essential for toxin production, is controlled by ecological factors, such as RH, temperature, and substrate. Rapid growth and subsequent aflatoxin production by *A. parasiticus* has been positively related to high storage RH [24,28]. In the present study, high RH was also positively related with higher levels of aflatoxin accumulation and fungal population in rice grains. However, unlike the fungal population, aflatoxin production was suppressed at 40 °C. Maximum aflatoxin production, among the tested temperatures was observed at 30 °C. Gqaleni et al. [32] reported that the optimum temperature for maximum aflatoxin production by *A. flavus* was 30 °C, with further temperature increases resulting in a gradual suppression of toxin production. Similarly, other studies [33,34] have shown that aflatoxin production is reduced at temperatures >30 °C. Although the optimum temperature for aflatoxin production may vary between *A. flavus* isolates, Kheiralla et al. [35] showed that most aflatoxins are produced by *A. flavus* isolates between 25 and 30 °C. The mycelial dry weight in that study, as an indicator of the fungal growth, was continuously enhanced with increasing temperature, with maximum growth at 35 °C; however, aflatoxin production was not related to fungal growth at temperatures >30 °C, as observed in our present study.

The characterization of biosynthetic and regulatory genes after the complete sequencing of aflatoxin genes has facilitated a better understanding of the biosynthetic pathway and the roles of different genes governing aflatoxin production [36,37]. Expression of aflatoxin biosynthetic genes depends on

temperature. Using microarray analysis, O'Brian et al. [38] reported reduced expression of aflatoxin biosynthetic genes at 37 °C and induced expression at 28 °C coupled with suppression and induction of aflatoxin production, respectively. Subsequently, Schmidt-Heydt et al. [39] showed that reduced expression of *aflS* and *aflJ* (regulatory genes of the aflatoxin production pathway) at temperatures >37 °C was related to reduced aflatoxin production, unlike the observations of O'Brian et al. [38] regarding the expression levels of these two genes at high temperature. Using RNA-sequencing technology, Yu et al. [40] observed the expression of >50% of aflatoxin production-related rRNA at 30 °C, which was associated with higher levels of aflatoxin production. Additionally, the transcriptional regulatory genes, *aflR* and *aflS*, were expressed at higher levels at 30 °C than at 37 °C. These findings may explain the observed significant reduction in aflatoxin production by *A. flavus* in rice grains at 40 °C in our present study.

Taken together, our results suggest that different temperatures and RHs affect the growth of *Aspergillus* and *Penicillium* spp. predominant in rice grains. Although these environmental factors similarly affected *A. flavus* KCCM 60330 growth and aflatoxin production, *P. protegens* AS15 consistently inhibited fungal population and aflatoxin production in stored rice grains under the same conditions. To our knowledge, this is the first report showing the consistent biocontrol activity of *P. protegens* against *A. flavus* population and aflatoxin production in stored rice grains under various environmental conditions of temperature and RH.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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