

# The control effect of a multifunctional bacterial agent fit for straw amendment against wheat soil-borne diseases

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**Abstract** Soil-borne diseases of wheat are getting more and more serious in the wheat/maize rotation growing system in northern China. A multifunctional microorganic strain called B1514 was found to have an inhibitory effect against major pathogens of winter wheat soil-borne diseases, have the ability to decompose maize straw, and have the ability to utilize the straw for multiplication. The strain was processed into bacterial agent HAD-1. Field experiments were conducted from 2008 to 2010 to test the control effect of HAD-1 on the major wheat soil-borne diseases, on decomposing ability to maize straw, and on reproductive capacity. Results showed that HAD-1 had significant control effects on sharp eyespot, take-all, and root rot on wheat. The control efficacy at wheat jointing stage was 59.63% to 72.59%, 57.64% to 59.29%, and 54.48% to 63.25%, respectively. The yield loss decreased by 8.67% to 11.70%. The population numbers of the strain B1514 increased  $2.68 \times 10^7$ – $4.83 \times 10^7$  times during the wheat growing season. HAD-1 significantly accelerated the decomposition rate of maize straw in the soil. The decomposition rate increased by 18.7% to 24.3% during wheat growing season.

**Keywords** Straw amendment, bacterial agent, winter wheat, soil-borne disease, bio-control

## Introduction

During the past 10 years, in the rotation cropping region of winter wheat and summer maize in North China, the new farming modes of no-tillage and straw returning have been widely used to increase soil organic matter, shorten the time spent on farming, and improve the efficiency of machine operations (Jiang et al., 2001; Lao et al., 2002; Liu et al., 2005; Tian et al., 2010). However, these new farming modes weaken the farming barrier between the two periods of wheat and corn in the field ecosystem; no-tillage protects the habitat from soil-borne pathogens, and straw returning takes the diseased remains back to the field, which in turn causes the accumulation of pathogenic sources then becoming conducive to disease occurrence and damage (Cao and Zhu, 2006; Jin et al., 2007). For example, the secondary disease sheath blight that sporadically occurs in the winter wheat region of Hebei, became more and more severe after implementation of

the no-tillage and straw returning farming systems. The area of sheath blight occurrence rose to 102.08 hm<sup>2</sup> in 2000 (accounting for 41% of sown area) and then became the second largest disease damaging wheat in Hebei. The root rot and take-all on wheat were also found to tend to be significantly aggravated (Zhang et al., 2005; Fang et al., 2007; Zhang, 2007; Cheng, 2010). To alleviate the adverse influence caused by straw returning, the multifunction strain B1514 was screened and obtained at the “Biological Control of Crop Pests and Diseases Engineering Technology Center”, a key laboratory in Hebei Province. The multifunction strain B1514 can make use of nutrients from decomposed straw to rapidly propagate, and it has strong inhibitory effects on the main soil-borne pathogens of wheat and strong ability to decompose wheat and corn stalks. B1514 was used as a functional strain to develop a type of multifunctional agent, HAD-1. HAD-1 has the function of both controlling soil-borne diseases and decomposing straw. In this study, the control effect of HAD-1 on wheat soil-borne disease, the ability of HAD-1 to decompose maize straw, and the reproductive capacity of B1514 were tested by plot trials, which may provide a reference and lay a basis for the biological control of soil-borne diseases of wheat.

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## Materials and methods

### Trail plot

The plot trail of HAD-1 was conducted in the experiment station located in the west campus of the Agricultural University of Hebei from October, 2008 to June, 2009 and from October, 2009 to June, 2010. The previous crop was maize and the topsoil was medium loam. Each plot area was 7.2 m<sup>2</sup>, with inter-districts of 1.5 m depth, made of concrete slabs separated closely.

### Pathogens

*Rhizoctonia cerealis* Vander Hoeven, *Gaeumannomyces graminis* (Sacc.) Arx et Oliver and *Bipolaris sorokiniana* (Sacc.) Shoem were isolated, conserved, and provided by the Research Center for Biocontrol Techniques against Pests on Crops of Hebei Province. The pathogen was inoculated on the grain medium, sterilized intermittently 3 times for 1 h at 121°C, and then was cultured in 25°C for 30 d and air-dried as the inoculum (Zhao and He, 2002).

### Multifunctional agent HAD-1

The functional strain B1514 was *Bacillus subtilis*. The save number was CGMCC No. 2752. The culture materials were corn stalk flour: wheat bran: corn meal = 4:3:2 (mass ratio); fermentation conditions were 28°C, with 20% water content, an initial pH8, and a culture time of 36 h (Zhang, 2007). When per gram (dry weight) culture material contained B1514  $\geq 2 \times 10^8$  CFU, the material was naturally air-dried into HAD-1.

### Determination of the control effect of HAD-1 to wheat soil-borne diseases

After corn was harvested and placed outside the district, 2.4 kg of pathogen inoculums were spread evenly in each plot through tillage. Three pathogens were inoculated from 2008 to 2009, combined at a mass ratio of 1:1:1, and inoculated from 2009 to 2010. The crushed corn stalks (9000 kg/hm<sup>2</sup>) and HAD-1 were mixed evenly and spread on the soil surface after tillage, buried in topsoil after irrigation for 7 d, and then the soil and plant wheat were prepared. The plot with inoculated pathogen but without use of the HAD-1 was designated as CK1 and that without any treatment as CK2; 4 replicates were set for each treatment, and the plots were set in random order. At each growth stage of wheat, samples were collected at 5 points, disease severity was investigated (rated by 6 levels) (Wu, 1993), and the disease index, control effect, the processing yield components, and yield and yield impairment rates were calculated.

Disease index =  $\Sigma$  (number of diseased plants  $\times$  disease level of each disease level)/(number of total plants  $\times$  the highest disease level)  $\times$  100

Control effect (%) = [(disease index of CK-disease index of treatment)/(disease index of CK)]  $\times$  100.

Production impairment rate (%) = (HAD-1 treatment yield-CK1 yield)/CK2 yield  $\times$  100.

### Determination of decomposition ability of HAD-1 to corn stalks and strain B1514 multiplication ability in field

Crushed corn stalks were dried at 80°C for 48 h. 100 g of dried corn stalks were uniformly mixed with topsoil, put into the network equipment bag, and buried in the topsoil before the sowing of wheat, with 4 bags buried per plot. 1 bag was taken out per plot at different periods. The weight of straw was calculated by the drying method to test the decomposition rate of straw. Sampling was done at 5 sites in each growth period. A 5–20 cm layer of strain B1514 was isolated with the selective medium and by the dilution plate method, proliferation rate was calculated as:

Proliferation ratio (times) = (the number of colonies at the end of a reproductive stage)/(number of colonies at the beginning stages).

### Statistical analysis

All data were analyzed by SPSS 13.0 for Windows; the significant difference between the treatments was determined by LSD analysis of multiple tests in one-way ANOVA.

## Results and analysis

### The control effects of HAD-1 to wheat soil-borne diseases under the conditions of straw turnover

In the plot experiment, the severity of three main soil-borne diseases with HAD-1 treatment at different periods was significantly lower than the control, with only the pathogen inoculated in the two growing seasons from 2008 to 2010. It indicated that the HAD-1 had significant control effects on the three diseases (shown in Table 1). During the period from 2008 to 2009, HAD-1 gave the highest control effect of 78.04% to sharp eyespot (*Rhizoctonia cerealis*) before overwintering, and gave the highest control effect of 72.59% and 79.99%, respectively, to take-all (*Gaeumannomyces graminis*) at the jointing stage and filling period. From 2009 to 2010, HAD-1 exhibited a similar control effect to the three wheat soil-borne diseases after mixed inoculation. The results from two growing seasons showed that HAD-1 lowered the hazard levels of the soil-borne diseases and the numbers of wheat spike and grain was significantly higher than the control plot. The grain weight in HAD-1 treatment was also significantly increased, except for the treatment inoculated with *Rhizoctonia cerealis* from 2008 to 2009. The yield loss in the HAD-1 treatment plot decreased by 11.70%, 9.40% and 10.35%, respectively, after inoculation with *Rhizoctonia cerealis*, *Gaeumannomyces graminis*, and

**Table 1** Control effect of the bacterial agent HAD-1 to three wheat soil-borne diseases in the field

Year	Stages	Treatments	Take-all		Sharp eyespot		Root rot	
			Disease index	Control efficacy (%)	Disease index	Control efficacy (%)	Disease index	Control efficacy (%)
2008–2009	Pre-overwintering	HAD-1	3.76a	51.30	3.47a	78.04	5.62a	64.63
		Control	7.72b	—	15.80b	—	15.89b	—
	Jointing	HAD-1	3.90a	72.59	12.03a	57.64	10.36a	63.25
		Control	14.23b	—	28.40b	—	28.19b	—
	Filling	HAD-1	5.24a	79.99	8.63a	68.03	8.20a	70.51
		Control	26.19b	—	26.99b	—	27.81b	—
2009–2010	Pre-overwintering	HAD-1	07.4a	48.47	5.34a	69.66	7.12a	53.49
		Control	14.36b	—	17.60b	—	15.31b	—
	Jointing	HAD-1	6.62a	59.63	7.78a	59.29	7.32a	54.48
		Control	16.4b	—	19.11b	—	16.08b	—
	Filling	HAD-1	6.18a	64.79	10.80a	61.04	10.16a	59.42
		Control	17.55b	—	27.72b	—	25.04b	—

Each datum in the table is an average of four replicates. Data followed by the different letters indicate the significant difference between HAD-1 treatment and CK at  $P=0.05$ .

*Bipolaris sorokiniana* from 2008 to 2009, and the loss was reduced by 8.67% from 2009 to 2010 (shown in Table 2).

### The proliferation of multifunctional strain B1514 in HAD-1 and its decomposition ability to maize straw

HAD-1 and crushed corn stalks were mixed and placed on the surface of the soil for 7 d before plowing. The functional strain B1514 was put into the soil in the form of a combination of ‘agents-straw’, which enabled it to colonize successfully and proliferate in the soil. The results in the two planting seasons from 2008 to 2010 showed that the proliferation rate of B1514 fluctuated as the wheat growth stages varied, reached the highest level during the jointing to maturity stage, and lowered to its minimum in the overwintering period. The difference was mainly due to the difference in soil temperature and humidity in different growth stages. The proliferation rate of B1514 in the whole growth period of wheat was  $2.68 \times 10^7$ – $4.83 \times 10^7$  times from 2008 to 2009 and  $4.37 \times 10^7$  times from 2009 to 2010 (shown in Table 3).

The strain B1514 made use of the nutrients from decomposition of straw, and significantly increased the decomposition rate of maize straw in soil. The decomposition rate to corn stalk with HAD-1 treatment was significantly ( $P=0.05$ ) different from that of the control at different growth stages. The straw decomposition rate with HAD-1 treatment in the whole growth period of wheat increased by 18.94%, 20.89% and 16.29%, compared with that of the control from 2008 to 2009, and increased by 18.70% from 2009 to 2010. Corn straw decomposition rate in all HAD-1 treatments in different growth stages was more than 10%, and was maximum in the jointing to maturity period at 11.99%–16.04% and 14.27%, respectively, in those two years (shown in Table 4).

## Discussion

Over the past years, soil-borne disease control in the field has been a major problem in the production of crops. Biological control is the focus in our current research on soil-borne

**Table 2** Effect of application of bacterial agent HAD-1 on wheat yield

Year	Pathogen	Treatment	Spike No. (million/hm <sup>2</sup> )	Grain No. (number/spike)	1000-grain weight (g)	Grain yield (kg/hm <sup>2</sup> )	Yield loss rate (%)
2008–2009	<i>G. graminis</i>	HAD-1	652.9a	35.8a	41.6a	8265.4a	11.70
		CK1	630.3b	35.0b	38.4b	7211.8b	—
	<i>R. cerealis</i>	HAD-1	708.1a	34.8a	41.4a	8669.0a	9.40
		CK1	612.0b	33.6b	44.8a	7821.5b	—
	<i>B. sorokiniana</i>	HAD-1	643.7a	35.5a	43.3a	8406.4a	10.35
		Control	638.5a	34.8b	39.5b	7474.1b	—
2009–2010	Mix pathogen	untreated	720.8	36.6	40.1	9008.5	—
		HAD-1	692.3a	39.0a	38.8a	8236.0a	8.67
	CK2	untreated	670.2	38.3	39.1	8426.5	—
		HAD-1	605.4b	38.1b	37.9b	7804.7b	—

Each datum in the table is the average of four replicates. Data followed by the different letters indicate significant difference between HAD-1 treatment and CK at  $P=0.05$ .

**Table 3** Proliferation rates of multifunctional strain B1514 at different stages of wheat

Year	Pathogen	Proliferation rate of B1514 (times)				
		Sowing to pre-winter	Overwintering stage	Greenup to jointing stage	Jointing to maturity stage	Growing season
2008–2009	<i>R. cerealis</i>	102.38	27.46	112.20	122.36	$3.86 \times 10^7$
	<i>B. graminis</i>	98.96	19.82	103.34	132.46	$2.68 \times 10^7$
	<i>G. graminis</i>	104.88	32.49	110.02	128.94	$4.83 \times 10^7$
2009–2010	Mixed pathogens	101.10	33.45	107.49	120.31	$4.37 \times 10^7$

**Table 4** Degradation rate of the multifunctional bacterial agent HAD-1 to maize straw

Year	Pathogen	Treatment	Degradation rate (%)				
			Sowing to pre-winter	Over wintering stage	Green up to jointing stage	Jointing to maturity stage	Growing season
2008–2009	<i>R. cerealis</i>	HAD-1	11.97a	3.88a	11.76a	14.77a	42.38a
		CK1	6.72b	2.32b	5.93b	6.52b	21.49b
	<i>B. sorokiniana</i>	HAD-1	12.72a	3.87a	10.87a	16.06a	43.52a
		CK1	5.95b	2.61b	6.47b	9.55b	24.58b
	<i>G. graminis</i>	HAD-1	12.56a	3.02a	12.36a	11.99a	39.93a
		CK1	6.83b	2.05b	7.43b	7.33b	23.64b
2009–2010	Mixed pathogens	HAD-1	12.42a	3.59a	11.66a	14.27a	41.94a
		CK1	6.50b	2.33b	6.61b	7.80b	23.24b

Each datum in the table is an average of four replicates. Data followed by the different letters indicate significant difference between HAD-1 treatment and CK at  $P = 0.05$ .

diseases because biocontrol agents have such advantages such as strong selectivity and not causing fungal resistance to agrichemicals (Fravel, 1988; Levy et al., 1992; Ge et al., 2004; Wang and Raaijmakers, 2004; Ji et al., 2006). Nowadays, biocontrol bacterial strains such as *Bacillus* spp., *Pseudomonas* spp., and agrobacterium radiobacter, etc are widely applied in production (Shen and Zhang, 2000; Cheng et al., 2003; Du et al., 2004; Zhou and Fan, 2005). Some progress has been made in studies on plant disease control by *Bacillus*, such as the application of *Bacillus* to control soil-borne diseases like rice sheath blight, tomato bacterial wilt, and the leaf spot disease wheat scab (Luo et al., 2002; Zheng et al., 2003; Shen et al., 2004; Zhang et al., 2005). However, the microbial control of crop soil-borne diseases also faces many problems. For example, after functional strains enter the soil in the form of living organisms, they form a dominant population with difficulty due to a lack of the matrix conducive to rapid colonization and proliferation. As a result, it is difficult for functional strains to give good biocontrol effect. It is difficult to achieve a combination of the antagonistic role of functional strains with the proliferation promotion role of functional strains (Shen et al., 2004; Zhang et al., 2005).

With production development in recent years, the amount of corn stalks has been increasing rapidly. The main components in stalks are cellulose, hemicellulose, and lignin; cellulose and lignin tend to form “wooden cellulose” which is not easily directly utilized as a carbon source by the majority of microorganisms (Xin, 2005), leading to slow degradation, topsoil voiding that seriously affects wheat sowing, emergence, and growth (Wang et al., 2009; Jia et al., 2010).

Therefore, the manual screening of some beneficial microorganisms with a certain antagonistic ability to wheat soil-borne diseases, and strong decomposition and adaptability to the soil environment with corn straw returning, may accelerate the decomposition of maize straw, improve soil character while not affecting crop growth, and to a certain extent, control soil-borne diseases.

The *Bacillus subtilis* strain B1514 used in this study, can not only speed up straw decomposition but also has strong antagonistic effects against soil-borne pathogens. The strain was prepared into a new type of multifunctional agent HAD-1. The results showed that the functional strain B1514 in agent HAD-1 can colonize and proliferate rapidly by utilizing corn stalks in the soil as matrix, and can accelerate straw decomposition, rapidly proliferate and form a dominant population by use of the nutrients produced by decomposition of corn stalks, inhibit the growth of soil-borne pathogens, and effectively control wheat soil-borne diseases. However, the mixed inoculation used in this study led to the phenomenon of a complex infection of several pathogens, which made it difficult to distinguish diseases. For determination of the proliferation, the use of the dilution plate count method may lead to some errors when detecting the amount of functional strain B1514. In the future, detection methods should be further improved.

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