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Isolation and Selection of Indigenous Bacterial Strains with Suppression Properties from the Rhizospheres of Potato and Wheat

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Authors' contributions

This work was carried out in collaboration between authors. Author SMA designed the study, analyzed the data, and wrote the protocol. Authors SMA, NH and NS performed the experiments, and wrote the manuscript. Authors MMZ and AG read and approved the final version for publication.

Research Article

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ABSTRACT

Soil-borne fungal and bacterial root pathogens can cause serious losses to agricultural crops. The enhancement of disease suppressive properties of soils will limit disease development, thus, being of great importance for sustainable agriculture as well as organic farming systems. The aim of the present study is to isolate, identify and to select indigenous bacterial strains with antifungal activity from the potato and wheat rhizospheres. 111 bacterial strains were obtained in the preliminary screening, from the antagonism test plates, 50 from potato and 61 from wheat. About 55% were $Gram^+$ and about 46% were $Gram^-$. Fourteen bacterial strains from potato revealed an antagonistic activity *in vitro* against the phytopathogenic fungi, *Phytophthora infestans*, *Fusarium oxysporum* f. sp. *albedinis* and *Fusarium solani* var. *coeruleum* with a percentage of inhibition varying from 0 to 92.30%. Twenty four bacterial strains from wheat had an antagonistic activity *in vitro* against the studied fungi with a range from 0 to 87%. This shows a promising beginning for detecting suppressive soils in Sétif aria.

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1. INTRODUCTION

Healthy soil is not only a fertile soil that provides a good physicochemical environment to plants but also provides a good biotic environment that allows plants to resist against pathogens. This soil reflects the appropriate balance between the functions of the microbial community which result in suppression of plant diseases. Susceptibility to the disease is highly specific to the pathogen and is probably dependent in: differentiation of resources needed for the pathogen, sensitivity of the pathogen to the reactions of plant resistance, and all of these factors at once [1]. Diseases caused by plant pathogens are causing the imbalance in the microbial community which provides the later development of deleterious microorganism populations; this disturbance is usually caused by human. Plant diseases are rare in natural vegetation probably because of: vegetal diversity, genetic heterogeneity of host plants populations, host resistance, and interactions with various antagonistic microorganisms. So in suppressive soil (healthy soil) the incidence remains low despite the presence of the pathogen and the environmental conditions, that are favorable for the disease expression on susceptible host plant [2], such as: culture techniques (monoculture), the application of a very high frequency of plantations, pesticides, and fertilizers.

Suppressive soils are already known for various pathogens as *Fusarium oxysporum*, *Gaeumannomyces graminis* var. *tritici*, *Pythium sp.*, *Rhizoctonia solani*, *Streptomyces scabies* [2, 3]. In these soils, pathogens are limited in their ability to establish or to produce disease symptoms. Soil suppressiveness can be due to the soil physico-chemical characteristics such as texture, pH, and Ca contents [4]. Soil biota (antagonistic microorganisms) can play a key role in soil suppressiveness too, by controlling the pathogen through competition, antibiosis, parasitism or enhancement of plant resistance. So far, most studies have dealt with the fuorescent *Pseudomonas* spp., because many *Pseudomonas* strains are involved in biological control of various soil-borne plant pathogens [3, 5]; as well as many *Bacillus* strains express activities that suppress necrotizing pathogens/parasites or otherwise promote plant growth [6]. Antibiotic-producing *Pseudomonas* isolates have also been used as wheat inoculant for biological control of the pathogen *Gaeumannomyces graminis* var. *tritici* [7,8,9].

The aim of this study is the isolation and selection of indigenous bacterial strains with antifungal activity from the potato and wheat rhizospheres, in the east of Algeria.

2. MATERIALS AND METHODS

2.1 Fungal Strains

Five phytopathogenic fungi were used in the study, *Fusarium oxysporum* f. sp. *albedinis* (INRA Algiers), *Fusarium solani* (LMA, Sétif Algeria), *Fusarium oxysporum* f. sp. *Lycopersici* (LMA, Setif Algeria), *Pythophthora infestans* (LMA, Sétif Algeria) and *Fusarium solani* var. *coeruleum* (Institut Pasteur, Paris France), and a referenced bacterium *Pseudomonas aureofaciens* 30-84 which was a gift of Pr HAAS (Lausanne, Switzerland).

2.2 Sampling, Isolation and Selection of Bacteria

Soil rhizosphere samples were obtained from potato and wheat crops fields located in Sétif, Algeria, known for their high outputs of wheat production (the samples were taken according to a random sampling). Soil samples were taken at 15-20 cm depth in the upper profile; the sampling was done by separating the different soil strata: rhizosphere soil, spermosphere and clinging soil to potato tubers or wheat roots. The collected samples were placed in sterile plastic bags until use; the soil was first dried at 28ºC for 24h, then sieved (1.5 mm mesh) to remove small stones and plant roots. First, the soil pH was determined according to [10]. The screening of bacteria with antifungal activity was performed as described by [11], with some modifications. One gram of rhizospheric soil sample was suspended in 5 ml of 0.9% (w/v) NaCl, and then subjected to agitation in a rotary shaker for 15mn. After decantation, a loopful of the supernatant fraction from a serial dilution (10^{-4}) was streaked on one edge of potato dextrose agar or King B agar plates, on the other edge a plug(6mm) taken from 5 days culture of pathogenic fungi mycelium growing on PDA plates at room temperature was placed. Plates were incubated at 27ºC for a week. Then, bacteria were purified from plates where hyphal growth inhibition was observed. The selected strains were then examined for Gram reaction, spore formation, and production of fluorescent pigments on King's B agar plates.

2.3 Antagonism *in vitro* against Pathogenic Fungi

The purified bacterial isolates were tested against *Fusarium solani*, *Fusarium solani* var. *coeruleum*, *Fusarium oxysporum* f. sp. *albedinis*, and *Phytophtora infestans*. The hyphal inhibition was measured by comparing the growth diameter in treatment with control, as previously described by [11].

3. RESULTS AND DISCUSSION

The screening strategy carried out in this work consisted of the isolation of culturable bacteria strains capable of antagonistic activity against the studied phytopathogenic fungi (Deuteromycetes and Oomycetes).

A primary selection was made from the antagonism plates where the confluent growth of bacteria from the studied rhizospheres inhibited the development of fungal mycelia (Fig. 1). This resulted in a group of bacteria able to survive in the presence of other microorganisms and display and suppress telluric fungal growth [11,12].

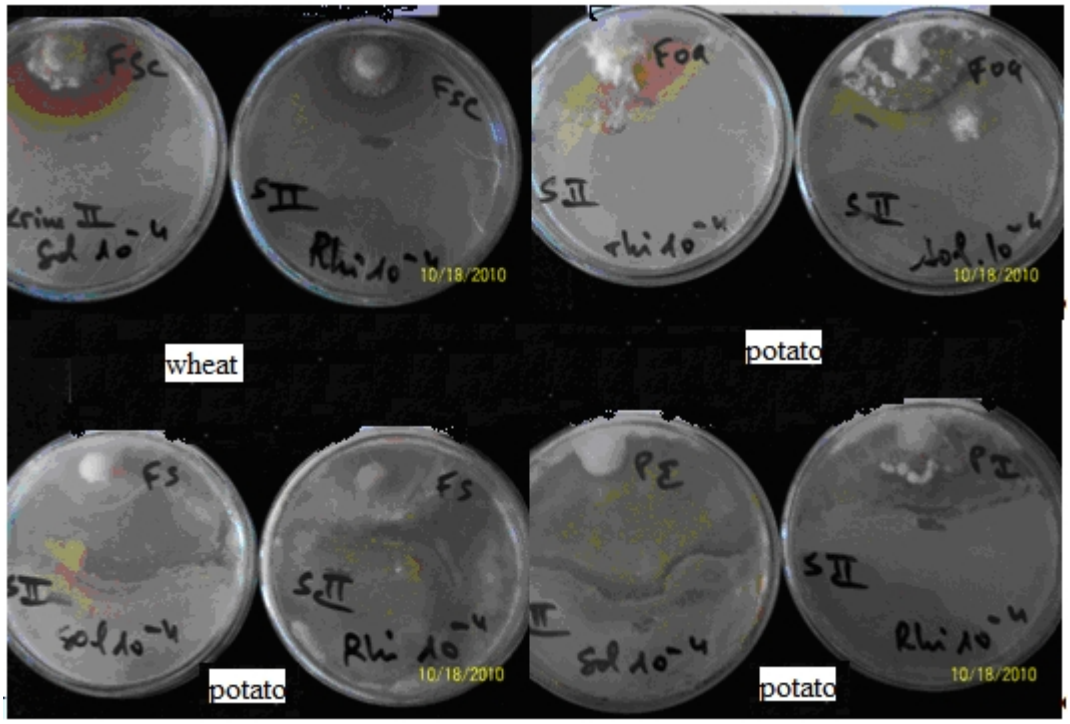

Fig. 1. Primary selection of antagonistic bacteria

$Rhi10^{-4}$ and $Sol10^{-4}$: supernatants dilutions respectively obtained from rhizosphere and spermosphere of potato and wheat; Fsc: Fusarium solani var. coeruleum, Foa : Fusarium oxysporum f. sp. albedinis, Fs: Fusarium sp., PI : Phytophthora infestans, SII, SekrineII and II: crop fields.

111 bacterial strains which were obtained in the preliminary screening, from the antagonism test plates are as follows, 50 were from potato and 61 from wheat. The isolates from potato are composed by, 50% $Gram^{+ve}$ among them 91% were spore-formers, 46% $Gram^{-ve}$ and 4% failed to grow (Table 1, Fig 2); while those of wheat are 55.73 % $Gram^{+ve}$ (43.9% spore-formers) and 45.27 $Gram^{-ve}$ (Table 2, Fig. 3). Pure bacterial cultures isolated from those plates were tested for fungal antagonism.

Table 1. Characteristics of potato isolates

Z	pH	Strains	G	Inhibition rates %			Cell shape
				Mitosporic fungi			
				Fsc	Foa	Pi	
1		2^a	+	37.5	53.48	Nd	Bacilli
	7.50- 7.83	5^a	–	34.7	76.74	Nd	Bacilli
		9^a	–	6.94	6.97	Nd	Bacilli
		8^b	+	0	53.84	Nd	Bacilli
		11^b	+	6.25	37.5	0	Bacilli
2		16^b	–	41.25	42.5	0	Ovoid bacilli
	<7	17^b	–	85	1.25	30	Ovoid bacilli
		18^b	–	46.2	Nd	34.21	Ovoid bacilli
		20^b	–	82.5	92.30	63.15	Ovoid bacilli
		22^b	–	52.5	43.75	0	Bacilli
		24^b	–	32.5	32.5	2.5	Small bacilli
		2^c	+	Nd	Nd	35.75	Big bacilli
	8.26- 8.60	6^c	–	Nd	Nd	86.25	Small bacilli
		9^c	–	Nd	Nd	7.5	Bacilli

Z: zone (1: spermosphere, 2: rhizosphere); pH: soil pH; G: Gram; Fsc: Fusarium solani var. coeruleum, Foa : Fusarium oxysporum f. sp. albedinis, PI : Phytophthora infestans; Nd: not determined; 2^a, 8^b, 16^b, 22^b, 24^b…..9^c: bacterial affiliated numbers, a:from the first field, b: from the second field and c: from the third field.

We focused on bacterial genera that are often found in large populations in soils with general disease suppression [11,12], such as positive spore-forming Gram-positive species belonging to the *Bacillus* genus and Gram-negative ones belonging to *Pseudomonas* [11]. Our results are consistent with the early raised hypothesis that this group of microorganisms is responsible for this kind of phenomenon in the soil, and supported by the several reports [11,13,14].

During preliminary screening of rhizospheric bacterial isolates, only 38, 28% and 39 % from potato and wheat were found to inhibit the growth of pathogens *in vitro*. Among the selected strains as "antagonists", 28.57% were $Gram^{+ve}$ from Potato 54.16% $Gram^{+ve}$ from Wheat, and 71.42% and 45.83% $Gram^{-ve}$ from Potato and Wheat respectively (Table 1,Fig. 2 and Table 2; Fig. 3).

These isolates were selected as bacterial antagonists, they were found to inhibit growth of the pathogen significantly with mean inhibition diameter of 10.2 to 56 mm. This procedure resulted in inhibition more than 50% with respect to the fungi alone. Looking for bacteria with a wide range of antifungal action, the fourteen bacterial strains from potato revealed an antagonistic activity *in vitro* against the phytopathogenic fungi, *Phytophthora infestans*, *Fusarium oxysporum* f. sp*. albedinis* and *Fusarium solani* var. *coeruleum*. The percentage of inhibition varied between 0 and 92.30% depending on isolates and the considered pathogen. Accordingly, three isolates showed an interesting activity against the phytopathogenic fungi tested (Table 1; Fig. 2). The 38 bacterial antagonists that were studied *in vitro* for their antagonistic activity against the pathogen have shown different degrees of antagonism. Soil suppressiveness to fusarium wilt or flax was conditioned not only by the density but also by the composition of the population of microorganisms. Similar results were reported by [15], especially for non-pathogenic *F. oxysporum*, moreover their activity was not influenced by the physical or chemical characteristics of the tested soil samples.

Table 2. Characteristics of wheat isolates

Z	pH	Strains	G	Inhibition rates %			Cell shape
				Mitosporic fungi		Oomycete	
				Fsc	Foa	Pi	
1	7.65	1(1)	+	46.25	41.25	Nd	Ovoid bacilli
	7.90	4(1)	+	62.5	41.25	Nd	Bacilli
		2(2)	–	Nd	35	Nd	Bacilli
	6.46	1(3)	--	62.5	41.25	0	Bacilli
	7.66	2(4)	+	50	Nd	Nd	Ovoid bacilli
		3(4)	+	76.2	21.25	36.25	bacilli
		4(4)	–	57.5	15	32.5	Ovoid bacilli
		5(4)	+	76.2	16.25	25	bacilli
		6(4)	–	87.5	Nd	11.25	Ovoid bacilli
	7.98	1	+	0	0	85	bacilli
		3	-	0	0	57	Ovoid bacilli
		4	-	0	78	Nd	Ovoid bacilli
		6	+	0	0	46.25	bacilli
	7.65	7(1)	–	50	37.51	Nd	Bacilli
		8(1)	+	0	32.5	0	Bacilli
	7.90	7(2)	+	8.75	41.25	17.5	Bacilli
		8(2)	–	43.7	Nd	Nd	Bacilli
		10(4)	–	13.75	Nd	Nd	Ovoid bacilli
2	7.66	11(4)	+	81.2	30	38.75	Bacilli
		12(4)	+	43.7	Nd	26.25	Ovoid bacilli
		13(4)	+	57.5	11.25	25	Ovoid bacilli
		14(4)	--	83.7	23.75	25	Ovoid Bacilli
	7.92	17	-	0	0	54.25	Ovoid bacilli
		20	-	0	0	57.5	Bacilli

Z: zone (1: spermosphere, 2: rhizosphere); pH: soil pH; Fsc: Fusarium solani var. coeruleum, Foa : Fusarium oxysporum f. sp. albedinis, PI : Phytophthora infestans; Nd: not determined; 10(4), 8(1), 7(2), 20: bacterial affiliated numbers, (1) (2) (3) and (4):first, second, third and the forth field.

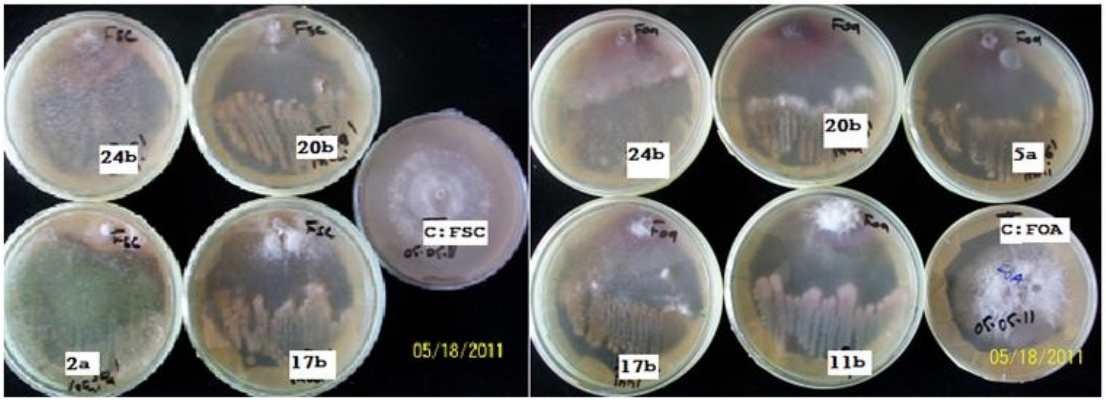

Fig. 2. Antagonistic activity by dual culture of Potato rhizospheric bacteria against phytopathogenic fungi

C: control, FSC: Fusarium solani var. coeruleum, FOA: Fusarium oxysporum f. sp. albedinis, 24b, 20b, 2a ……..: bacterial isolates, a:from the first field, b: from the second field and c: from the third field.

While the 24 isolates from wheat, the percentage of inhibition varied between 0 and 87.5%. The isolates noted 11(4) and 14(4) have shown the highest degree of inhibition *in vitro* (Fig. 3). The isolate noted [11(4)] was identified as *Bacillus* sp. while the isolate noted [14(4)] was identified as *Pseudomonas* sp. (which produces siderophores on KB medium, data not shown). *Pseudomonas aureofaciens* 30-84 (positive control) has inhibited *Phytophthora infestans* with 55.3 %, *Fusarium oxysporum* f. sp. *albedinis* with 41.17 % and *Fusarium solani* var. *Coeruleum* with 59.17% (Fig. 4). 30% of the fluorescent *Pseudomonads* isolated inhibited mycelial growth of *Phytophthora infestans in vitro*. Similar results have already reported about bacteria belonging to the same genera, against *Pythium ultimum*; another soil borne Oomycete. While bacteria belonging to the genera *Bacillus* inhibited *Phytophthora infestans* as well as *Fusarium oxysporum* f. sp. *albedinis*, *Fusarium solani* var. *coeruleum* but with less activity [1,11,16].

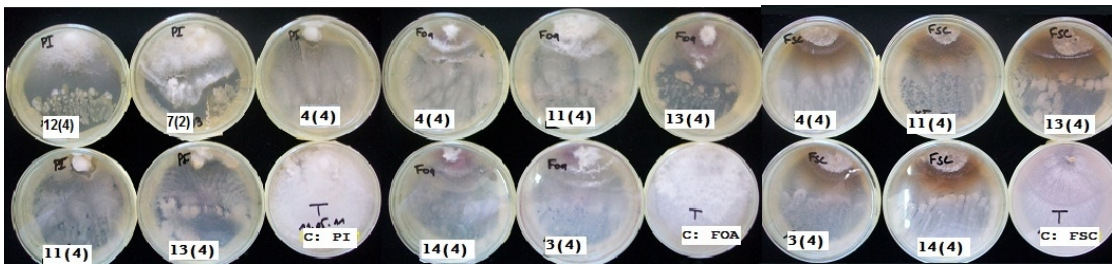

Fig. 3. Antifugal activity by dual culture of Wheat rhizospheric bacteria against phytopathogenic fungi

C: control, PI: Phytophthora infestans, FOA: Fusarium oxysporum f. sp. albedinis, FSC: Fusarium solani var. coeruleum, 12(4), 7(2), 11(4) ……..: bacterial isolates, (1) (2) (3) and (4):first, second, third and the forth field.

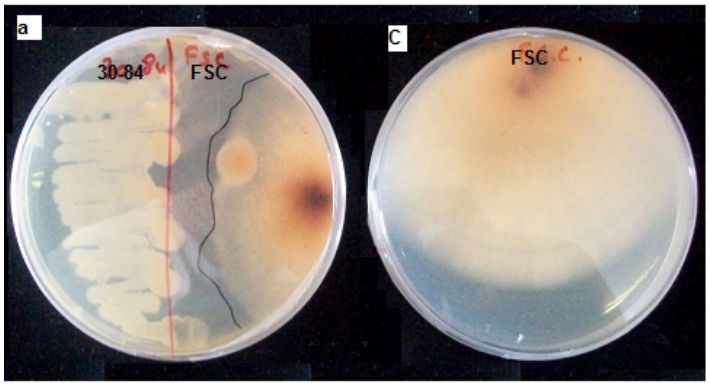

Fig. 4. Antifugal activity by dual culture of *Pseudomonas aureofaciens* 30-84 against FSC

a: dual culture of Pseudomonas aureofaciens 30-84 *and FSC; C: control, FSC: Fusarium solani var. coeruleum*

Our results are consistent with those of [17], they had selected 16 isolates of fluorescent *Pseudomonas* exhibiting the characteristic to colonize the rhizosphere of tomato, among which a single isolate effectively inhibited four of the five fungi tested. [11] had reported that within 150 initial isolates that inhibited *Fusarium solani* BNM400 more than 40% with respect to the fungi growing alone, the number of selected isolates was then reduced to 80. Looking for bacteria with a wide range of antifungal action, six out of the 80 isolates were reselected after testing them against a panel of phytopathogenic fungi.

Among the Gram positive isolates obtained from the potato field, 14.28% inhibited *Fusarium solani* var. *coeruleum*, 21.42% inhibited *Fusarium oxysporum* f. sp. *albedinis* whereas 14.28% inhibited *Phytophthora infestans*. While among the Gram^{-ve}, 57.14% inhibited *Fusarium solani* var. *coeruleum*, 50% inhibited *Fusarium oxysporum* f. sp. *albedinis* and 35.71% inhibited *Phytophthora infestans*. From the seven Gram^{-ve} inhibitory strains colonizing the rhizosphere, only one strain inhibited the three phytopathogenic tested fungi. Concerning Wheat rhizosphere isolates, among the 11 Gram^{-ve} isolates 45.45% inhibited *Phytophthora infestans,* 54.54% inhibited *Fusarium solani* var. *coeruleum*, and only 45.45% inhibited *Fusarium oxysporum* f. sp. *albedinis.* On the other hand, 84.61% of the Gram^{+ve} inhibited *Phytophthora infestans,* 73.92% inhibited *Fusarium solani* var. *coeruleum* and 73.92% inhibited *Fusarium oxysporum* f. sp. *albedinis*.

Variations in the indigenous bacterial populations may be related to the soil characteristics such as the soil pH. [18] showed that an increase of the soil pH by the amendment with clay (montmorillonite or illite) induced a significant increase of the density of the endogenous populations of fluorescent *Pseudomonads*. Studying Châteaurenard and the Dijon soils which differ in their pH (respectively 8.05 and 6.91), [19] had also speculated that this differences may affect fluorescent *Pseudomonads* populations.

Otherwise, the difference between the selected populations could be also ascribed to variations in the amounts and compositions of the compounds exuded by the cultivated plant species (potato and wheat), the availability of the root exudates may vary with different clay contents [19,20,21,22].

The pH values of wheat rhizosphere roots and spermosphere are neutral to slightly basic respectively, 7.27 and 7.98; while those from potato are , slightly acidic to basic respectively from <7 to 8.60.

A study of the relations between the pH of soils and their level of conduciveness to *F.solani* var. *coeruleum* and *F. roseum* var. *sambucinum* has been carried out by [10].They observed a direct relation between the low values of pH and the suppresiveness of soils to *F.solani* var. *coeruleum*. The critical value between conducive and suppressive soils is about pH 5.3; under this, most soils are suppressive. They speculate that the pathogen persists in the conductive soils with chlamydospores whereas in the suppressive soils, the pathogen is completely lysed and the formation of resting spores is inhibited. The pH of the soil studied here is higher, that's why the *F.solani* var. *coeruleum* is not inhibited in antagonistic tests.

Several bacterial mechanisms have been described for the protection of plants against fungal diseases. Independently, of the mode of action used, evidence exists correlating the efficacy of bacterial strains with control soil-borne pathogen with their ability to competitively colonize and survive in the root system of the plant to be protected [23].

4. CONCLUSION

The screening procedure demonstrated to be very effective. The main goal of this work was accomplished and it shows a promising beginning for detecting suppressive soils in Sétif aria. The practical significance of this type of studies acquires its real importance when considering the need to replace chemical control procedures for the treatment of soil and/or plant diseases. This implies that the antagonists isolated have potential to be used as treatment to seeds and tubers.

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COMPETING INTERESTS

Authors declare that no competing interests exist with other people or organizations that could inappropriately influence our work.

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