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Antagonistic Activity of Probiotic Strains Against Brucella spp. In Vitro

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Abstract **-** Brucellosis remains a significant challenge for public health and livestock management in many countries. The search for alternative strategies to combat this disease, including the use of probiotics, has garnered considerable interest. This study aimed to evaluate the antagonistic activity of four lactic acid bacterial strains (*Lactobacillus plantarum* 14d/19, *L. plantarum* 14d/87, *L. brevis* Б-3/43, *L. acidophilus* 27w/77) and their associations against *Brucella melitensis*, *B. abortus*, and *B. suis* using three in vitro methods.

Keywords**:** *Brucella*, lactic acid bacteria, probiotic, antagonistic activity, disk diffusion, agar overlay, stab culture

1. Introduction

Brucellosis is a zoonotic infection of global importance, posing risks to both animal productivity and human health. Kazakhstan is among the countries where the disease remains endemic [4]. Traditional treatment relies on antibiotics, yet rising resistance and chronic forms necessitate alternative control strategies. Recent attention has turned to probiotics, particularly lactic acid bacteria (LAB), due to their potential antimicrobial properties [1], [2], [3]. This study investigates the in vitro antagonistic effect of selected LAB strains and their combinations against three clinically relevant *Brucella* species.

2. Materials and Methods

2.1. Bacterial Strains

LAB strains used were *L. plantarum* 14d/19, *L. plantarum* 14d/87, *L. brevis* Б-3/43, and *L. acidophilus* 27w/77. Pathogenic strains tested included *Brucella melitensis*, *B. abortus*, and *B. suis*.

2.2. Antagonistic Assays

Three methods were employed:

- **Disk diffusion method**: Cell-free supernatants of LAB cultures were applied to sterile paper disks placed on Brucella-inoculated agar (Figure 1).
- **Stab culture method**: LAB were stab-inoculated into agar, overlaid with Brucella after 24 h of incubation (Figure 2).
- **Agar overlay method**: Drop inoculation of LAB was followed by an overlay of soft agar containing Brucella.

Each LAB strain and two associations (A1 and A2) were tested at serial dilutions (undiluted to 1:10,000). Diameters of inhibition zones were measured after 24–48 hours.

3. Results and Discussion

All methods yielded consistent trends. Among the tested combinations, **A2** (14d/19 + Б-3/43 + 14d/87) exhibited the strongest antagonistic activity across all Brucella strains and dilutions.

Table 1 shows results for *B. melitensis*, where A2 maintained activity even at 1:10,000 dilution (zone: 24±1 mm).

№	Strains lactic acid bacteria	Diameters of growth inhibition zones (mm) for *B. melitensis* by lactic acid bacteria at different dilutions				
		Initial	1:10	1:100	1:1000	1:10000
1	Б-3/43	15±1	0	0	0	0
2	14д/87	17±1	16±1	17±1	18±1	0
3	*27w/77*	16±1	15±1	18±1	15±1	19±1
4	14д/19	0	0	0	0	0
5	А-1	21±1	17±1	15±1	15±1	21±1
6	А-2	24±1	19±1	19±1	17±1	24±1

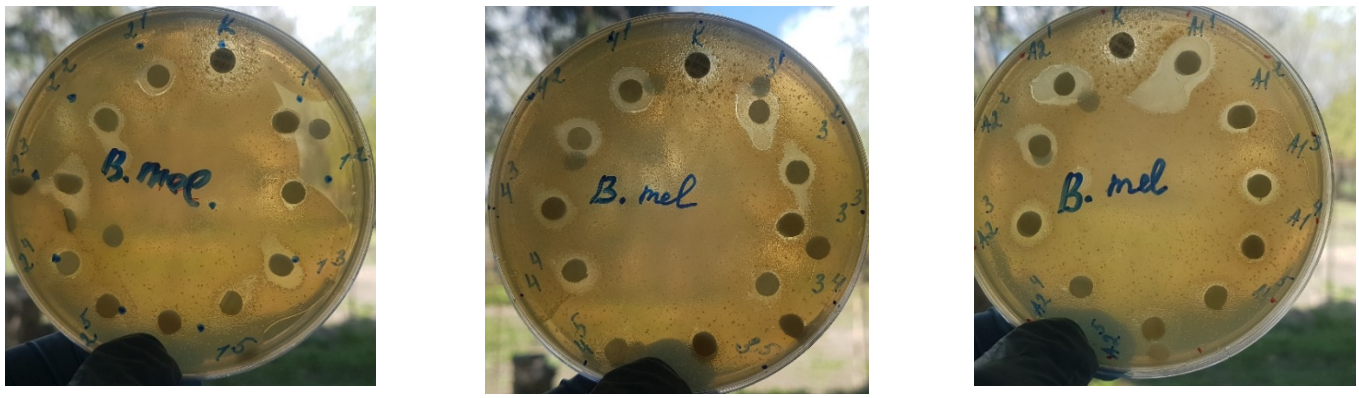
Table 2 presents inhibition of *B. abortus*; again, A2 demonstrated superior and stable activity (zones: 20–22 mm across dilutions).

№	Strains lactic acid bacteria	Diameters of growth inhibition zones (mm) for *B.abortus* by lactic acid bacteria at different dilutions				
		Initial	1:10	1:100	1:1000	1:10000
1	Б-3/43	18±1	16±1	17±1	18±1	15±1
2	14д/87	17±1	16±1	14±1	15±1	15±1
3	*27w/77*	16±1	15±1	14±1	15±1	15±1
4	14д/19	0	0	0	0	0
5	А-1	19±1	17±1	17±1	19±1	17±1
6	А-2	20±1	22±1	22±1	21±1	22±1

Table 3 highlights the challenge of inhibiting *B. suis*, where only A2 showed activity at all dilutions (15–18 mm), while individual LAB showed none.

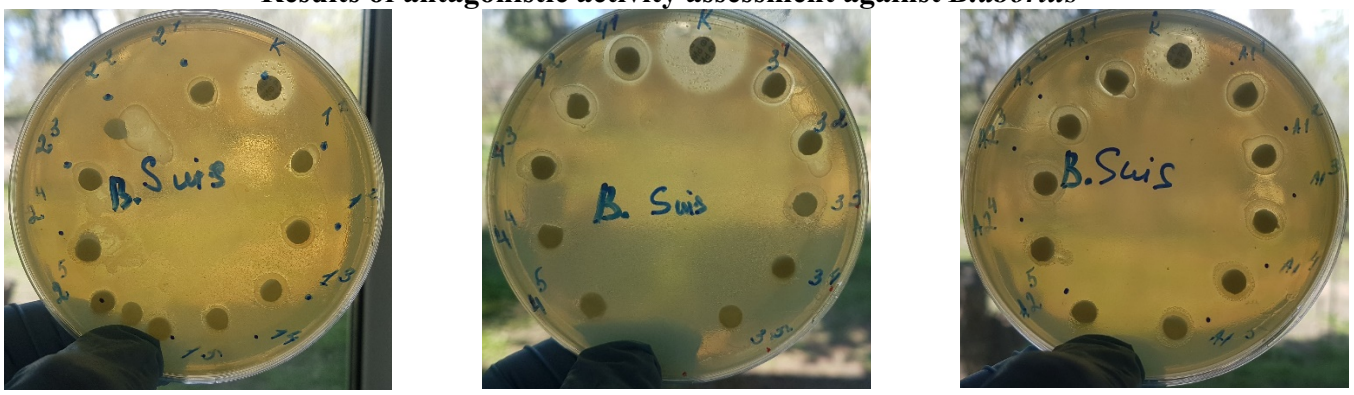
№	Strains lactic acid bacteria	Diameters of growth inhibition zones (mm) for *B.suis* by lactic acid bacteria at different dilutions				
		Initial	1:10	1:100	1:1000	1:10000
1	Б-3/43	0	0	0	0	0
2	14д/87	0	0	0	0	0
3	*27w/77*	0	0	0	0	0
4	14д/19	0	0	0	0	0
5	A_1	16±1	0	0	0	0
6	A_2	18±1	15±1	17±1	17±1	15±1

These results indicate synergistic effects in the A2 combination. Disk diffusion and overlay methods were the most sensitive (figure 1), while stab culture allowed observation of persistent effects(figure 2). The pronounced in vitro efficacy against *Brucella* spp. supports the potential role of specific probiotic formulations in brucellosis management.



Results of antagonistic activity assessment against *B. melitensis*

Results of antagonistic activity assessment against *B.abortus*



Results of antagonistic activity assessment against *B.suis*

Fig 1 : Antagonistic activity of LAB against *Brucella* spp. via disk diffusion.

Fig 2: Antagonistic activity via perpendicular stab culture method.

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4. Conclusion

The LAB combination A2 displayed consistent and high antagonistic activity against all tested *Brucella* strains in vitro. These findings suggest promise for probiotic-based interventions in brucellosis control. Future studies should evaluate in vivo efficacy, immunomodulatory effects, and safety in livestock.

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