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Screening and Phenotypic Characterization of Presumptive Avirulent *Ralstonia*-Like Isolates from Tomato Rhizosphere Soils for Bacterial Wilt Management in Northern Tanzania

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Abstract

Bacterial wilt caused by the *Ralstonia solanacearum* species complex is among the most damaging constraints to tomato (*Solanum lycopersicum* L.) production in Tanzania, and available management options give only partial control. Naturally occurring isolates that have lost pathogenicity are of interest for biological control because they occupy the same niche as virulent strains. This study aimed to recover and characterize isolates with a non-fluidal colony phenotype from tomato rhizosphere soils in bacterial wilt-affected areas of Arusha District Council. Thirty samples (27 composite rhizosphere soil samples from asymptomatic plants and three symptomatic plants) were collected from nine fields in three wards and plated on triphenyl tetrazolium chloride (TZC) medium. Twelve small, dry, deep-red, non-fluidal isolates were recovered from soil and three fluidal presumptive virulent isolates from symptomatic stems. None of the 12 caused wilt on the tomato varieties Tanya and Tengeru 97 in a screen house up to 35 days after inoculation, whereas the presumptive virulent control caused severe wilt (mean severity 4.50 and 3.50 on a 0 - 5 scale). Inference was confined to wilt incidence, which separated the positive control from all other treatments (Fisher's exact test, $p < 0.001$). In an agar well diffusion assay of seven isolates, two (BWN A1 and BWN A5) produced mean total inhibition zones of 16.4 and 16.2 mm against the presumptive virulent test isolate (BWN P1), numerically larger than the 15.4 mm zone of the antibiotic control (Ampicillin-cloxacillin) but not significantly different from it (Welch's t-test with Holm adjustment), whereas five produced none. These isolates are candidates for further characterization; their molecular identity and their abil-

ity to suppress disease in plants remain unconfirmed and require molecular identification and replicated pot and field evaluation.

Keywords

Avirulent Isolates, Bacterial Wilt, *Ralstonia solanacearum*, Colony Morphology, Antagonism

1. Introduction

Tomato (*Solanum lycopersicum* L.) is the second most important vegetable crop in Tanzania after potato and a major source of cash income for smallholder households [1]. Its production is constrained above all by bacterial wilt, caused by members of the *Ralstonia solanacearum* species complex (RSSC), soil-borne bacteria that colonize and occlude the xylem and cause rapid, irreversible wilting [2] [3].

The disease is widely distributed in tropical, subtropical and warm temperate regions, where it is a major constraint on the production of many crops [4], and *R. solanacearum* is ranked among the ten most important plant pathogenic bacteria in molecular plant pathology [5]. Losses of up to 100% have been reported from affected Tanzanian fields where management was ineffective [6].

The pathogen persists in soil, irrigation water, crop debris and symptomless hosts, and the species complex comprises genetically diverse phylotypes differing in host range and aggressiveness [7] [8]. On the basis of polyphasic and genomic evidence, the complex has been proposed to be divided into three species: *R. pseudosolanacearum* (phylotypes I and III), *R. solanacearum* (phylotype II) and *R. syzygii* (phylotype IV) [9] [10]. Few management options are available, and those available suppress soil-borne inoculum inconsistently and lie beyond the economic reach of smallholders, who therefore rely mainly on sanitation, roguing, mulching, organic amendments and tolerant cultivars; these reduce losses but do not prevent them reliably under continuous solanaceous cropping [6].

Biological control is consequently receiving increasing attention. Rhizosphere-competent bacteria and fungi have been evaluated widely, but many are strongly antagonistic *in vitro* and inconsistent in the field, partly because introduced strains compete poorly with the resident soil community [11]; consistent suppression appears to depend less on *in vitro* antibiosis than on root colonization and shifts in the resident community [12]. Native avirulent strains may therefore be promising, because they colonize niches similar to those of virulent strains and may interfere with pathogen establishment or induce plant resistance [13]. The use of avirulent derivatives of the pathogen itself is not new: avirulent mutants were proposed for the control of tomato bacterial wilt as early as reported [14], and genetically engineered Hrp^- mutants, whose colonization of tomato was restricted to the roots and lower stem, were associated with reduced disease severity under challenge inoculation [15]. More recently, the strain FJAT-1458 reduced

disease by 100% in pots and 77.5% in the field [16], and combining an avirulent strain with a microbial restoration substrate gave better control than either alone [17]. Suppression has been attributed to niche pre-emption and priming of host defense [18], mechanisms demonstrated under particular conditions that cannot be assumed to apply elsewhere.

Tanzanian bacterial wilt populations have recently been characterized at phylotype level, and phylotype I *R. pseudosolanacearum* predominates among strains from tomato in the main producing regions [19] [20]. That work addressed virulent strains from diseased plants; the avirulent component of the local population has not been surveyed in northern Tanzania. It was hypothesized that tomato-growing soils in affected areas contain naturally occurring *Ralstonia* isolates with reduced or absent pathogenicity and potential antagonistic activity. The objective was to recover isolates with the non-fluidal colony phenotype from three affected wards of Arusha District Council and to characterize them by colony morphology, by pathogenicity on two locally grown tomato varieties and by *in vitro* antagonism against a virulent isolate from the same fields. This is, to the authors' knowledge, the first description of candidate native non-pathogenic *Ralstonia*-like isolates from Tanzanian tomato soils.

2. Materials and Methods

2.1. Study Area and Sampling

Sampling was carried out during the February-March 2025 tomato cropping season, and all laboratory and screen-house experiment was done at Tanzania Agricultural Research Institute, Tengeru in Arusha, Tanzania. Samples were collected from three tomato-producing wards of Arusha District Council (**Table 1**), an area with bimodal rainfall and loamy to sandy clay soils under year-round irrigation.

Three fields were selected purposively per ward, giving nine; a field was eligible if tomato was grown intensively, if the farmer reported wilt-related losses, and if symptomatic and asymptomatic plants were both present.

Within each field, three composite rhizosphere soil samples were taken, giving 27 composites, each pooling the rhizosphere soil of several asymptomatic plants along a diagonal transect; plants were classed as asymptomatic if free of wilting, leaf yellowing, stunting and stem discoloration. Plants were uprooted carefully, loosely adhering soil shaken off, and soil remaining firmly attached to the roots taken as rhizosphere soil [21]. One symptomatic plant was also collected from one field in each ward. The 30 samples were bagged individually, transported in a cool box and processed on arrival.

The field, not the composite sample, is the independent sampling unit: the three composites within a field are subsamples, so the 27 composites represent nine fields rather than 27 independent sites. They were processed separately to maximize recovery of distinct colony types, not as replicates, and no inference about variation among fields or wards is drawn. Isolate counts per ward (**Table 1**) are descriptive.

2.2. Isolation and Phenotypic Characterization

Casamino acid-peptone-glucose (CPG) medium was used as the basal medium, and triphenyl tetrazolium chloride (TZC) medium was prepared from the same base by amending it after autoclaving with filter-sterilized triphenyl tetrazolium chloride to 0.005% (w/v), that is 50 $mg \cdot l^{-1}$ [22].

Symptomatic plants were subjected to the stem-streaming test, in which a slimy bacterial exudate from cut stem sections is characteristic of bacterial wilt [3]; vascular tissue was then surface-sterilized, macerated and dilution-streaked onto CPG and TZC plates. For soil, 1 g of each composite was suspended in 9 ml of sterile distilled water and a tenfold dilution series prepared to 10^{-6} [21]. Aliquots of 0.1 ml from the 10^{-3} and 10^{-5} dilutions were spread on TZC plates, three per dilution per sample, and incubated at 28˚C for 48 - 72 h; lower dilutions gave confluent growth and 10^{-6} too few colonies. Colonies were purified by three successive single-colony transfers, and colony form, margin, elevation, texture, colour and fluidity recorded on three TZC plates per isolate after 48 h at 28˚C. Isolates were coded by ward (BWN, MLN, SMS), then A for the non-fluidal soil phenotype or P for the fluidal phenotype from plants, and a serial number.

Colony morphology on tetrazolium medium supports only preliminary phenotypic grouping; it does not establish species identity or phylotype within the species complex, which requires sequence-based molecular identification, nor virulence status, which is assessed separately by plant pathogenicity assays under specified conditions. The groups are therefore designated presumptive virulent and presumptive avirulent throughout, and the isolates described as *Ralstonia*-like rather than assigned to a species.

2.3. Pathogenicity Test

Inoculum was prepared from 24 h nutrient agar cultures and adjusted to an optical density of 0.1 at 600 nm, approximately 10^8 colony-forming units $(CFU) \cdot ml^{-1}$. Seedlings of the tomato varieties Tanya and Tengeru 97 were raised in sterilized soil to the four-true-leaf stage at three weeks, root-tip wounded, transplanted singly into pots of sterilized soil and inoculated by drenching the root zone with 30 ml of suspension. Plants were held in a screen house at 28˚C and irrigated at two-day intervals.

The experiment comprised 14 treatments: the 12 presumptive avirulent isolates, a presumptive virulent isolate BWN P1 as positive control and sterile distilled water as negative control, each applied to both varieties, giving 28 treatments by variety combinations. It was a randomized complete block design with four blocks, the blocking factor being position along the screen-house bench, which accounts for the gradient in light and temperature. Each experimental unit was one pot with one seedling, giving four plants per combination and 112 plants in total.

Severity was assessed at 7, 14, 21, 28 and 35 days after inoculation on a 0 - 5 scale, where 0 = no symptoms; 1 = 1% - 25% of leaves wilted; 2 = 26% - 49%; 3 = 50% - 74%; 4 = 75% - 100%; and 5 = plant dead [23]. Final severity at 35 days was

analyzed. Plants were then uprooted, stems sectioned longitudinally to record vascular discoloration, and re-isolation carried out on TZC medium from stem tissue and pot soil: for the positive control, to satisfy Koch's postulates, and for the symptomless plants inoculated with the candidate isolates, to determine whether the inoculated isolates had persisted in the plant and soil medium.

2.4. *In Vitro* Antagonism Assay

Antagonism was assessed by the agar well diffusion method, with modifications [24]; this widely used screening method measures inhibition mediated by diffusible compounds and is not a dual culture assay [25]. A lawn of the presumptive virulent test isolate BWN P1 was established by spreading 0.1 ml of a suspension of approximately 10^8 CFU·ml^{-1} over a CPG plate, and four wells of 6 mm diameter were punched at equidistant positions.

The assay comprised nine treatments: seven presumptive avirulent isolates representing all three wards BWN A1, BWN A5, MLN A1, MLN A2, SMS A1, SMS A2 and SMS A3were selected because they showed rapid colonization during re-isolation in pathogenicity test; the five isolates not tested were BWN A2, BWN A3, BWN A4, MLN A3 and SMS A4), ampicillin-cloxacillin as positive control, and sterile distilled water as negative control. Each bacterial treatment was applied as 20 µl per well of a suspension of approximately 10^8 CFU·ml^{-1}, matching the lawn inoculum; the antibiotic control was applied as 20 µl per well of a filter-sterilized 1 mg·ml^{-1} aqueous stock, delivering 20 µg per well. Because living suspensions and a fixed antibiotic dose are not equivalent quantities, no equivalence of concentration between them is implied.

The four wells on a plate received the same treatment and were technical replicates, averaged to give one value per plate; the plate was the experimental unit and three replicate plates per treatment were the experimental replicates, giving n = 3 per treatment and 27 plate values. Individual wells were never treated as replicates. Plates were incubated at 28˚C for 48 h in a completely randomized design, and the total diameter of the clear zone, including the 6 mm well, was measured to the nearest 1 mm. All diameters reported follow this convention.

2.5. Data Analysis

Analyses were done in R version 4.3.3 with significance at $p < 0.05$, and descriptive results are given as mean ± SD. Severity at 35 days could not be analyzed by two-way ANOVA because it was zero in 13 of the 14 treatments on both varieties, leaving no within-group variance and completely separated groups, so severity is reported descriptively only. Inference was based on wilt incidence (severity > 0): for each variety, the positive control was compared with the pooled 13 remaining treatments by Fisher's exact test, and positive control severity was compared between varieties by the exact Wilcoxon rank-sum test. Treatment-by-treatment comparisons were not reported because, with four plants per combination, the smallest possible p-value (0.029) would not survive correction for 13 comparisons.

Inhibition zone diameters (n = 3, nine treatments) were analyzed by one-way ANOVA and confirmed with the Kruskal-Wallis test, which supports only an overall treatment difference. Because variances were unequal, Tukey's test was replaced by Welch's t-test with Holm adjustment, comparing the two active isolates with the antibiotic control and with each other, and excluding the six treatments with no inhibition zone.

3. Results

3.1. Isolation and Recovery of Isolates

Twenty-seven bacterial isolates were purified from the soil samples: 11 from Bwawani, seven from Mlangarini and nine from Sambasha (Table 1). Twelve had small, dry, circular, uniformly deep-red, non-fluidal colonies on TZC medium: five from Bwawani (BWN A1 - A5), three from Mlangarini (MLN A1 - A3) and four from Sambasha (SMS A1 - A4). Of the remaining 15, seven had large, fluidal, irregular, creamy-white colonies with a pink centre and eight had characteristics consistent with *Bacillus* spp. All three symptomatic plants gave a milky-white ooze in the stem-streaming test, and isolation from their vascular tissue yielded three fluidal isolates, coded BWN P1, MLN P1 and SMS P1.

Table 1. Location, number of fields sampled and recent cropping history of the three wards of Arusha District Council sampled during the February-March 2025 tomato cropping season, with the numbers of samples collected and isolates recovered per ward.

Ward	Latitude	Longitude	Fields (no.)	Recent cropping history	Composite soil samples (no.)	Symptomatic plants (no.)	Isolates from soil (no.)	Presumptive avirulent (no.)	Other isolates (no.)
Bwawani (BWN)	3˚37'07.3"S	36˚50'14.9"E	3	Tomato-maize rotation	9	1	11	5	6
Mlangarini (MLN)	3˚17'26.8"S	36˚40'26.6"E	3	Eggplant-tomato rotation	9	1	7	3	4
Sambasha (SMS)	3˚24'29.0"S	36˚47'07.2"E	3	Tomato, chilli pepper and potato	9	1	9	4	5
Total	–	–	**9**	–	**27**	**3**	**27**	**12**	**15**

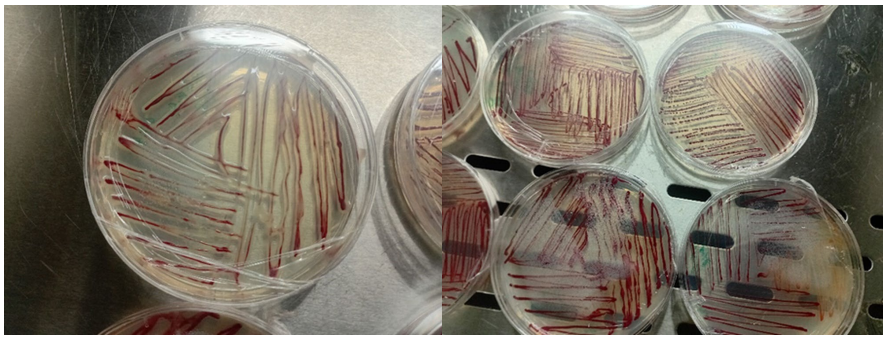
3.2. Colony Characteristics of the Two Phenotypic Groups

Table 2. Colony characteristics of the two phenotypic groups on triphenyl tetrazolium chloride medium after 48 h at 28˚C.

Phenotypic group	Form and margin	Elevation and texture	Colour	Fluidity	Isolate codes
Presumptive virulent	Irregular, undulate margin	Raised, mucoid	Creamy white with a pink to pale-red centre	Fluidal	BWN P1, MLN P1, SMS P1 (n = 3)
Presumptive avirulent	Circular, entire margin	Convex, dry, butyrous	Uniformly deep red	Non-fluidal	BWN A1 - A5, MLN A1 - A3, SMS A1 - A4 (n = 12)

Two colony phenotypes were recognized on TZC medium after 48 h at 28˚C and differed in form, margin, elevation, texture, colour and fluidity (Table 2, Figure

1). The fluidal phenotype, designated presumptive virulent, comprised the three isolates from symptomatic plants; the non-fluidal phenotype, designated presumptive avirulent, comprised the 12 soil isolates. Characteristics were consistent across the three replicate plates per isolate.



(a) (b)

Figure 1. Colony morphology of the two phenotypic groups on triphenyl tetrazolium chloride medium after incubation at 28˚C for 48 h. (a) Fluidal, irregular, creamy-white colonies with a pink centre, characteristic of the presumptive virulent phenotype recovered from symptomatic vascular tissue; (b) small, circular, dry, uniformly deep-red, non-fluidal colonies, characteristic of the presumptive avirulent phenotype recovered from rhizosphere soil.

3.3. Pathogenicity of the Presumptive Avirulent Isolates

None of the 12 presumptive avirulent isolates produced wilt symptoms on either variety at any assessment up to 35 days: mean severity was 0.00 ± 0.00 and wilt incidence 0 of 4 plants for every isolate on both varieties (Table 3). Plants treated with sterile distilled water likewise remained symptomless. The presumptive virulent control caused severe wilt on both varieties, all four plants wilting on each, with mean severity 4.50 ± 0.58 on Tanya and 3.50 ± 0.58 on Tengeru 97.

Table 3. Mean disease severity and wilt incidence on the tomato varieties Tanya and Tengeru 97 at 35 days after inoculation.

Treatment	Phenotypic group	Tanya: mean severity ± SD	Tanya: wilt incidence (wilted/tested)	Tengeru 97: mean severity ± SD	Tengeru 97: wilt incidence (wilted/tested)
12 soil isolates (BWN A1 - A5, MLN A1 - A3, SMS A1 - A4), each assessed separately	Presumptive avirulent	0.00 ± 0.00	0/4 for every isolate	0.00 ± 0.00	0/4 for every isolate
Presumptive virulent isolate (positive control)	Presumptive virulent	4.50 ± 0.58	4/4	3.50 ± 0.58	4/4
Sterile distilled water (negative control)	–	0.00 ± 0.00	0/4	0.00 ± 0.00	0/4

Wilt incidence in the positive control (4 of 4) differed from the pooled incidence of the 13 remaining treatments (0 of 52) on both varieties (Fisher's exact test, $p = 2.7 \times 10^{-6}$). The experiment therefore demonstrates complete separation between the positive control and the candidate isolates under the conditions

tested, and no measurable variation among the candidates themselves. Because every candidate and the negative control scored zero at every assessment, the data contain no information with which to rank the candidates, and no such comparison is attempted.

Mean severity in the positive control was numerically higher on Tanya than on Tengeru 97, but with four plants per variety the difference was not statistically supported (exact Wilcoxon rank-sum test, $W = 14$, $p = 0.114$), so no conclusion is drawn about relative susceptibility.

Plants inoculated with the presumptive virulent isolate developed vascular browning and their stem sections released a milky exudate (Figure 2). Re-isolation from these plants and their pot substrate yielded colonies morphologically identical to the inoculated isolate, satisfying Koch's postulates; no *Ralstonia*-like colonies were re-isolated from the negative control.

Re-isolation from the symptomless plants and pot soil inoculated with the 12 candidate isolates were recovered from Tengeru 97 with codes, BWN A1, BWN A5, MLN A1, MLN A2, SMS A1, SMS A2 and SMS A3 had 3/4 plants. Recovery of the inoculated isolate indicates that the absence of wilt reflected an inability to cause disease after establishment, whereas failure to recover it leaves open that the isolate did not persist, in which case absence of disease cannot be attributed to avirulence.



(a) (b)

Figure 2. Response of tomato plants 35 days after root-drench inoculation with 30 ml of a suspension of approximately 108 colony-forming units·ml^{-1}. (a) Plants inoculated with a presumptive avirulent isolate, showing no wilt symptoms; (b) plants inoculated with the presumptive virulent isolate, showing wilting and collapse.

3.4. *In Vitro* Antagonism against the Presumptive Virulent Isolate

Seven of the 12 presumptive avirulent isolates, representing all three wards, were tested. Two produced clear inhibition zones: BWN A1, 16.4 ± 0.5 mm, and BWN A5, 16.2 ± 0.4 mm (Table 4, Figure 3). The ampicillin-cloxacillin control produced 15.4 ± 0.3 mm.

Table 4. Mean total diameter of inhibition zones, including the 6 mm well, produced by seven presumptive avirulent isolates and two controls against the presumptive virulent test isolate in an agar well diffusion assay on casamino acid-peptone-glucose medium after 48 h at 28˚C. Means sharing the superscript letter a do not differ significantly (Welch's t-test with Holm adjustment, $p > 0.05$); no zone was detected in the remaining treatments, which had zero variance and were not entered in pairwise tests.

Treatment	Ward of origin	Total inhibition zone diameter, including well (mm; mean ± SD)
BWN A1	Bwawani	16.4 ± 0.5[a]
BWN A5	Bwawani	16.2 ± 0.4[a]
Ampicillin-cloxacillin (positive control)	–	15.4 ± 0.3[a]
MLN A1	Mlangarini	0.0 ± 0.0
MLN A2	Mlangarini	0.0 ± 0.0
SMS A1	Sambasha	0.0 ± 0.0
SMS A2	Sambasha	0.0 ± 0.0
SMS A3	Sambasha	0.0 ± 0.0
Sterile distilled water (negative control)	–	0.0 ± 0.0



Figure 3. Agar well diffusion assay of isolate BWN A1 and BWN A5 against the presumptive virulent test isolate on casamino acid-peptone-glucose medium after 48 h at 28˚C, showing a clear inhibition zone. Wells were 6 mm in diameter and received 20 µl of a suspension of approximately 10^8 colony-forming units·ml^{-1}. Diameters in **Table 4** are total and include the well.

All values are total diameters and include the 6 mm well. No zone was recorded for MLN A1, MLN A2, SMS A1, SMS A2, SMS A3 or the negative control. Treatments differed significantly ($F = 3447$; $df = 8, 18$; $p < 0.001$), confirmed by the tie-corrected Kruskal-Wallis test ($H = 25.9$; $df = 8$; $p = 0.001$). These tests show an overall difference among treatments but do not identify which treatments differ. In pairwise comparisons by Welch's t-test with Holm adjustment, neither BWN

A1 (difference 1.0 mm; [adjusted p = 0.16]) nor BWN A5 (difference 0.8 mm; [adjusted p = 0.16]) differed significantly from the antibiotic control, and the two isolates did not differ from each other ([adjusted p = 0.62]) (**Table 4**). The numerically larger zones of the two isolates therefore cannot be distinguished from the antibiotic control with three plates per treatment.

4. Discussion

The results support the first part of the hypothesis, that isolates with the non-fluidal, deep-red phenotype are present in local soils, and the second only partially, since antagonism was detected in a minority of isolates and only *in vitro*.

Recovery of the two phenotypes from different sample types is consistent with the known ecology of the species complex, in which soil populations are generally low and unevenly distributed [7], although the design permits no quantitative comparison of their abundance. Rhizosphere isolates from diseased fields have in some cases proved useful for disease suppression [26]. Phylotype I *R. pseudosolanacearum* predominates among virulent strains from tomato in Tanzanian production regions [19] [20], and any candidate proposed for local deployment would have to be evaluated against that resident population.

The two phenotypes differed consistently, in agreement with the classical description of virulence-related colony variation on tetrazolium medium [22]. In phenotype conversion, the loss of extracellular polysaccharide production and of virulence is in most strains associated with loss of expression or function of the regulator PhcA [27], so a reduced polysaccharide output is a plausible explanation for the non-fluidal phenotype, but this is a hypothesis to be tested rather than a finding of the present study. Afluidal colonies are also not proof of avirulence, since polysaccharide-defective strains that remained highly virulent have been described [28]. The symptomless isolates may equally be genuinely avirulent, hypo virulent under the conditions used, or members of related non-pathogenic taxa sharing the same appearance on this medium. The stability of the non-pathogenic phenotype also needs to be verified, because reversion from non-pathogenic to pathogenic forms through alterations in *phcA* has been reported after passage through host plants [29]. Avirulent strains characterized elsewhere suppress wilt severity [13] [16] [17], but their identity was confirmed by molecular means.

The pathogenicity experiment is a screen rather than a comparative experiment. The positive control wilted every plant, whereas none of the 12 candidates or the negative control showed symptoms, so the data provided separate the candidates from the virulent control but contain no information with which to rank the candidates, to detect differences among them, or to support a reliable conventional test of a treatment by variety interaction. A single root-drench assay on two varieties, at one inoculum concentration, over 35 days and with four plants per combination, can exclude gross pathogenicity only under the conditions tested; it cannot detect hypo virulence expressed under other conditions, and whether the symptomless outcome reflects true avirulence or failure of the inoculated isolates

to persist depends on the re-isolation results. This is why the isolates are described as candidates rather than as biological control agents.

Absence of a zone in five isolates indicates only that inhibition by diffusible compounds was not detected, not absence of biological control potential: competition for nutrients and colonization sites, niche exclusion and induced systemic resistance have been proposed elsewhere to account for suppression by avirulent strains showing no *in vitro* antibiosis [12] [30], and were not tested. The two differ in diffusion behaviour, mode of action and time course, so the numerically larger zones produced by BWN A1 and BWN A5, which did not differ significantly from the antibiotic control, do not indicate greater efficacy *in planta*; the antibiotic served as an assay performance check, not an efficacy criterion.

The most important limitation is that identification rests on phenotype alone. Further limitations are the small number of isolates; sampling restricted to three wards in a single season; testing of only seven of the 12 candidates for antagonism, so that the proportion inhibitory (two of seven) cannot be inferred to all 12 and may be affected by the selection criterion; and assessment of antagonism *in vitro* only and at one-time point.

5. Conclusions

Rhizosphere soils of tomato fields in three bacterial wilt-affected wards of Arusha District Council yielded 12 *Ralstonia*-like isolates whose colony characteristics on tetrazolium medium differed consistently from those of isolates from symptomatic vascular tissue. None caused wilt on Tanya or Tengeru 97 up to 35 days after inoculation, and two of the seven tested for antagonism inhibited the virulent test isolate in an agar well diffusion assay.

These findings show that isolates with a non-pathogenic phenotype, under the conditions tested, are present in local tomato soils and can be recovered by routine methods. They do not show that the isolates can manage bacterial wilt: no disease management experiment was conducted, and their molecular identity remains unconfirmed, while absence of virulence has been shown only under the conditions of a single screen-house assay. They are therefore reported as candidates for further investigation. Further work should confirm their identity and phylotype, verify the stability of the non-pathogenic phenotype and assess root colonization, persistence and biosafety, before pot and field experiments determine whether they reduce bacterial wilt in production.

Author Contributions

Conceptualization and Methodology-Muya, Mbega, Mrema and Aloyce, Writing-Original draft preparation-Muya, Mrema and Aloyce, Writing—review and Editing—Muya, Mbega, Mrema and Aloyce.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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