

Dagna MACULEWICZ¹

BINUCLEATE *Rhizoctonia* spp. AS A BIOCONTROL AGENTS AGAINST PLANT PATHOGENS

MOŻLIWOŚCI ZASTOSOWANIA DWUJĄDROWYCH IZOLATÓW *Rhizoctonia* spp. W BIOLOGICZNEJ OCHRONIE ROŚLIN

Abstract: *Rhizoctonia* spp. is a large, diverse, ubiquitous soil inhabiting group of fungi. They are generally regarded as plant pathogens causing root rot and other plant diseases which results in crop losses of many economically important plant species, but they can also enter into symbiotic associations with orchids.

Binucleate species commonly cause root, stem and foliar diseases, but due to its wide range of host plants and frequent causing losses in important agricultural crops, probably the most studied *Rhizoctonia* species is multinucleate *Rhizoctonia solani* Kühn.

Crops, including fruits and vegetables, are exposed to diseases caused by microorganisms, especially pathogenic fungi, during growth and after harvest. The occurrence of plant diseases results in lower harvest, in worsens their quality and raises production costs. The use of chemical plant protection results in the contamination of soil and increase pathogen resistance to commonly used fungicides. Biological control is an alternative and safe for the environment method of plant protection.

Hypovirulent BNR isolates from different anastomosing groups can be successfully used as biocontrol agents in plant protection against pathogenic *Rhizoctonia* and fungi from other species. They may occupy the same ecological niches as pathogens, compete successfully for nutrients, induce plant resistance and promote plant growth.

Keywords: mechanism of biocontrol, binucleate *Rhizoctonia*

Fungal diseases are a numerous and, from an economic point of view, the most important group of plant diseases. Plant protection most often involves the use of fungicides, which represent 15 % of pesticide sales. Residues of chemicals are a threat to human health. Moreover, they cause reduction of beneficial organisms and pathogen resistance to chemicals. This is the reason for the increased interest in biological control agents (BCA). When conventional pesticides cannot be used *eg* in production of organic food, BCA can be used. However, biocontrol agents represent only 1 % of agricultural chemical sales [1].

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The “biological control” and its abbreviated synonym “biocontrol” regarding to plant protection means the use of one or more antagonistic microorganisms to suppress plant diseases. The organism that suppresses the pathogen is called the biological control agent. Biocontrol organisms belong to different taxonomic groups – viruses, bacteria, fungi or protozoa [2].

Usually their action involves the prevention of the infection, reduction in the colonization of host tissues or disruption of life and sporulation of the pathogen. Efficient prevention requires the presence of the antagonist on the plant surface before the infection [3].

Many of BCA exist naturally in the plant roots and leaves as a saprotrophs in a particular ecological niche. BCA should have high capacity to colonization, high growth rate and greater ability to survive and development in a wide range of pH, temperature and humidity than pathogen. It should be effective even in low concentrations and have wide spectrum of activity. It is particularly important not to produce metabolites toxic for humans and not to exert adverse effects on raw material [3].

Biocontrol agents generally protect against specific pathogens from infecting the target plant. Hypovirulent or avirulent strains can infect the host without causing disease symptoms. The protection of plants by BCA is the result of interaction of many different mechanisms. Protection may be direct (antagonism against the pathogen), or indirect (through competition for ecological niches, or enhance the host defence through induced resistance) [4, 5].

Direct antagonism by obligate parasites of a plant pathogen requires a high degree of selectivity for the pathogen, called hyperparasitism. The pathogen is directly attacked by a specific BCA that attack living hyphae or its propagules [3, 6].

Microbial predation is more general than hyperparasitism, pathogen is non-specific and provides less predictable levels of disease control. Sometimes BCAs exhibit predatory behavior under nutrient-limited conditions, such activity generally is not expressed under typical growing conditions. For example, some species of *Trichoderma* produce chitinase that are directed against cell walls of *R. solani* when concentration of readily available cellulose decreases [3].

Some binucleate nonpathogenic-*Rhizoctonia* isolates directly parasitized hyphae of *Pythium ultimum*, *P. oligandrum* and *P. ultimum* var. *sporangiferum* [7].

Indirect antagonism includes the production of enzymes or antibiotics, competition and induced-resistance mechanisms [3].

Many microorganisms have the ability to secrete enzymes which destroy cells walls of other organisms and use their protoplasmic material as a source of nutrients. These chemical substances are highly specific and have the ability to inhibit the growth and metabolism of selected microorganisms or kill them. Antimicrobial activity was exhibited eg by *Aspergillus* sp. against *Escherichia coli*, *Penicillium* sp. against *Bacillus* sp. and *Cladosporium* sp. against *Escherichia coli* [8].

Non-pathogenic *Rhizoctonia* (NpR) and pathogens can compete for nutrients, and this is a very important process for limiting disease incidence and severity. The most of them are generally thought to protect the plant by rapid colonization and exhausting the limited available nutrients so that none are available for pathogens to grow [3].

The colonization of the plant or substances secreted by avirulent isolate can activate plant resistance responses, which, in the case of a subsequent infection by pathogens, are faster and more efficient. The first of pathways is termed systemic acquired resistance (SAR), mediated by salicylic acid (SA). SA is produced following pathogen infection and typically leads to the expression of pathogenesis-related (PR) proteins, which include a variety of enzymes. They may lyse pathogen cells, reinforce cell walls to resist infections, or induce cell death in the infection site. The second pathway is induced systemic resistance (ISR), mediated by jasmonic acid (JA) and/or ethylene which causes changes in cell wall composition, production of chitinases and glucanases and synthesis of phytoalexins. Once stimulated plant, exhibits an enhanced resistance upon inoculation with a pathogen [5, 6].

Binucleate *Rhizoctonia* spp. in plant protection

Rhizoctonia spp. is a group of soil fungi with diverse morphology and pathogenicity. This genus was established in 1815 by de Candolle [9]. *Rhizoctonia* spp. can be divided into two groups – binucleate (BNR) and multinucleate (MNR). Multinucleate isolates may display a large variation of number of nuclei in cells (more than 4) and belong to *Rhizoctonia solani* with *Thanatephorus cucumeris* teleomorph [10].

Binucleate *Rhizoctonia* spp. include various fungi which can be divided into groups with hyphal anastomosis. Ogoshi et al [11] described 13 anastomosis groups (from AG-A to AG-O). AG-B has been further divided into AG-Ba, AG-Bb and AG-Bo subgroups. Currently 19 AGs are described (from AG-A to AG-U). Perfect states of BNR correspond with *Ceratobasidium* sp. [12, 11].

Rhizoctonia spp. are fungi with a wide range of trophic strategies. They can be plant pathogens, saprotrophs or enter into symbiotic mycorrhizal associations with orchids [13].

Several genera of fungi, including binucleate *Rhizoctonia* [4, 14], *Trichoderma* [15,16], *Gliocladium* [16], or *Cladorrhinum* [17], can control the development of pathogenic isolates of *R. solani*. Binucleate *Rhizoctonia* spp. controlled *Rhizoctonia* diseases on creeping bentgrass [18], bean [4], potato [19], bedding plants [20], sugarbeet [21], cotton [22], cabbage [23], cucumber [24], and many more. BNR also have been shown to control *Pythium* and *Alternaria* diseases [25, 22].

Protective capacity in relation to plants exhibit representatives of many AGs (A, Ba, Bb, B(o), F, G, H, J, K, L, M, N, O, P, R and S). They can protect plants of various species against phytopathogenic fungi belonging to the genus *Rhizoctonia* and other species. The induction of host defenses may be associated with the appearance of small disease symptoms caused by the hypovirulent isolate and isolates causing minor damage to plants are potentially the best for biological plant protection. In addition to the inability to cause severe disease symptoms, hypovirulent isolates have, usually similar to those virulent, ability to colonize and occupy the same ecological niches in tissues and on the plant surface [26].

The authors of one of the first reports of the possibility of plant protection against pathogens by avirulent isolates of *Rhizoctonia* spp. are Burpee i Goultly [18]. They

studied BNR effect on pathogenic isolates of *R. solani* on turfgrasses and in all three experiments they observed the suppression of brown patch.

Muslim et al [27] demonstrated that BNR causes a significant reduction in disease symptoms caused by *Fusarium oxysporum* on tomatoes, Cardoso and Echandi [4] observed significant protection by BNR against *R. solani* on bean, root exudates from bean seedlings colonized with a binucleate np-R inhibited virulent *R. solani* *in vitro*, and fewer infection cushions were formed by the pathogen on the np-R-colonized seedlings as compared to that on control seedlings [4, 26]. Olson and Benson [28] reported induction of resistance against *Botrytis cinerea* on geranium. Xue et al [24] observed increase of the activity of peroxidases in the presence of BNR in all bean tissues in the absence of disease symptoms.

Four isolates of hypovirulent binucleate *Rhizoctonia* were used for control of *Fusarium* wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici*. BNR isolates could significantly reduce foliar symptoms and discoloration inside the stem [27].

Villajuan-Abgona et al [24] reported that three isolates of BNR (AG-A and AG-Ba) provided infection of 58 to 71 % against pathogenic *R. solani* AG2-2 on cucumber seedlings. BNR (*Ceratobasidium albatensis*) has been shown to control *R. solani* induced damping-off in *Pinus* spp. [29]. When BNR was inoculated onto soybean prior to *R. solani*, there was a significant reduction in disease severity, even when BNR had grown for only 24 h [30]. Sneh and Ichielevich-Auster [31] reported > 60 % protection of the cabbage and 73–95 % of the cucumber seedlings by BNR AG-Bo and AG-P against *R. solani* AG1-IA and AG4. Biocontrol of BNR fungi provided effective control of preemergence damping-off of impatiens caused by *R. solani*. Disease control of either BNR isolate was similar to the standard fungicide treatment of thiophanate-methyl and better than that with the commercial biocontrol product derived from *Gliocladium virens* [14].

Hypovirulent BNR may have a positive effect on plants also by improving their growth and development [32]. Some NpR show slower growth of mycelium compared to the pathogens. This problem can be eliminated by early inoculation of NpR mycelium *eg* on grains and their application near the sowing. The rich food base strengthens their growth, gives them the possibility of early colonization of plant tissues, which in turn gives them an advantage over pathogens occurring naturally in the environment. The earlier colonization by NpR gives better plant protection.

Np-R isolates can survive on the crop roots and after harvest, in plant debris, but it depends on the type of substrate, soil depth and humidity. Propagule density is increased on fresh organic substrates or when germinating seeds are available [26, 33].

Sneh et al [7] observed that there was no correlation between *in vitro* growth rate of hypovirulent isolates and their disease protection ability, but a higher growth rate would be advantageous for faster and more extensive colonisation of the infection sites on the host surface before the approaching hyphal tips of the pathogen.

BNR protective effects against *R. solani* are usually obtained following BNR pre-inoculation before *R. solani* attack, either after short periods of preinoculation *eg* 24 h or 48 h and up to 7 or more days of pre-inoculation [5]. Pre-incubation of BNR or

delayed inoculation of pathogen provided an increased protection on cucumber seedlings [24].

Poromarto et al [30] examined the interactions of intermingling hyphae of BNR AG-K and *R. solani* AG 4 in vitro and on the surface of the host. There was no evidence of lysis, mycoparasitism, inhibition of growth, or any other form of antagonism between hyphae. The results of these studies strongly suggest that induced resistance is the mechanism of biocontrol of *R. solani* on soybean by BNR. The inhibition of hyphal growth of *R. solani* on the surface of soybean tissue preinoculated with BNR appears to be a novel characteristic of induced resistance.

Xue et al [34] did not observe evidence of hyperparasitism or antibiosis between BNR and *R. solani* AG-4 or *Colleotrichum lindemuthianum* in vitro tests. Many researchers discounted that mechanisms as likely mechanisms of suppression of *R. solani* by BNR. They reported that BNR AG-G induced systemic resistance and protection of bean plants against *R. solani* AG-4 and *C. lindemuthianum*.

In the absence of pathogenic isolates an acceleration of growth of radish, lettuce, carrot, cotton, wheat, pepper and some ornamental plants were observed [26]. Muslim et al [27] observed a significant increase in fresh weight of stem and leaf of tomato in the presence of BNR.

BNR AG-K and AG-F can increase *Capsicum annuum* shoot mass and elongation and protect against weak pathogens [35]. Root colonisation with BNR increased cucumber seedlings tolerance to low soil moisture levels [31].

Xue et al [34] described the anatomical and cytological changes in tissues of bean seedlings protected with a nonpathogenic BNR isolate prior to their inoculation with a virulent isolate of *R. solani* which was associated with a deposition of cell wall material rich in lignin, suberin, and phenols. These compounds provide a physical or chemical barrier to the pathogen. NpR are inducers of peroxidases, 1,3 β -glucanases, and chitinases at the local and the systemic level in beans. It shows that induced resistance is multicomponent.

These studies demonstrate a significant protective role of binucleate *Rhizoctonia*, but only few of them specify their membership to anastomosis groups or subgroups. Khan et al [36] emphasize that BNR have the protective potential, but they require a more accurate testing, in particular in the field.

Isolates from orchids

Some *Ceratobasidium* species are mycorrhizal symbionts of orchids, whose seeds require an association with a fungus to obtain sufficient nutrition for germination. Orchid mycorrhizae are unlike other types of mycorrhizae in that the fungus probably receives little or nothing from the plant in most cases [2, 13, 37].

Mosquera-Espinosa et al [37] tested the biocontrol ability of BNR isolates from Colombian orchid roots. They caused low severity disease on rice and protected rice plants from pathogenic *R. solani* AG1-1A.

Disease protection by ectomycorrhizal fungi may involve multiple mechanisms including antibiosis, synthesis of fungistatic compounds by plant roots in response to mycorrhizal infection and a physical barrier of the fungal mantle around the plant root [2].

Microbes that contribute most to disease control are most likely those that could be classified as competitive saprophytes, facultative plant symbionts and facultative hyperparasites. These can generally survive on dead plant material, but they are able to colonize and express biocontrol activities while growing on plant tissues. A few, like avirulent *Fusarium oxysporum* and binucleate *Rhizoctonia*-like fungi, are phylogenetically very similar to plant pathogens but lack active virulence determinants for many of the plant hosts from which they can be recovered. Others, like *Pythium oligandrum* are currently classified as distinct species. However, most are phylogenetically distinct from pathogens and, most often they are subspecies variants of the same microbial groups [2].

Combination of biocontrol fungi and bacteria

Mixtures of some non-pathogenic bacteria and fungi may enhance the level of biocontrol of different plant pathogens [38]. Eken and Yuen [39] reported that BNR isolate BN-8 in combination with *Lysobacter enzymogenes* strain C3 reduced disease severity of wheat cultivars (Russ and Alsen) caused by *Bipolaris sorokiniana* and *Rhizoctonia solani* AG-4.

The cucumber seedlings grown on water agar and treated with mycelia disks of hypovirulent BNR isolate W7 two days prior to inoculation of the virulent *R. solani* isolate C4 showed a low disease severity, which is highly significant compared with hypocotyls of seedling inoculated with virulent *R. solani* without non-pathogenic BNR. The BNR isolate provided 63.2 % protection to the seedling against the pathogen. The cucumber seedling inoculated with the non-pathogenic BNR alone exhibited low disease severity which indicate faint lesion and slight browning of the root portion and can be considered as a non-pathogenic reaction [40].

Summary and conclusions

In recent years the interest in biological control of plant has been growing. Chemicals become insufficient, and their use carries the risk of environmental pollution, changes in global climate, the emergence of novel, more virulent pathogens and threat to human health. Fungi can be effectively and safely used as biocontrol agents. A great attention is paid to the fungi of the genus *Rhizoctonia*, especially binucleate isolates which are common in the plant rhizosphere. They are considered the microorganisms usable as an alternative to chemical pesticides. Many literature data confirm the use of these microorganisms to plant protection by competition for nutrients with pathogens and thus they inhibit their growth. BNR can promote plant growth and stimulate plant resistance against different phytopathogens.

Research conducted for about 25 years led to a commercial use of the registered biological agents for controlling plant diseases.

Further study of mechanisms of biocontrol and the ecology of the NpR should aid in the selection and development of the most effective biocontrol agents from within these fungi.

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MOŻLIWOŚCI ZASTOSOWANIA DWUJĄDROWYCH IZOLATÓW *Rhizoctonia* spp. W BIOLOGICZNEJ OCHRONIE ROŚLIN

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Abstrakt: *Rhizoctonia* spp. to duża, zróżnicowana i powszechnie występująca w glebie grupa grzybów. Zwykle są one patogenami wielu ważnych gospodarczo gatunków roślin. Powodują zgnilizny korzeni, są także często przyczyną chorób łodyg i liści. Mają szeroki zakres roślin żywicielskich. Obok możliwości oddziaływania patogenicznego mogą one również wchodzić w związki symbiotyczne, np. ze storczykami. Gatunki dwujądrowe *Rhizoctonia* są dość częste w środowisku i występują obok innego ważnego patogena roślin, którym jest wielojądrowy *Rhizoctonia solani* Kühn.

Występowanie chorób roślin skutkuje niższymi i o gorszej jakości plonami, a ochrona przed skutkami chorób roślin podnosi koszty produkcji. W celu ograniczenia strat możliwe jest stosowanie chemicznych środków ochrony roślin, to jednak powoduje skażenie gleby i przyczynia się do wykształcania u patogenów odporności na fungicydy. Alternatywą jest bezpieczna dla środowiska biologiczna ochrona roślin z wykorzystaniem hypowirulentnych izolatów dwujądrowych *Rhizoctonia* (BNR). W tym celu stosowane są izolaty z różnych grup anastomozowych. Mogą one być wykorzystywane jako czynniki biologiczne w ochronie roślin przeciw różnym grzybom chorobotwórczym, w tym także przeciw *Rhizoctonia solani*. Jest to możliwe, dlatego że zajmują one te same nisze ekologiczne, co patogeny. Dodatkowo izolaty BNR mają zdolność wzbudzania naturalnej odporności roślin i stymulowania ich wzrostu.

Słowa kluczowe: biologiczna ochrona, dwujądrowe *Rhizoctonia*

