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LUTTE CONTRE *PYTHIUM ULTIMUM* CHEZ LA TOMATE DE SERRE: UNE APPROCHE MICROBIENNE

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RÉSUMÉ COURT

Au cours de ces travaux, 237 micro-organismes ont été isolés de la rhizosphère de plants de tomate cultivés en laine de roche ou en substrats organiques. Quarante de ces micro-organismes ont démontré un effet antagoniste *in vitro* envers le champignon pathogène *Pythium ultimum*, parmi lesquels huit (*Penicillium brevicompactum, P. solitum* souche 1, *Trichoderma atroviride, Pseudomonas fluorescens, P. fluorescens* sous-groupe G souche 2, *P. marginalis, P. putida* sous-groupe B souche 1 et *P. syringae* souche 1) ont permis de réduire de façon marquée le développement de la pourriture pythienne causée par *P. ultimum* chez la tomate de serre cultivée en conditions hydroponiques. La réduction de la maladie était associée, dans la plupart des cas, à un meilleur développement de la plante résultant en une augmentation significative du rendement en fruits et de la croissance végétative. Parmi ces micro-organismes, la bactérie *P. putida* sous-groupe B souche 1 et le champignon *T. atroviride* ont également augmenté significativement la croissance reproductive des plants de tomate sains cultivés en hydroponie. Cet effet stimulant pourrait être attribuable, à tout le moins en partie, à une régulation de la concentration d'auxines au niveau de la rhizosphère par ces micro-organismes montrant une activité 1-aminocyclopropane-1-carboxylate (ACC) désaminase et capables de synthétiser et de dégrader l'acide indole-acétique (AIA). Les travaux ont, par ailleurs, montré que *P. ultimum* est en mesure de produire des composés auxiniques (notamment l'AIA) et que l'AIA appliqué au niveau des racines ou des parties aériennes influence le développement des symptômes de pourriture pythienne chez la tomate.

RÉSUMÉ LONG

Au cours de cette étude, 237 micro-organismes ont été isolés de cinq substrats utilisés pour la culture de la tomate de serre. De ceux-ci, 40 micro-organismes ont réduit la croissance mycélienne *in vitro* de *Pythium aphanidermatum* et de *Pythium ultimum* parmi lesquels plusieurs permettaient de réduire la fonte des semis causée par ces deux agents pathogènes. Des travaux ont par la suite été entrepris afin de tester la capacité de ces micro-organismes à réduire la pourriture pythienne (*P. ultimum*) chez des plants de tomate matures cultivés en conditions hydroponiques. Les résultats obtenus ont montré que *Penicillium brevicompactum, P. solitum* souche 1, *Pseudomonas fluorescens, P. marginalis, P. putida* sous-groupe B souche 1, *P. syringae* souche 1 et *Trichoderma atroviride* ont réduit le taux d'infection par *P. ultimum*, amélioré l'ancrage dans le substrat et augmenté le rendement vendable des plants cultivés dans de la laine de roche. Les expériences effectuées dans un substrat organique contenant de la tourbe, de la sciure de pin et du compost (*v/v/v*; 60/30/10) ont également démontré la capacité de la plupart de ces micro-organismes à réduire la pourriture pythienne et à augmenter l'ancrage des plants.

P. putida sous-groupe B souche 1 et *T. atroviride* ont par ailleurs augmenté significativement la croissance reproductive des plants sains cultivés en conditions hydroponiques, et ce aussi bien en laine de roche qu'en substrat organique. Cet effet stimulant pourrait être attribuable, à tout le moins en partie, à une régulation de la concentration d'auxines au niveau de la rhizosphère par ces micro-organismes et à une production microbienne de 1-aminocyclopropane-1-carboxylate (ACC) désaminase. À cet effet, les travaux réalisés suggèrent que la synthèse microbienne d'acide indole-acétique (AIA) à partir de tryptophane serait impliquée dans la stimulation du développement de plantules de tomate d'une part ; ils démontrent d'autre part que la présence de *P. putida* et *T. atroviride* au niveau de la rhizosphère réduit l'effet inhibiteur de l'AIA sur l'élongation racinaire.

Les travaux ont également montré que *P. ultimum* est en mesure de produire des composés auxiniques (notamment l'AIA) et que l'AIA appliqué au niveau des racines ou des parties aériennes influence le développement des symptômes de pourriture pythienne chez la tomate. Plus spécifiquement, les travaux ont montré que de faibles (0 à 0,1 µg/ml) concentrations d'AIA exogène au niveau de la rhizosphère ont augmenté la sévérité des symptômes causées par *P. ultimum,* tandis que de fortes concentrations (10 µg/ml), appliquées soit au niveau du substrat ou au niveau des parties aériennes, ont réduit les dommages causés par cet agent pathogène.

Cette étude a permis d'identifier des micro-organismes capables d'une part de limiter les dommages attribuables au champignon *P. ultimum* chez la tomate de serre et d'autre part de stimuler la croissance des plants de tomate. Considérant l'importance de cet agent pathogène responsable de pertes substantielles dans la culture de la tomate de serre en milieu hydroponique, ces micro-organismes bénéfiques pourraient trouver des applications dans la culture commerciale de ce légume.

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INTRODUCTION GÉNÉRALE

Au Canada, la production de tomates (*Lycopersicon esculentum* Mill.) de serre s'est développée rapidement au cours des dernières années, atteignant 445 hectares en culture en 2005 (Statistique Canada, 2005). La culture de la tomate de serre se fait principalement en systèmes hydroponiques, lesquels présentent de nombreux avantages tels que la stérilité du substrat en début de culture ainsi qu'un contrôle constant de l'irrigation et de la fertilisation. Les cultures hydroponiques utilisant des substrats inertes s'avèrent toutefois propices au développement de certaines maladies causées par des agents pathogènes bien adaptés aux milieux humides (Zinnen, 1988 ; Stanghellini et Rasmussen, 1994 ; Paulitz et Bélanger, 2001). *Pythium ultimum* Trow, l'agent pathogène responsable de la pourriture pythienne chez la tomate de serre, se développe et se propage rapidement dans de tels milieux. Cette maladie, devenue problématique en raison du manque de moyens de contrôle efficaces, cause des pertes importantes du rendement en fruits résultant de la dégénérescence graduelle du système racinaire infecté. Une avenue intéressante pour lutter contre cet agent pathogène consiste en l'utilisation d'agents microbiens bénéfiques bien adaptés aux conditions qui prévalent en cultures hydroponiques.

De nombreuses bactéries (Amer et Utkhede, 2000 ; Patten et Glick, 2002) ainsi que plusieurs champignons (Kleifeld et Chet, 1992 ; Yedidia et al., 2001 ; Caron et al., 2002) sont reconnus pour avoir un effet bénéfique sur le développement des plantes. Généralement, cet effet bénéfique sur la croissance de la plante hôte résulte d'une activité antagoniste envers certains agents pathogènes, réprimant ainsi le développement d'infections, ou d'un effet stimulant sur la croissance de la plante (Whipps, 2001 ; Persello-Cartineaux et al., 2003). La production de régulateurs de croissance, en particulier d'auxines notamment l'acide indole-acétique (AIA), est un mécanisme

souvent associé à l'effet bénéfique attribué à certains micro-organismes (Patten et Glick, 2002 ; Vessey, 2003 ; Antoun et Prévost, 2005).

Dans le contexte actuel où sont valorisées des approches respectueuses de l'environnement, les micro-organismes bénéfiques revêtent un intérêt particulier. L'objectif de cette thèse était d'identifier des micro-organismes bénéfiques capables d'une part de réduire les dommages causés par *Pythium* spp. chez la tomate de serre et d'autre part de stimuler la croissance des plants. Les travaux visaient également d'étudier dans quelle mesure l'AIA est impliquée dans l'effet bénéfique observé.

Cette thèse est divisée en cinq chapitres. Le premier chapitre consiste en une revue bibliographique traitant de la tomate de serre, des maladies causées par *Pythium* spp. chez cette culture et leur contrôle ainsi que des micro-organismes bénéfiques. Le deuxième chapitre décrit le processus de sélection d'agents antagonistes envers *P. ultimum* et *Pythium aphanidermatum* (Edson) Fitz. Le troisième chapitre présente l'étude de l'effet des micro-organismes sur le développement de la pourriture pythienne et la croissance des plants infectés. Le quatrième chapitre porte sur l'effet stimulant de micro-organismes sélectionnés sur des plants sains et sur les mécanismes impliqués. Enfin, le cinquième chapitre de cette thèse décrit l'effet de l'AIA sur le développement des symptômes causés par *P. ultimum* chez la tomate.

CHAPITRE 1

REVUE DE LITTÉRATURE

1.1 Tomate de serre

Au Canada, la production en serre s'est développée rapidement au cours des vingt dernières années pour atteindre une superficie de près de 1900 hectares en 2005 (Statistique Canada, 2005). L'industrie canadienne de la serriculture s'est principalement développée en Colombie-Britannique, en Ontario et au Québec. En 2005, la valeur des produits de serre a atteint 2,1 milliards de dollars, dont 30% provenait des productions légumières (Statistique Canada, 2005), la tomate de serre étant la principale culture légumière. De 1998 à 2005, une augmentation de 27% a été observée au niveau des rendements en fruits des plants de tomate atteignant 210 000 tonnes pour une valeur de 384,6 millions de dollars pour l'ensemble du Canada (Statistique Canada, 2005). En 2005, le Québec représentait environ 12% de la superficie totale canadienne allouée à la tomate de serre pour une valeur de la production atteignant 39 millions de dollars (Statistique Canada, 2005). Bien que la majeure partie des tomates produites en champ est utilisée pour la transformation, les tomates produites en serre, considérées comme des produits de luxe, se vendent principalement pour le marché frais. Sa consommation sous forme crue ou cuite est très répandue, en grande partie en raison de ses qualités nutritives. En effet, la tomate est une bonne source d'antioxydants tels la vitamine C et le lycopène (Leonardi et al., 2000 ; Stewart et al., 2000 ; Toor et Savage, 2005 ; Toor et al., 2006).

1.1.1 Conditions de croissance en serre

Plusieurs techniques mises en place ont permis d'améliorer la culture en serre de la tomate. L'éclairage artificiel, les systèmes de chauffage et l'enrichissement en CO_2 en sont quelques exemples (McAvoy et Janes, 1984 ; Yelle et al., 1987). L'amélioration de la planification des cultures par le développement de techniques, telles que les cultures intercalées, permet maintenant d'obtenir un approvisionnement en tomates constant durant toute l'année grâce à une utilisation optimisée des ressources (Dorais et al., 1991).

1.1.2 Substrats

La production de tomates en serre se fait selon deux types de culture soit la culture en plein sol et la culture en hydroponie avec ou sans substrat. Parmi les substrats utilisés pour la culture de la tomate de serre, la laine de roche et la fibre de coco sont les plus utilisées par les producteurs canadiens. La facilité de manipulation, leur grande capacité de rétention en eau, leurs composantes non assimilables par la plante et sa stérilité (en début de production) expliquent sa popularité (Papadopoulos, 1991). La fertilisation des plants dans les systèmes hydroponiques se fait au moyen d'une solution nutritive. L'utilisation à grande échelle de la laine de roche pose deux problèmes majeurs. D'une part ce matériel non recyclable engendre de grandes quantités de déchets et d'autre part, le grand volume de solution nutritive rejeté dans le cas des systèmes en drainage libre peut devenir une importante source de contamination du sol (Fricke, 1998). Ceci pousse de plus en plus les producteurs à opter pour des substrats organiques. La sciure de bois est un exemple de substrat organique dont la popularité est grandissante. Cette popularité est attribuable, en grande partie, à leur facilité d'approvisionnement au Canada. Dans ces substrats, la fertilisation se fait le plus souvent par l'entremise du système d'irrigation (Papadopoulos, 1991). Les systèmes hydroponiques en recirculation sont, également, de plus en plus populaires. Ces systèmes permettent une économie d'échelle d'eau d'irrigation et de fertilisants.

1.2 Maladies de la tomate de serre

Les conditions environnementales à l'intérieur de la serre, plus particulièrement la température et l'humidité relative, peuvent s'avérer propices au développement de certaines maladies foliaires (Paulitz et Bélanger, 2001). De plus, l'uniformité des plants et la densité de plantation permettent une propagation rapide d'agents pathogènes d'un plant infecté à un plant sain (Paulitz, 1997). Les agents pathogènes aériens peuvent être introduits par le système de ventilation alors que les agents pathogènes telluriques peuvent l'être par l'intermédiaire du substrat ou d'outils contaminés (Jarvis, 1992; Paulitz, 1997 ; Paulitz et Bélanger, 2001). Certains agents pathogènes sont, quant à eux, introduits et dispersés par l'eau d'irrigation (Stanghellini et Rasmussen, 1994). Les agents pathogènes bien adaptés à de forts taux d'humidité sont, par conséquent, favorisés par les conditions de culture en systèmes hydroponiques.

1.2.1 Fonte des semis

La fonte des semis est une maladie qui se caractérise par un faible taux de levée et un affaissement des plantules avant qu'elles aient atteint la maturité (Richard et Boivin, 1994). Les graines peuvent être infectées avant la germination ou suite à l'émergence hors du substrat (Fig. 1). Chez la tomate, la fonte des semis est causée par plusieurs espèces de *Pythium* dont *P. aphanidermatum* et *P. ultimum.*

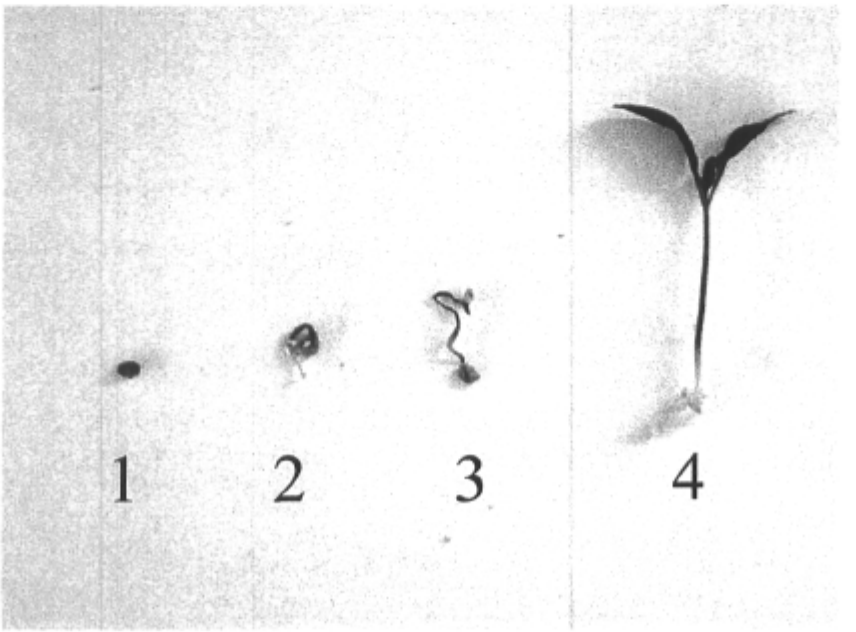

Figure 1. Dommages causés par *Pythium ultimum* sur des semis de tomate: (1) pourriture de la graine, (2) fonte des semis en pré-levée, (3) fonte des semis en post-levée, (4) plantule saine.

1.2.2 Pourriture pythienne de la tomate

La pourriture pythienne chez la tomate de serre est principalement causée par *P. ultimum*. Toutefois, *P. aphanidermatum* peut également être responsable de cette maladie. Le flétrissement des plants de tomate en période de chaleur et d'ensoleillement intense est une indication de la présence de pourriture pythienne (Rankin et Paulitz, 1994). Les symptômes les plus courants au niveau du système racinaire sont des lésions brunes et humides. Dans les cas sévères, les racines deviennent visqueuses et flasques (Fig. 2). Cette dégradation du système racinaire affectera éventuellement l'ancrage des plants dans le substrat.

Figure 2. Pourriture pythienne (*Pythium ultimum*) chez la tomate de serre.

1.2.3 *P. ultimum* et *P. aphanidermatum*

1.2.3.1 Description et classification

Les *Pythium* spp. sont habituellement considérés comme des champignons aquatiques. Toutefois, du point de vue génétique, biochimique et morphologique, les oomycètes seraient plus près des algues chrysophytes que des vrais champignons. Actuellement, les classifications suivantes sont acceptées (Carlile et al., 2001 ; Agrios, 2005):

Règne : Chromista	Règne : Stramenopiles
Division : Oomycota	Division : Oomycota
Classe : Oomycètes	Classe : Oomycètes
Ordre : Péronosporales	Ordre : Péronosporales
Famille : Pythiaceae	Famille : Pythiaceae
Genres : *Pythium*	Genres : *Pythium*
Espèces : *ultimum* ou *aphanidermatum*	Espèces : *ultimum* ou *aphanidermatum*

Les différentes espèces de *Pythium* se développent sous la forme d'un mycélium blanc. La figure 3 montre le cycle vital des champignons du genre *Pythium*. En reproduction asexuée, les sporanges, formés directement sur les hyphes et non sur des sporangiophores, déversent les zoospores flagellées dans une vésicule qui éventuellement se rompera pour libérer les zoospores (Gilman, 1957). En reproduction sexuée, la fusion d'une anthéridie et d'une oogone résulte en la formation d'une oospore. Une nouvelle hyphe se développe suite à la germination des oospores ou des zoospores enkystées (Agrios, 2005).

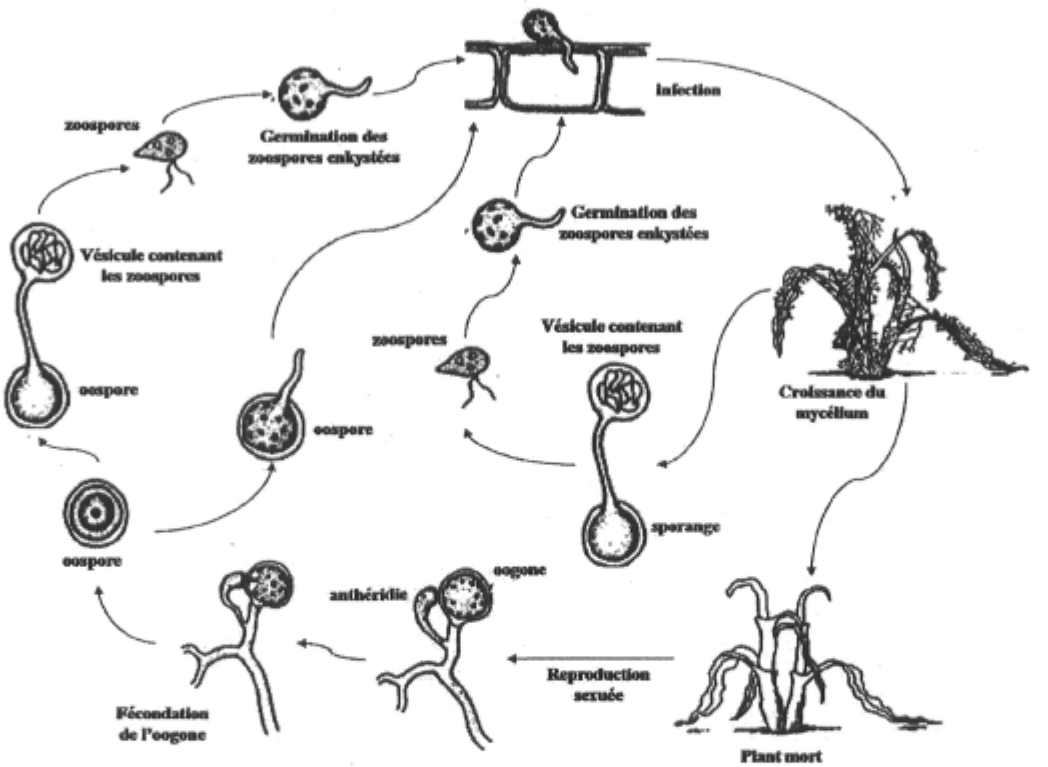

Figure 3. Cycle vital des champignons du genre *Pythium.* (Adapté de Agrios [2005])

1.2.3.2 Morphologie

Les hyphes de *P. aphanidermatum*, dont le diamètre se situe entre 2,8 µm et 7,3 µm, sont transparentes et non septées à l'exception des fructifications (Gilman, 1957). Les sporanges sont filamenteux et renflés. Les zoospores (12 µm par 7,5 µm) sont uniformes et portent deux flagelles disposés latéralement (Gilman, 1957). Les oogones sont sphériques et habituellement terminales. Les anthéridies sont typiquement intercalaires et mononucléiques.

Chez *P. ultimum,* les hyphes peuvent être septées, mais chez des cultures âgées uniquement (Gilman, 1957). Le diamètre des hyphes se situe entre 1,7 µm et 6,5 µm. Il est important de mentionner que *P. ultimum* var. *ultimum* ne produit pas de zoospores (Gilman, 1957; Francis et St. Clair, 1997). Les oogones sont lisses, sphériques et habituellement terminales. Les anthéridies originent de la base de l'oogone. Les oospores sphériques (14,7 µm à 18,3 µm de diamètre) ne remplissent généralement pas l'intérieur de l'oogone (Gilman, 1957).

1.2.3.3 Dissémination

P. aphanidermatum produit des zoospores motiles dans l'eau, ce qui le rend bien adapté aux conditions aquatiques (Agrios, 2005). En serre, la dissémination de cet agent pathogène se fait par conséquent principalement par la solution nutritive. Les zoospores sont attirées par chimiotaxie vers les exsudats racinaires et sont en mesure d'infecter la plante (Paulitz, 1997). Pour ce qui est de *P. ultimum*, la croissance mycélienne d'un plant infecté vers un plant sain constitue le principal moyen de dissémination. Les oospores servent habituellement de structures de survie en absence de plantes hôtes (Green et Jensen, 2000).

1.2.4 Autres maladies de la tomate de serre

En marge des maladies causées par *Pythium* spp., plusieurs maladies affectent la tomate de serre au Québec et au Canada. Parmi les maladies fongiques, la moisissure grise (*Botrytis cinerea* Pers:Fr), la brûlure tardive (*Phytophthora infestans* (Mont) de Bary) et la fusariose vasculaire (*Fusarium oxysporum* Schlechtend:Fr. f. sp. *lycopersici* W.R. Jarvis & Shoemaker) ou des racines (*F. oxysporum* Schlechtend:Fr. f. sp. *radicis-lycopersici* W.C. Snyder & H.N. Hans.) causent le plus de dommages (Richard et Boivin, 1994). Le chancre bactérien (*Clavibacter michiganensis* subsp. *michiganensis* (Smith) Davies et al.) est la maladie bactérienne la plus commune. La maladie bronzée de la tomate et la mosaïque de la tomate et du tabac sont les maladies virales les plus importantes (Richard et Boivin, 1994).

1.3 Moyens de lutte contre la fonte des semis et la pourriture pythienne

1.3.1 Pratiques culturales et sanitaires

Certaines pratiques culturales, telles la désinfection des instruments de travail et des espaces de culture ainsi que l'utilisation de matériel végétal exempt d'agents pathogènes, doivent être mises en place afin d'éviter l'introduction de l'inoculum dans le système (Jarvis, 1992). Toutefois, l'exclusion complète d'agents pathogènes est pratiquement impossible. Le choix du substrat a également une influence sur l'incidence des maladies. Les substrats inertes comme la laine de roche présentent l'avantage d'être relativement stériles en début de culture. Ils sont cependant plus vulnérables au développement de la pourriture pythienne (Stanghellini et Rasmussen, 1994 ; Zinnen, 1988). Cette vulnérabilité est attribuable au fait que ces substrats ne possèdent pas de tampon biologique qui diminue habituellement la dissémination et le développement des populations d'agents pathogènes non compétitifs dans le sol (Hendrix et Campbell, 1973 ; Paulitz, 1997). Juneau et collaborateurs (2006) ont démontré que l'utilisation d'un substrat organique à base de tourbe, de compost et de sciure de pin réduisait la pourriture

pythienne comparativement à la laine de roche chez des plants de tomate cultivés sous des conditions environnementales propices au développement de cette maladie. De façon générale, les pratiques culturales améliorant le développement du système racinaire aident à la prévention de la pourriture pythienne.

La désinfection de la solution nutritive, une source importante de contamination par *Pythium* spp. dans les systèmes hydroponiques, est considérée comme une méthode intéressante pour lutter contre ce pathogène. Des systèmes basés sur la pasteurisation à la chaleur, des traitements à l'ozone ou au peroxyde d'hydrogène ainsi que l'utilisation de membranes de filtration, de surfactants ou de traitements au rayons UV ont été adaptés pour une utilisation dans des systèmes de recirculation en culture hydroponique (Runia, 1995 ; Menzies et Bélanger, 1996 ; Stanghellini et Miller, 1997). Toutefois, l'efficacité de ces pratiques est variable. Par exemple, les traitements aux rayons UV diminuent les populations de *Pythium* spp. présentes dans la solution nutritive mais n'ont que peu d'effets sur l'apparition et le développement de la pourriture pythienne au niveau des plants de tomate en hydroponie (Zhang et Tu, 2000). Ce manque de répression semble lié à l'effet négatif de la désinfection sur les populations de bactéries et de champignons non pathogènes présentes au niveau de la rhizosphère des plants. La diminution de ces populations microbiennes crée un environnement où la compétition pour la niche écologique est moins importante et favorise ainsi l'établissement et le développement des propagules de *Pythium* spp. ayant survécu à la désinfection (Buyanovsky et al., 1981 ; Berger et al., 1996).

1.3.2 Lutte chimique

Pour les cultures ornementales en serre, le métalaxyl (fongicide) est utilisé comme traitement du substrat pour lutter contre les maladies causées par *Pythium* spp. (Paulitz et Bélanger, 2001). Toutefois, la lutte chimique est généralement limitée dans les cultures légumières en serre. En effet, aucun fongicide chimique n'est homologué pour ce type de cultures au Canada.

1.3.3 Lutte biologique

L'intérêt pour la lutte biologique s'est grandement développé au cours des dernières années. Les effets néfastes sur l'environnement de l'utilisation des pesticides chimiques (apparition de souches résistantes et accumulation de résidus de pesticides) ont mené à une plus grande conscientisation autant chez les producteurs que chez les consommateurs de l'impact de leur utilisation (Hunter et al., 1987 ; Nelson et Powelson, 1988 ; Malathrakis, 1989 ; Roberts, 1990). Il existe plusieurs définitions de la « lutte biologique ». Garrett (1965) définit celle-ci comme « toute condition ou pratique sous laquelle la survie ou l'activité d'un agent pathogène est réduite par l'entremise d'un autre organisme vivant (à l'exception de l'homme) avec pour résultat la réduction de la maladie causée par cet agent pathogène ». Baker et Cook (1974) la définissent plutôt comme « la réduction de la densité de l'inoculum ou de la maladie causée par un agent pathogène ou un parasite dans son stade actif ou dormant, par un ou plusieurs organismes, soit de façon naturelle ou par manipulation de l'environnement, de l'hôte ou d'un agent antagoniste ou par l'introduction massive d'un ou de plusieurs agents antagonistes ». Il existe également une définition plus simple de la lutte biologique énoncée par Cook et Baker (1983) : « la réduction de la quantité d'inoculum ou de la maladie causée par un agent pathogène résultant de l'action d'un ou plusieurs organismes autres que l'homme ». La lutte biologique peut être pratiquée de différentes façons : amendement des sols, utilisation de plantes inhibitrices, de plantes pièges ou de plantes hôtes alternatives, emploi de champignons mycorhiziens ou utilisation de micro-organismes antagonistes (Jeyarajan, 1989).

1.3.3.1 Lutte biologique avec des micro-organismes antagonistes

Un micro-organisme est considéré antagoniste lorsqu'il empêche le développement d'une maladie ou la survie d'un agent pathogène (Cook et Baker, 1983). Les modes d'action des micro-organismes antagonistes sont : la compétition, l'antibiose, le parasitisme ou l'induction des mécanismes de défense de la plante (Cook et Baker, 1983 ; Chaube et Singh, 1991 ; Whipps, 1997, 2001). Park (1960) définit la compétition comme la réduction de croissance résultant de la lutte entre organismes pour les nutriments, la

niche écologique ou tout autre facteur environnemental. Par exemple, certains micro-organismes sont en mesure de chélater les sources de fer sous la forme d'ions ferriques (Fe^{3+}) dans la rhizosphère en produisant des sidérophores, réduisant ainsi la disponibilité du fer pour l'agent pathogène (Loper et Buyer, 1991 ; O'Sullivan et O'Gara, 1992 ; Duijff et al., 1994 ; Whipps, 2001). L'antibiose, quant à elle, se définit comme l'inhibition d'un organisme par un produit métabolique d'un autre organisme, tel qu'un antibiotique ou l'acide cyanhydrique (HCN), par exemple (Castric, 1974 ; Baker et Cook, 1974). Un organisme peut également se servir d'un autre être vivant comme source de nutrition. Dans ce cas, il s'agit de parasitisme biotrophe s'il ne mène pas à la mort de l'hôte (Berry et Barnett, 1957) ou nécrotrophe si, au contraire, il entraîne la mort de l'hôte (Barnett et Binder, 1973). Enfin, certains micro-organismes exercent leur effet antagoniste en induisant chez la plante hôte des réactions de défense, ce qui implique la synthèse de différents composés par la plante. Ces composés agissent directement sur le développement de l'agent pathogène ou forment des barrières au niveau de la plante, réduisant ainsi l'infection (Campbell et Ellis, 1992 ; Benhamou, 1995).

Pour qu'un micro-organisme antagoniste puisse éventuellement être utilisé comme agent de lutte biologique il doit présenter certaines propriétés. Il doit être stable génétiquement, efficace à de faibles concentrations, en mesure de compétitionner avec les autres micro-organismes du milieu et survivre aux stress environnementaux (Wisniewski et Wilson, 1992). Un agent de lutte biologique doit être efficace sans toutefois affecter des organismes non visés, incluant l'humain (Kennedy et Kremer, 1996). Il devrait également être peu coûteux, facile d'utilisation et compatible avec les autres méthodes de lutte utilisées contre l'agent pathogène.

La culture en serre se prête bien à l'utilisation d'agents de lutte biologique (micro-organismes antagonistes) pour contrôler les maladies racinaires. L'environnement (température, humidité et conditions du substrat) est contrôlé de façon précise et peut être modifié pour favoriser l'établissement et le développement des agents de lutte biologique (Paulitz et Bélanger, 2001). Les systèmes de culture sont initialement stériles au départ, ce qui facilite grandement leur colonisation par l'agent de lutte biologique (Paulitz, 1997).

De plus, les produits serricoles sont des denrées à haute valeur économique, ce qui justifie les dépenses associées à l'application parfois plus coûteuse de ces technologies relativement nouvelles (Paulitz et Bélanger, 2001).

1.3.3.2 Micro-organismes antagonistes envers *Pythium* spp.

Plusieurs études ont démontré l'effet répressif de divers micro-organismes envers *P. aphanidermatum* et *P. ultimum* (Tableau 1). Deux biofongicides sont d'ailleurs homologués depuis peu au Canada pour lutter contre les maladies racinaires de la tomate de serre. Il s'agit de Mycostop™ (*Streptomyces griseoviridis* Anderson et al.) et de Rootshield™ (*Trichoderma harzianum* Rifai) qui peuvent être utilisés de façon préventive pour lutter contre les maladies affectant les cultures légumières en serre, incluant les maladies causées par *Pythium* spp. (RAP, 2006).

Tableau 1. Micro-organismes ayant démontré une activité antagoniste envers *Pythium aphanidermatum* et *Pythium ultimum* chez différentes plantes hôtes.

Agents pathogènes	Micro-organismes antagonistes	Plantes hôtes	Références
P. aphanidermatum	*Bacillus subtilis*	Laitue	Utkhede et al. (2000)
	Pseudomonas aureofaciens	Concombre	Chen et al. (1998)
	Pseudomonas chlororaphis	Poivron	Khan et al. (2003)
	Pseudomonas corrugata	Concombre	Zhou et Paulitz (1993)
			Rankin et Paulitz (1994)
			Chen et al. (1998)
	Pseudomonas fluorescens	Tomate	Zhou et Paulitz (1993)
			Ramamoorthy et al. (2002b)
		Concombre	Rankin et Paulitz (1994)
			Postma et al. (2001)
	Pythium oligandrum	Concombre	Postma et al. (2001)
	Streptomyces griseoviridis	Concombre	Postma et al. (2001)
	Trichoderma harzianum	Concombre	Postma et al. (2001)
P. ultimum	*Enterobacter cloacae*	Coton	van Dijk et Nelson (1998)
		Blé	Kageyama et Nelson (2003)
		Carotte	Kageyama et Nelson (2003)
		Laitue	Kageyama et Nelson (2003)
	Fusarium oxysporum	Concombre	Benhamou et al. (2002)
	Pseudomonas corrugata	Betterave	Georgakopoulos et al. (2002)
		Concombre	Georgakopoulos et al. (2002)
		Maïs	Pandey et al. (2001)
	Pseudomonas fluorescens	Concombre	Georgakopoulos et al. (2002)
			Carisse et al. (2003)
		Betterave	Thrane et al. (2000)
	Pseudomonas putida	Betterave	Shah-Smith et Burns (1996)
	Pythium oligandrum	Concombre	Ali-Shtayeh et Saleh (1999)
	Pythium periplocum	Concombre	Ali-Shtayeh et Saleh (1999)
	Trichoderma harzianum	Concombre	Caron et al. (2002)
		Tomate	Caron et al. (2002)

1.3.3.3 Rhizosphère : source de micro-organismes antagonistes

Le terme rhizosphère réfère au volume de sol qui entoure le système racinaire et qui est directement influencé par celui-ci. Il s'agit d'une zone où la plante, le sol, les micro-organismes et la microfaune sont en constantes interactions (Antoun et Prévost, 2005). Les micro-organismes présents dans la rhizosphère sont variés. Ils peuvent avoir un effet bénéfique, neutre ou délétère sur la plante. Dans le cas des maladies racinaires, il s'agit du lieu de rencontre entre l'agent pathogène et l'agent de lutte biologique. Ainsi, toutes les interactions possibles doivent être prises en considération afin de bien comprendre la complexité et les contraintes de la lutte biologique au niveau du sol (Whipps, 1997, 2001). La rhizosphère constitue une bonne source de micro-organismes antagonistes potentiellement efficaces comme agents de lutte biologique puisqu'ils sont bien adaptés aux conditions du milieu et que dans certains cas, comme par exemple pour les rhizobactéries, ils ont évolué avec la plante et sont capables de coloniser activement le système racinaire de celle-ci en présence d'une microflore compétitive (Schroth et Hancock, 1982 ; Antoun et Kloepper, 2001).

1.4 Micro-organismes stimulant la croissance des plantes

L'effet bénéfique de certains micro-organismes ne se limite pas dans tous les cas à une réduction des dommages causés par des agents pathogènes. Certains micro-organismes peuvent également avoir un effet bénéfique sur la croissance et le développement de la plante hôte. Le terme PGPR (Plant growth-promoting rhizobacteria) est utilisé pour décrire les bactéries qui, lorsqu'introduites dans un sol contenant une microflore compétitive, ont un effet positif sur la croissance de la plante (Kloepper et Schroth, 1978). On peut élargir ce concept afin d'inclure les champignons ayant un effet bénéfique sur la croissance de la plante. Ces champignons sont nommés PGPF (Plant growth-promoting fungi). Les mécanismes d'action de ces micro-organismes stimulants sont variés et rarement exclusifs. L'effet stimulant peut être indirect si l'augmentation de la croissance de la plante est due à la répression d'une maladie (Persello-Cartineaux et al., 2003) ou lorsque le micro-organisme dégrade des composés phytotoxiques dans un sol contaminé (Jacobsen, 1997). L'effet stimulant direct, quant à lui, implique soit l'amélioration de la

nutrition minérale de la plante (fixation du N_2, solubilisation du phosphore), soit la production de régulateurs de croissance (Vessey, 2003 ; Antoun et Prévost, 2005).

L'utilisation des PGPRs ou des PGPFs en agriculture pourrait mener à une réduction importante de la quantité de pesticides et de fertilisants chimiques utilisée. Ainsi la lutte contre certains agents pathogènes et l'amélioration des rendements des cultures pourraient se faire de façon simultanée et complémentaire (Antoun et Prévost, 2005).

1.4.1 Solubilisation du phosphore

La disponibilité du phosphore sous forme soluble dans le sol est souvent un facteur limitant pour la croissance et le développement des plants (Rodriguez et Fraga, 1999). La solubilisation du phosphore inorganique par les micro-organismes augmente la disponibilité de cet élément dans la rhizosphère et constitue un des mécanismes d'action les plus souvent impliqués dans la stimulation de la croissance des plantes cultivées en champ par les PGPRs (Richardson, 2001). De nombreuses études ont d'ailleurs démontré la capacité de bactéries des genres *Pseudomonas*, *Bacillus*, *Rhizobium* et *Erwinia* à solubiliser des sources insolubles de phosphore (Goldstein, 1986). Certains champignons, notamment des genres *Penicillium* et *Trichoderma*, possèdent également la capacité de solubiliser le phosphore (Illmer et Schinner, 1992 ; Altomare et al., 1999 ; Yedidia et al., 2001). Dans la plupart des cas rapportés, la solubilisation des sources de phosphore inorganiques insolubles par les micro-organismes telluriques semble résulter de la synthèse d'acides organiques (Banik et Dey, 1982 ; Halder et al., 1990 ; Illmer et Schinner, 1992).

1.4.2 Production d'acide indole-3-acétique (AIA)

Le développement des plantes, autant en phase végétative que reproductive, est contrôlé en majeure partie par des régulateurs de croissance. Parmi ceux-ci, l'acide indole-3-acétique (AIA) est l'auxine naturelle la plus abondante et la plus étudiée (Fig. 4). L'effet de l'AIA sur la croissance de la plante peut être positif ou négatif selon la concentration considérée. L'AIA semble impliquée dans la formation des fruits chez la tomate au

moment de l'expansion cellulaire ainsi que dans le développement final du fruit (Srivastava et Handa, 2005). L'application exogène d'AIA augmente la croissance de la tige jusqu'à une concentration de 10^{-5} M alors que la croissance des racines atteint son maximum à une concentration beaucoup plus faible, soit 10^{-9} M (Tanimoto, 2005). Une forte concentration d'AIA (de l'ordre de 10^{-6} M) réduit l'élongation des racines et augmente la formation de racines secondaires (Tanimoto, 2005). L'AIA affecte la croissance des plantes vraisemblablement en influençant la concentration d'éthylène, un important régulateur de croissance végétale (Xie et al., 1996 ; Glick et al., 1998). L'acide aminocyclopropane-1-carboxylique (ACC), le précurseur immédiat de l'éthylène, est synthétisé à partir du *S*-adénosylméthionine (SAM) sous l'action de l'ACC synthase (Czarny et al., 2006). L'AIA stimule l'activité de l'ACC synthase, ce qui induit une production accrue d'ACC (Kende, 1993). Une plus grande quantité d'éthylène peut donc être synthétisée à partir de l'ACC sous l'action de l'ACC oxydase, ce qui a pour effet de réduire l'élongation des racines (Kende, 1993 ; Glick et al., 1997, 1998 ; Hansen et Grossman, 2000).

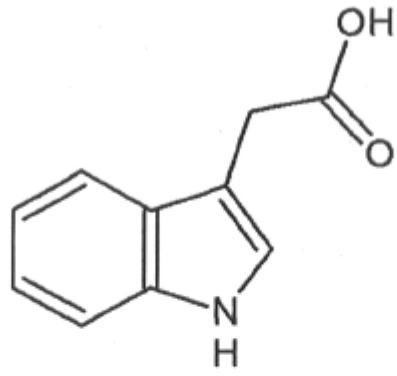

Figure 4. Structure moléculaire de l'acide indole-3-acétique (AIA).

La capacité de produire de l'AIA a déjà été rapportée chez plusieurs bactéries et champignons, autant bénéfiques que pathogènes (Tableau 2). Chez les micro-organismes bénéfiques, la production d'AIA est très souvent associée à leur effet bénéfique sur la croissance des plantes. Patten et Glick (2002) ont démontré que l'AIA produit par *Pseudomonas putida* (Trevisan) Migula GR12-2 joue un rôle important dans le développement optimal du système racinaire de la plante hôte. Des résultats similaires ont également été rapportés pour *Pythium oligandrum* Drechsler, un champignon antagoniste (Le Floch et al., 2003).

Tableau 2. Micro-organismes ayant la capacité de synthétiser l'acide indole-3-acétique (AIA).

Micro-organismes	**Références**
Azospirillum spp.	Pedraza et al. (2004)
Erwinia herbicola	Brandl et al. (1996)
Fusarium oxysporum	Hasan (2002)
Paenibacillus polymyxa	Lebuhn et al. (1997)
Pseudomonas fluorescens	Beyeler et al. (1999)
Pseudomonas putida	Patten et Glick (2002)
Pythium groupe F	Rey et al. (2001) Le Floch et al. (2003)
Pythium oligandrum	Le Floch et al. (2003)
Pythium ultimum	Rey et al. (2001)
Rhizobium leguminosarum	Antoun et al. (1998)

Plusieurs voies de synthèse de l'AIA ont été identifiées autant chez les plantes que chez les bactéries (Fig. 5). Chez les plantes supérieures, la principale voie de synthèse de l'AIA implique la formation, à partir du tryptophane, d'acide indole-3-pyruvique et d'indole-3-acétaldéhyde (Persello-Cartieaux et al., 2003). Cette voie de synthèse semble également être utilisée par certaines bactéries bénéfiques. Les bactéries phytopathogènes semblent, quant à elles, produire l'AIA à partir du tryptophane en passant par l'indole-3-acétamide (Costacurta and Vanderleyden, 1995). Cette voie de synthèse serait spécifique aux micro-organismes (Manulis et al., 1991). Bien que certaines voies de synthèse indépendantes du tryptophane aient été identifiées chez certains micro-organismes, la production microbienne d'AIA au niveau de la rhizosphère semble dépendante du tryptophane exsudé par la plante.

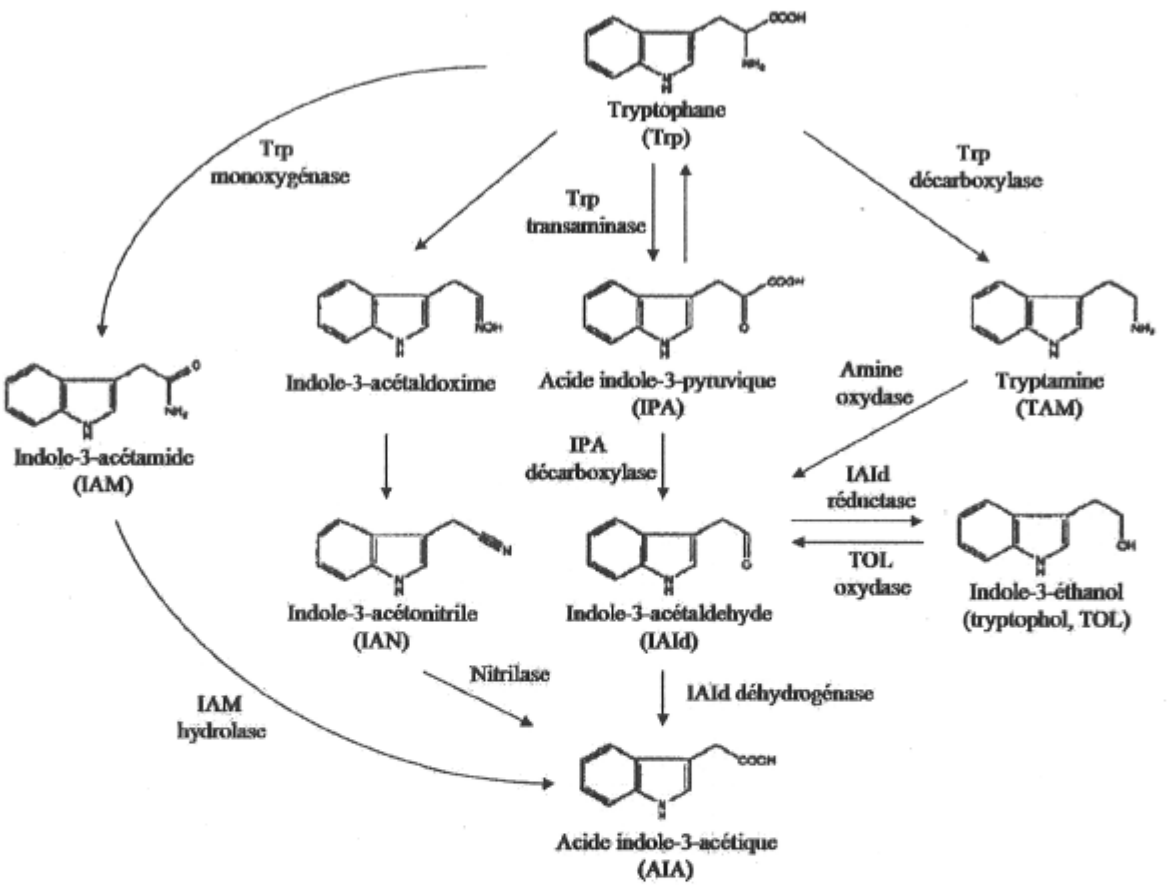

Figure 5. Voies de synthèse de l'acide indole-3-acétique (AIA) à partir du tryptophane (Adaptée de Bartel [1997]).

1.4.3 Dégradation de l'acide indole-acétique (AIA)

En plus d'avoir la capacité de produire de l'AIA, certains micro-organismes ont également la capacité de le dégrader (Proctor, 1958 ; Libbert and Risch, 1969). Leveau et Lindow (2005) ont d'ailleurs démontré que *P. putida* souche 1290 est en mesure d'utiliser l'AIA comme unique source de carbone et d'azote pour son développement. Les racines étant très sensibles à l'AIA (Scott, 1972), la présence de tels micro-organismes peut s'avèrer bénéfique pour le développement du système racinaire et de la plante en général en régulant la concentration de ce régulateur de croissance dans la rhizosphère.

1.4.4 ACC désaminase d'origine microbienne

L'effet stimulant des micro-organismes présents dans la rhizosphère peut également être lié à leur capacité de synthétiser l'ACC désaminase. Cette enzyme dégrade l'ACC en ammonium et en α-cétobutyrate par hydrolyse, ce qui réduit la quantité d'ACC dans la rhizosphère. Afin de maintenir un équilibre, la plante exsude une plus grande quantité d'ACC au niveau des racines, réduisant ainsi la quantité de ce précurseur pouvant être transformée en éthylène (Glick et al., 1998 ; Penrose et Glick, 2001). Une diminution de la concentration d'éthylène réduit l'inhibition de croissance causée par ce dernier sur le système racinaire et indirectement sur le développement de la plante (Glick et al., 1998 ; Wang et al., 2000 ; Penrose et Glick, 2001 ; Safronova et al., 2006).

1.5 AIA et maladies

L'effet de l'AIA d'origine microbienne influence habituellement le développement de symptômes chez la plante hôte. Certaines bactéries, telles *Agrobacterium tumefaciens* (Smith & Townsend) Conn, induisent la production de tumeurs chez l'hôte en affectant la régulation de la synthèse de certains régulateurs de croissance (auxines et cytokinines). La croissance accrue des cellules autour du site d'infection favorise la croissance

bactérienne et l'infection de l'hôte (White et Ziegler, 1991). L'AIA semble également impliqué de façon indirecte dans la pathogénécité de *Pythium* groupe F, un agent pathogène mineur de la tomate de serre (Rey et al., 2001). Les changements physiologiques au niveau du système racinaire résultant d'une augmentation de la concentration d'AIA semblent faciliter l'infection par certains micro-organismes pathogènes incluant *Pythium* spp. (Yamada, 1993).

Dans certains cas, l'AIA semble avoir un effet répressif sur le développement des maladies. Des études ont démontré que l'AIA réduit le développement de certains agents pathogènes *in vitro* (Martínez Noël et al., 2001 ; Sharaf et Farrag, 2004 ; Slininger et al., 2004 ; Ueno et al., 2004). On rapporte également que l'application d'AIA exogène réduit les dommages causés par *Phythophthora infestans* et *Gibberella pulicaris* (Fr.) Sacc. chez la pomme de terre (Martínez Noël et al., 2001 ; Slininger et al., 2004). L'effet répressif de l'AIA a, entre autres, été associé à la régulation de l'activité enzymatique de la glutathione S-transférase (GST) causant une nécrose des cellules avoisinant les cellules infectées (Hahn et Strittmatter, 1994). Des études menées chez la tomate et chez l'orge avec *Fusarium oxysporum* Schlechtend:Fr. (Sharaf et Farrag, 2004) et *Magnaporthe grisea* (Hebert) Barr (Ueno et al., 2004), respectivement, ont démontré que l'AIA serait impliqué dans la stimulation des mécanismes de défense de la plante hôte. Chez l'orge, la répression de *M. grisea* serait liée à une activité accrue des chitinases, peroxydases et de la phenylalanine ammonia lyase de la plante hôte (Ueno et al., 2004).

1.6 Hypothèses de recherche

Ce projet de doctorat était destiné à vérifier les hypothèses suivantes :

1. Certains micro-organismes naturellement présents au niveau de la rhizosphère de plants de tomate ont un effet répressif envers la fonte des semis (*P. aphanidermatum* et *P. ultimum*) et la pourriture pythienne (*P. ultimum*) de la tomate de serre cultivée en conditions hydroponiques.

2. Certains micro-organismes présents au niveau de la rhizosphère ont un effet stimulant sur la croissance des plants de tomate sains qui résulte de l'effet synergique de plusieurs mécanismes incluant l'amélioration de la nutrition minérale de la plante ou la régulation de la concentration de certains régulateurs de croissance.

3. L'AIA affecte le développement de symptômes liés à l'infection par *P. ultimum* chez des plants de tomate.

1.7 Objectifs de recherche

Afin de vérifier les hypothèses émises, les objectifs suivants ont été établis :

1. Isoler des micro-organismes présents au niveau de la rhizosphère de plants de tomate cultivés en milieux organiques et en laine de roche.

   1.1 Évaluer l'effet antagoniste de ces micro-organismes *in vitro* envers *P. ultimum* et *P. aphanidermatum*.

   1.2 Évaluer l'effet de ces micro-organismes sur la fonte des semis de la tomate de serre en laine de roche.

2. Évaluer l'effet de micro-organismes antagonistes sélectionnés sur le développement de la pourriture pythienne causée par *P. ultimum* chez la tomate de serre cultivée en hydroponie.

3. Démontrer l'effet stimulant de certains micro-organismes antagonistes sur la croissance et le développement des plants de tomate en conditions hydroponiques.

   3.1 Évaluer l'effet sur la nutrition minérale des plants de tomate des micro-organismes démontrant un effet stimulant.

   3.2 Évaluer le potentiel de production et de dégradation de l'AIA des micro-organismes démontrant un effet stimulant.

   3.3 Évaluer la présence de l'activité ACC désaminase chez les micro-organismes démontrant un effet stimulant.

4. Évaluer l'effet de l'AIA sur le développement de symptômes causés par *P. ultimum* chez des plants de tomate.

CHAPITRE 2

MICRO-ORGANISMES AYANT LA CAPACITÉ DE RÉDUIRE LA FONTE DES SEMIS CAUSÉE PAR *PYTHIUM* SPP. CHEZ LA TOMATE CULTIVÉE EN LAINE DE ROCHE

Sommaire : L'objectif des travaux présentés dans ce chapitre était d'identifier des micro-organismes antagonistes envers *Pythium aphanidermatum* et *Pythium ultimum* au moyen d'essais *in vitro* et *in vivo*. Les travaux réalisés ont permis d'identifier, à partir d'une collection de 237 micro-organismes isolés de cinq substrats utilisés pour la culture de la tomate de serre, 40 micro-organismes capables de réduire la croissance mycélienne *in vitro* de *P. aphanidermatum* et *P. ultimum*. La capacité de ces micro-organismes à contrôler la fonte des semis a, par la suite, été testée en laine de roche. Les résultats ont montré que *Pseudomonas corrugata* souches 1 et 3, *Pseudomonas fluorescens* sous-groupes F et G souches 1, 2, 3, 4 et 5, *Pseudomonas marginalis, Pseudomonas putida* sous-groupe B souche 1, *Pseudomonas syringae* souche 1 et *Pseudomonas viridiflava* ont significativement réduit la fonte des semis causée par *P. ultimum* ou *P. aphanidermatum*. *Pseudomonas marginalis* est le seul micro-organisme qui a réduit la fonte des semis causée par l'un ou l'autre de ces deux agents pathogènes.

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Antagonist microorganisms with the ability to control *Pythium* damping-off of tomato seeds in rockwool

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Abstract: A total of 237 microorganisms were isolated from five different greenhouse tomato growing media. Of those, 40 microorganisms reduced the *in vitro* mycelial growth of both *Pythium aphanidermatum* and *Pythium ultimum*. The ability of these microorganisms to control damping-off was then tested in rockwool. As a result, *Pseudomonas corrugata* strains 1 and 3, *Pseudomonas fluorescens* subgroups F and G strains 1, 2, 3, 4 and 5, *Pseudomonas marginalis, Pseudomonas putida* subgroup B strain 1, *Pseudomonas syringae* strain 1 and *Pseudomonas viridiflava* significantly reduced damping-off caused by *P. ultimum* or *P. aphanidermatum*. *Pseudomonas marginalis* was the only microorganism that significantly reduced damping-off caused by both pathogens.

Key words: biological control, greenhouse culture, hydroponic, seed decay

2.1 Introduction

Damping-off is an important disease of numerous crops, namely greenhouse tomato, causing important losses in nurseries where young susceptible transplants are produced. In Canada, this disease is mainly caused by *Pythium ultimum* and *Pythium aphanidermatum*, which are responsible for seed decay as well as pre- and postemergence damping-off of tomato seedlings. Presently, none of the tomato cultivars used in greenhouse crops are resistant to *Pythium* spp. and in Canada no chemical fungicides are registered to control this pathogen in greenhouse tomato crops (Paulitz and Bélanger, 2001). Consequently, control and management of the disease rely on cultural practices such as proper control of irrigation and UV sterilization of the nutritive solution but the effectiveness is often questionable (Paulitz, 1997). Since cultural practices alone are not always sufficient to effectively control the disease, alternative strategies are needed.

In Europe and Canada, the rockwool system of production is widely used by commercial greenhouse tomato growers. In such a system, the number of resident potentially competitive microorganisms are extremely low, facilitating dissemination of the pathogen through the nutritive solution (Stanghellini and Rasmussen, 1994) and allowing biocontrol agents with a low competitive ability to become established (Rankin and Paulitz, 1994). Few works have reported on successful biocontrol of *Pythium* in greenhouse hydroponic production systems. Rankin and Paulitz (1994) published the first report of reduction of *Pythium* disease with rhizobacteria in rockwool. They showed that application of specific strains of *Pseudomonas corrugata* and *P. fluorescens* reduced *P. aphanidermatum* root rot of cucumbers. Beneficial effects of application of *P. corrugata* (strain 13) and *P. fluorescens* (strains 63-49, 63-28, 15) on rockwool-grown cucumbers was subsequently reported by McCullagh et al. (1996). *P. fluorescens* 63-28 was also reported to increase the marketable tomato fruit yield and to inhibit efficiently *P. ultimum* in peat-based substrates (Gagné et al., 1993). To our knowledge, no specific microorganisms were reported for their ability to control *Pythium* disease on rockwool-grown tomato.

As part of ongoing research aimed to test different organic material such as peat, barks and composts as alternatives to rockwool for greenhouse production, the objectives of this study were (1) to isolate microorganisms from these materials and (2) to test their ability to protect tomato seedlings against *Pythium* damping-off.

2.2 Materials and methods

2.2.1 Fungi

Virulent strains of *Pythium ultimum* and *P. aphanidermatum* isolated from infected tomato roots were provided by the laboratory of Dr Richard Bélanger (Université Laval, Québec, Québec, Canada). The fungi were grown on potato dextrose agar (PDA; Difco Laboratories, Becton Dickinson, Sparks, MD) at 24°C. PDA disks covered with *P. ultimum* or *P. aphanidermatum* mycelium conserved in sterile distilled water at 24°C served as stock cultures. Considering that, at room temperature, zoospores are not produced by *P. ultimum* (van der Plaats-Niterink, 1981), the propagule suspension of *P. ultimum* consisted of hyphae and oospores. The propagule suspension of *P. aphanidermatum* consisted of zoospores, hyphae and oospores. They were both prepared from liquid cultures (250 ml flasks) containing 100 ml of potato dextrose broth (PDB; Difco Laboratories) incubated on a rotary shaker (150 rpm) at 24°C for 1 week. PDA disks covered with actively growing mycelium of either *P. ultimum* or *P. aphanidermatum* were used to inoculate the flasks.

2.2.2 Isolation of microorganisms

Microorganisms were isolated, using serial dilutions and spread on selective media in Petri dishes, from rockwool, a mixture of peat and compost (9:1, v:v), a mixture of peat, pine bark and compost (6:3:1, v:v:v), a mixture of peat, pine bark and compost (3:6:1, v:v:v) and a mixture of pine bark and compost (9:1, v:v) in which tomato was previously cultivated. Isolation of microorganisms was performed according to Michaud et al.

(2002). One gram of each substrate was added to 10 ml of distilled sterile water. Six dilutions 1/10 were made following homogenization of the mixture. Fungi were isolated on malt agar (Difco Laboratories) containing streptomycin (0.3 g l^{-1}; Sigma-Aldrich, Mississauga, Ontario) and on czapek agar (Sigma-Aldrich). Bacteria were isolated on the following media: tryptic soy agar (TSA; Sigma-Aldrich), TSA amended with crystal violet (0.002 g l^{-1}; Sigma-Aldrich), and pseudomonas agar F (Difco Laboratories) amended with chloramphenicol (0.125 g l^{-1}; Sigma-Aldrich) and ampicillin (0.5 g l^{-1}; Sigma-Aldrich). Crystal violet was added to TSA to facilitate the isolation of gram-negative bacteria, while ampicillin and chloramphenicol were added to pseudomonas agar F to improve *Pseudomonas* spp. bacteria isolation. Petri dishes were incubated at 24°C following the spread of the dilutions. Morphologically distinct microorganisms were isolated and grown in pure culture on TSA (bacteria) or PDA (fungi).

2.2.3 Effect of microorganisms on mycelial growth of *P. ultimum* and *P. aphanidermatum*

The effect of microorganisms on the growth of the pathogens was performed *in vitro* according to Brown et al. (1987). A drop of each bacterial suspension (4×10^7 bacteria ml^{-1}) was placed on PDA at one side of a Petri dish. A mycelial disk (7 mm in diameter) of either *P. ultimum* or *P. aphanidermatum* was placed on the opposite side of the Petri dish, 3 cm away from the bacteria. Bacterial suspensions were obtained from pure cultures grown in 10 ml of tryptic soy broth (TSB; Sigma-Aldrich) for 24 h on a rotary shaker at 24°C. Fungal isolates were grown on PDA in pure culture. A PDA disk covered with actively growing mycelium of each fungus (7 mm in diameter) was placed on PDA at one side of a Petri dish 24 h prior to adding *P. ultimum* or *P. aphanidermatum* as described before. The five following controls were used: *P. ultimum* against *P. ultimum*, *P. ultimum* against *P. aphanidermatum*, *P. aphanidermatum* against *P. aphanidermatum*, *P. ultimum* against a drop of TSB medium and *P. aphanidermatum* against a drop of TSB medium. Triplicate Petri dishes were incubated at 24°C for 48 h. The presence (+) or the absence (-) of an inhibition zone between the pathogen and the microorganism tested was then recorded.

2.2.4 Effect of microorganisms on damping-off incidence

Microorganisms which inhibited the *in vitro* mycelial growth of *P. ultimum* and *P. aphanidermatum* were tested for their effect on the incidence of damping-off of tomato seedlings. Tomato seeds (cv. Trust F1; De Ruiter Seeds, Columbus, Ohio) were soaked in distilled water for 6 h, planted in multicellular blocks of rockwool and subsequently received either 1 ml of a bacterial suspension (4×10^7 bacteria ml^{-1}) or 1 ml of a fungal spore suspension (1×10^4 spores ml^{-1}). The seeds received subsequently 1 ml of either propagule (hyphae and oospores) suspension (1×10^4 propagules ml-ı) of *P. ultimum* or propagule (zoospores, hyphae and oospores) suspension (1×10^4 propagules ml^{-1}) of *P. aphanidermatum* (prepared from liquid cultures) and were then covered with vermiculite and placed in a growth chamber (16-20°C, 80 % relative humidity and 16 h photoperiod) for 2 weeks. During that period, the rockwool blocks were watered daily with a nutritive solution (1%) of 20-20-20 (N-P-K). Noninfected (non-infected control) and infected (infected control) seeds treated with distilled water were used as controls. Two weeks after sowing, seeds were rated for emergence. The rate of emerged seedlings was then calculated as follows: [number of emerged seedlings/number of seeds planted] × 100. The rate of decayed seeds was also evaluated and expressed in percent. A completely randomized experimental design with four replicates was used.

2.2.5 Antagonist identification

Bacteria and fungi which inhibited the *in vitro* mycelial growth of both *P. ultimum* and *P. aphanidermatum* were identified using the BIOLOG Identification System (BIOLOG Inc., Hayward, CA) or by 16S rDNA sequencing by Dr Anne-Marie Simao-Beaunoir (Université de Sherbrooke, Sherbrooke, Québec, Canada).

2.2.6 Statistical analysis

Data on the effect of microorganisms on damping-off incidence were analyzed with SAS (SAS Institute, Cary, NC), using the general linear models procedure. Data percent values were arcsin transformed before analysis. When significant ($P<0.05$), treatment

means were compared using the Fisher protected LSD test. Each experiment was performed twice. Considering that same trends were obtained in both experiments, results of both experiments were pooled.

2.3 Results

A total of 237 microorganisms (160 bacteria and 77 fungi) were isolated from five tomato growing media. They were tested for their ability to inhibit the *in vitro* mycelial growth of the pathogens. In dual cultures on agar, forty microorganisms (19 bacteria; 21 fungi) were shown to reduce the mycelial growth of both *P. ultimum* and *P. aphanidermatum,* as indicated by the presence of an inhibition zone (Table 1). Most of the antagonistic bacteria and fungi were from the genus *Pseudomonas* and *Penicillium,* respectively. No gram-positive bacteria isolates were shown to reduce the mycelial growth of either *P. ultimum* or *P. aphanidermatum.*

The isolates that presented an inhibiting effect *in vitro* on both pathogens were then tested for their ability to reduce damping-off of tomato seedlings under growth chamber conditions. Compared to infected-control seeds, *Fusarium oxysporum* strain 2, *Pseudomonas corrugata* strains 1 and 3, *P. fluorescens* subgroup F, *P. fluorescens* subgroup G strains 1, 2, 3, 4 and 5, *P. marginalis, P. putida* subgroup B strain 1, *P. resinovorans, P. syringae* strain 1, *P. viridiflava* and *Trichoderma longibrachiatum* reduced significantly the rate of decayed seeds (Table 2) resulting from *P. ultimum* inoculation. Among these microorganisms, *P. corrugata* strain 3, *P. fluorescens* subgroup F, *P. fluorescens* subgroup G strains 1, 2 and 4, *P. marginalis, P. syringae* strain 1 and *P. viridiflava* significantly increased the percentage of emerged tomato seedlings (Table 2).

The application of microorganisms also influenced significantly the percentage of *P. aphanidermatum*-decayed seeds (Table 3). *F. oxysporum* strain 2, *Penicillium janthinellum* strain 4, *P. corrugata* strains 1 and 3, *P. fluorescens* subgroup G strain 3, *P.*

marginalis, P. putida subgroup B strain 1, *P. resinovorans, P. syringae* strain 3 and *P. viridiflava* were shown to reduce significantly the rate of decayed seeds. Among these microorganisms, *P. corrugata* strain 1, *P. fluorescens* subgroup G strain 3, *P. marginalis* and *P. putida* subgroup B strain 1 increased the percentage of emerged *P. aphanidermatum* inoculated seedlings.

P. marginalis was the only microorganism out of the forty tested that significantly reduced damping-off incidence caused by both pathogens with 62.5% and 87.5% of emerged seedlings when inoculated with *P. ultimum* and *P. aphanidermatum,* respectively. None of the fungi tested significantly increased the percentage of emerged seedlings.

2.4 Discussion

In this study, a two-step procedure involving *in vitro* and *in vivo* assays was used to select antagonist microorganisms with the ability to control *Pythium* damping-off of tomato seeds (Okamoto et al., 2000; Dal Bello et al., 2003). In order to have a reasonable number of microorganisms for *in vivo* tests, the 237 microorganisms isolated from the different greenhouse tomato growing media were tested for their ability to inhibit the *in vitro* mycelial growth of both pathogens, *P. ultimum* and *P. aphanidermatum.* Of those, 40 microorganisms were shown to inhibit the growth of both *P. ultimum* and *P. aphanidermatum* presumably by producing antifungal compounds. These microorganisms were identified as *Acremonium potronii, Acrodontium griseum, Enterobacter cloacae, Fusarium oxysporum, F. tumidum, Penicillium brevicompactum, P. griseofulvum, P. janthinellum, P. restrictum, P. simplicissimum, P. solitum, Pseudomonas corrugata, P. fluorescens* subgroups F and G, *P. marginalis, P. putida* subgroup B, *P. resinovorans, P. syringae, P. viridiflava, Trichoderma atroviride* and *T. longibrachiatum.* Three fungi were not further identified. These microorganisms were then tested *in vivo* for their ability to reduce damping-off caused by both pathogens. Among these microorganisms, *P. corrugata* strains 1 and 3, *P. fluorescens* subgroups F and G strains 1, 2, 3, 4 and 5, *P. marginalis*, *P. putida* subgroup B strain 1, *P. syringae*

strain 1 and *P. viridiflava* reduced damping-off caused by *P. ultimum* or *P. aphanidermatum*. These microorganisms were previously reported for their ability to reduce the development of various pathogens mainly in soil.

Pseudomonas putida is a bacterium frequently isolated from soil which received a lot of attention for its capacity to degrade complex aromatic molecules (Koshelava et al., 2000; Sultana et al., 2001). In this study, two strains of *P. putida* subgroup B were isolated and showed *in vitro* inhibition of both pathogens. However, only *P. putida* strain 1 reduced damping-off of tomato caused by *P. aphanidermatum.* Previous works have reported the biocontrol ability of this bacterium against *Pythium* damping-off of sugarbeet (Shah-Smith and Burns, 1996) and safflower (Liang et al., 1996), durum wheat tan spot caused by *Pyrenophora tritici-repentis* (da Luz et al., 1998), potato silver scurf caused by *Helminthosporium solani* (Elson et al., 1997; Martinez et al., 2002) and potato soft rot caused by *Erwinia carotovora* (Colyer and Mount, 1984). *P. marginalis* isolated from a mixture of peat and compost strongly reduced the incidence of damping-off caused by both pathogens. To our knowledge, no study has previously reported the antagonistic activity of *P. marginalis* against Oomycetes, but such an activity was reported against *Colletotrichum graminicola* on sorghum (Michereff et al., 1994).

Pseudomonas corrugata was reported to display antagonism against various plant pathogens in different substrates including soil (Pandey et al., 2001), peat moss (Georgakopoulos et al., 2002) and rockwool (Rankin and Paulitz, 1994). Antagonism against *P. ultimum* in maize (Pandey et al., 2001), cucumber and sugar beet (Georgakopoulos et al., 2002), *P. aphanidermatum* in cucumber (Rankin and Paulitz, 1994) and *Gaeumannomyces graminis* var. *tritici* in wheat (Ryder and Rovira, 1993) has been observed. *P. syringae* was previously reported for its biocontrol activity against apple blue (*Penicillium expansum*) and gray (*Botrytis cinerea*) mold (Zhou et al., 2001; 2002) and against the foodborne pathogen *Escherichia coli* (Janisiewicz et al., 1999) on apple. In the present study, *P. corrugata* and *P. syringae* reduced significantly damping-off of tomato caused by *P. ultimum*. However, their potential use as biocontrol agents of tomato pathogens must be viewed with caution. Indeed, *P. corrugata* and *P. syringae* pv.

tomato are well known tomato pathogens causing pith necrosis and bacterial speck, respectively (Richard and Boivin, 1994).

Pseudomonas fluorescens has been extensively studied for its ability to stimulate the growth of plants (Howie and Echandi, 1983; Kloepper et al., 1988; Kurek and Jaroszuk-Ścisel, 2003) and to reduce the development of various plant pathogens (Paulitz et al., 1992; Ramamoorthy et al., 2002a). Numerous studies have demonstrated the potential of different strains of *P. fluorescens* as biocontrol agents of different pathogens including *Fusarium sambucinum* (Schisler et al., 2000), *Pythium* sp. group G (Bardin et al., 2003) and *P. ultimum* (Carisse et al., 2003). The ability of isolates of *P. fluorescens* subgroups C and E to reduce cucumber root rot caused by *P. aphanidermatum* has also been reported (Paulitz et al., 1992). Of particular interest, studies have reported the biocontrol potential of specific strains of *P. fluorescens* against *Fusarium* wilt of tomato grown in soil (Larkin and Fravel, 1998), *P. aphanidermatum* damping-off of tomato grown in soil (Ramamoorthy et al., 2002b) and *P. ultimum* damping-off of tomato seedlings grown in Petri dish bioassays (Hultberg et al., 2000). Our results showed the efficiency of different strains of *P. fluorescens* of the subgroups F and G to reduce the incidence of tomato damping-off caused by either *P. ultimum* or *P. aphanidermatum* in rockwool.

Biocontrol agents exert disease suppression by different modes of action including competition, direct parasitism, antibiosis and induction of plant resistance mechanism (Whipps, 2001). None of the mechanisms are necessarily mutually exclusive and frequently several modes of action are exhibited by a single biocontrol agent. The mechanism by which *P. fluorescens* exerts its antagonism against *Pythium* sp. has been extensively studied and appears to involve the production of a variety of antibiotic compounds (Howell and Stipanovic, 1980; Sharifi-Tehrani et al, 1998; Thrane et al, 2000), competition (Mohamed and Caunter, 1995; Ellis et al., 1999) and induced resistance (Benhamou et al., 1996; Ramamoorthy et al., 2002a). *P. putida*, *P. corrugata* and *P. marginalis* were also reported to exert an antagonistic activity through the production of antimicrobial compounds (de Freitas et al., 1991; Harris et al., 1997), competition (Paulitz et al., 1992; Fukui et al., 1994) or induction of plant resistance

mechanism (Chen et al., 1998; Ongena et al., 2000; 2002). In this study, the presence of an inhibition zone occurring between the pathogens and *P. fluorescens*, *P. putida*, *P. corrugata* and *P. marginalis* in dual culture on agar plate suggests that the production of antibiotic compounds is involved in the antagonism observed *in vivo*. However, other modes of action such as competition and induced resistance can not be ruled out.

It is interesting to note that certain microorganisms allowed to reduce the incidence of seed decay caused by either *P. ultimum* or *P. aphanidermatum* but did not allow to decrease the incidence of damping-off. For instance, *F. oxysporum* strain 2 reduced seed decay incidence caused by both pathogens but failed to reduce the incidence of damping-off. Such a phenomenon which was also observed with *T. longibrachiatum, P. janthinellum* and *P. resinovorans* suggests that some microorganisms protected the seed from decay but failed to protect the epicotyl leading to emergence.

In this study, a two-step procedure including *in vitro* screening, has allowed us to identify several microorganisms with the ability to reduce damping-off caused by *P. aphanidermatum* or *P. ultimum*. *In vitro* screening is often used as a first filter to identify potential biocontrol agents (Okamoto et al., 2000; Dal Bello et al., 2003). However, microorgansims showing no antagonistic activity *in vitro* may display a biocontrol ability *in vivo* (Knudsen et al., 1997). For this reason, the 197 microorganisms showing no antagonistic activity *in vitro* will be tested in future work for their ability to control damping-off in rockwool.

Very few works have reported on successful biocontrol of *Pythium* in greenhouse hydroponic production systems. The results presented in this study have led to the identification of several microorganisms displaying the ability to control tomato damping-off caused by either *P. ultimum* or *P. aphanidermatum* in rockwool. These findings may open the way for new avenues of research in the biological control of *Pythium* diseases in hydroponic systems.

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Table 1. Effect of microorganisms isolated from different tomato growth substrates on the *in vitro* mycelial growth of *Pythium ultimum* and *Pythium aphanidermatum*

Microorganisms	Source[a] medium	Inhibiting zone[b]	
		P. ultimum	*P. aphanidermatum*
Acremonium potronii	B	+[c]++	+++
Acrodontium griseum	D	++[d]+	+++
Enterobacter cloacae	D	+++[e]	+++
Fungus 2-8-8 [f]	B	+++	+++
Fungus 5-12-6 [f]	B	+++	+++
Fungus 8-13-1 [f]	C	+++	+++
Fusarium oxysporum strain 1	C	+++	+++
Fusarium oxysporum strain 2	B	+++	+++
Fusarium tumidum	D	+++	+++
Penicillium brevicompactum	B	+++	+++
Penicillium griseofulvum	C	+++	+++
Penicillium janthinellum strain 1	A	+++	+++
Penicillium janthinellum strain 2	C	+++	+++
Penicillium janthinellum strain 3	C	+++	+++
Penicillium janthinellum strain 4	B	+++	+++
Penicillium janthinellum strain 5	B	+++	+++
Penicillium restrictum	C	+++	+++
Penicillium simplicissimum strain 1	B	+++	+++
Penicillium simplicissimum strain 2	A	+++	+++
Penicillium solitum	A	+++	+++
Pseudomonas corrugata strain 1	R	+++	+++
Pseudomonas corrugata strain 2	A	+++	+++
Pseudomonas corrugata strain 3	R	+++	+++
Pseudomonas fluorescens subgroup F	C	+++	+++
Pseudomonas fluorescens subgroup G strain 1	C	+++	+++
Pseudomonas fluorescens subgroup G strain 2	R	+++	+++
Pseudomonas fluorescens subgroup G strain 3	D	+++	+++
Pseudomonas fluorescens subgroup G strain 4	C	+++	+++
Pseudomonas fluorescens subgroup G strain 5	D	+++	+++
Pseudomonas fluorescens subgroup G strain 6	R	+++	+++
Pseudomonas marginalis	A	+++	+++
Pseudomonas putida subgroup B strain 1	C	+++	+++
Pseudomonas putida subgroup B strain 2	C	+++	+++
Pseudomonas resinovorans	R	+++	+++
Pseudomonas syringae strain 1	R	+++	+++
Pseudomonas syringae strain 2	B	+++	+++
Pseudomonas syringae strain 3	C	+++	+++
Pseudomonas viridiflava	R	+++	+++
Trichoderma atroviride	A	+++	+++
Trichoderma longibrachiatum	B	+++	+++
Pythium aphanidermatum		---	---
Pythium ultimum		---	

[a]Microorganisms were isolated from rockwool [R], a mixture of peat and compost (9:1, v:v) [A], a mixture of peat, pine bark and compost (6:3:1, v:v:v) [B], a mixture of peat, pine bark and compost (3:6:1, v:v:v) [C] and a mixture of pine bark and compost (9:1, v:v) [D].

[b]Presence (+) or absence (-) of an inhibition zone between the pathogen and the microorganism.

[c,d,e] Replicate 1, 2 and 3, respectively.

[f]Not identified.

Table 2. Effect of antagonistic microorganisms on the emergence of tomato seedlings and the decay of tomato seeds inoculated with *Pythium ultimum* in rockwool

Microorganisms	Emerged seedlings (%)	Decayed seeds (%)
Infected control	0 a	100 e
Non-infected control	100 d	0 a
Acremonium potronii	0 a	87.5 de
Acrodontium griseum	0 a	75 cde
Enterobacter cloacae	0 a	50 abcde
Fungus 2-8-8	12.5 ab	62.5 bcde
Fungus 5-12-6	0 a	75 cde
Fungus 8-13-1	0 a	62.5 bcde
Fusarium oxysporum strain 1	0 a	75 cde
Fusarium oxysporum strain 2	25 abc	37.5 abcd
Fusarium tumidum	0 a	75 cde
Penicillium brevicompactum	0 a	62.5 bcde
Penicillium griseofulvum	0 a	62.5 bcde
Penicillium janthinellum strain 1	0 a	75 cde
Penicillium janthinellum strain 2	0 a	62.5 bcde
Penicillium janthinellum strain 3	0 a	62.5 bcde
Penicillium janthinellum strain 4	0 a	50 abcde
Penicillium janthinellum strain 5	0 a	50 abcde
Penicillium restrictum	0 a	100 e
Penicillium simplicissimum strain 1	12.5 ab	50 abcde
Penicillium simplicissimum strain 2	0 a	87.5 de
Penicillium solitum	12.5 ab	62.5 bcde
Pseudomonas corrugata strain 1	37.5 abc	37.5 abcd
Pseudomonas corrugata strain 2	50 abcd	50 abcde
Pseudomonas corrugata strain 3	62.5 bcd	12.5 ab
Pseudomonas fluorescens subgroup F	62.5 bcd	25 abc
Pseudomonas fluorescens subgroup G strain 1	62.5 bcd	12.5 ab
Pseudomonas fluorescens subgroup G strain 2	75 cd	12.5 ab
Pseudomonas fluorescens subgroup G strain 3	25 abc	37.5 abcd
Pseudomonas fluorescens subgroup G strain 4	75 cd	12.5 ab
Pseudomonas fluorescens subgroup G strain 5	50 abcd	25 abc
Pseudomonas fluorescens subgroup G strain 6	12.5 ab	62.5 bcde
Pseudomonas marginalis	62.5 bcd	25 abc
Pseudomonas putida subgroup B strain 1	50 abcd	12.5 ab
Pseudomonas putida subgroup B strain 2	25 abc	50 abcde
Pseudomonas resinovorans	50 abcd	37.5 abcd
Pseudomonas syringae strain 1	62.5 bcd	12.5 ab
Pseudomonas syringae strain 2	25 abc	75 cde
Pseudomonas syringae strain 3	50 abcd	50 abcde
Pseudomonas viridiflava	62.5 bcd	37.5 abcd
Trichoderma atroviride	0 a	87.5 de
Trichoderma longibrachiatum	25 abc	25 abc

In each column, means followed by the same letter are not significantly different at (P>0.05) according to the Fisher protected LSD test (n = 8).

Table 3. Effect of antagonistic microorganisms on the emergence of tomato seedlings and the decay of tomato seeds inoculated with *Pythium aphanidermatum* in rockwool

Microorganisms	Emerged seedlings (%)	Decayed seeds (%)
Infected control	0 a	100 d
Non-infected control	100 d	0 a
Acremonium potronii	0 a	62.5 abcd
Acrodontium griseum	0 a	50 abcd
Enterobacter cloacae	25 ab	50 abcd
Fungus 2-8-8	0 a	62.5 abcd
Fungus 5-12-6	25 ab	62.5 abcd
Fungus 8-13-1	0 a	87.5 cd
Fusarium oxysporum strain 1	0 a	62.5 abcd
Fusarium oxysporum strain 2	0 a	25 abc
Fusarium tumidum	0 a	50 abcd
Penicillium brevicompactum	0 a	100 d
Penicillium griseofulvum	0 a	100 d
Penicillium janthinellum strain 1	0 a	75 bcd
Penicillium janthinellum strain 2	0 a	75 bcd
Penicillium janthinellum strain 3	0 a	75 bcd
Penicillium janthinellum strain 4	37.5 abc	25 abc
Penicillium janthinellum strain 5	12.5 ab	37.5 abcd
Penicillium restrictum	0 a	87.5 cd
Penicillium simplicissimum strain 1	0 a	37.5 abcd
Penicillium simplicissimum strain 2	0 a	75 bcd
Penicillium solitum	25 ab	62.5 abcd
Pseudomonas corrugata strain 1	62.5 bcd	12.5 ab
Pseudomonas corrugata strain 2	25 ab	37.5 abcd
Pseudomonas corrugata strain 3	50 abcd	12.5 ab
Pseudomonas fluorescens subgroup F	25 ab	50 abcd
Pseudomonas fluorescens subgroup G strain 1	12.5 ab	62.5 abcd
Pseudomonas fluorescens subgroup G strain 2	12.5 ab	37.5 abcd
Pseudomonas fluorescens subgroup G strain 3	62.5 bcd	12.5 ab
Pseudomonas fluorescens subgroup G strain 4	12.5 ab	50 abcd
Pseudomonas fluorescens subgroup G strain 5	62.5 bcd	37.5 abcd
Pseudomonas fluorescens subgroup G strain 6	12.5 ab	75 bcd
Pseudomonas marginalis	87.5 cd	0 a
Pseudomonas putida subgroup B strain 1	62.5 bcd	25 abc
Pseudomonas putida subgroup B strain 2	12.5 ab	62.5 abcd
Pseudomonas resinovorans	50 abcd	25 abc
Pseudomonas syringae strain 1	0 a	75 bcd
Pseudomonas syringae strain 2	12.5 ab	75 bcd
Pseudomonas syringae strain 3	25 ab	0 a
Pseudomonas viridiflava	25 ab	25 abc
Trichoderma atroviride	0 a	87.5 cd
Trichoderma longibrachiatum	0 a	100 d

In each column, means followed by the same letter are not significantly different at ($P>0.05$) according to the Fisher protected LSD test (n = 8).

CHAPITRE 3

UTILISATION DE MICRO-ORGANISMES BÉNÉFIQUES COMME MOYEN DE LUTTE CONTRE LA POURRITURE PYTHIENNE (*PYTHIUM ULTIMUM*) CHEZ LA TOMATE DE SERRE EN CONDITIONS HYDROPONIQUES

Sommaire : Les travaux présentés au chapitre 2 ont permis de sélectionner plusieurs micro-organismes démontrant une forte activité antagoniste envers *Pythium ultimum.* Vingt-huit de ces micro-organismes ont été testés afin d'évaluer leur capacité à réduire la pourriture pythienne (*P. ultimum*) chez des plants de tomate matures cultivés en conditions hydroponiques. De ceux-ci, *Penicillium brevicompactum, Penicillium solitum* souche 1, *Pseudomonas fluorescens*, *Pseudomonas marginalis, Pseudomonas putida* sous-groupe B souche 1, *Pseudomonas syringae* souche 1 et *Trichoderma atroviride* ont réduit le taux d'infection, amélioré l'ancrage dans le substrat et augmenté le rendement vendable des plants cultivés en laine de roche en présence de *P. ultimum.* Les expériences effectuées dans un substrat organique contenant de la tourbe, de la sciure de pin et du compost (*v/v/v*; 60/30/10) ont également démontré la capacité de la plupart de ces micro-organismes à réduire la pourriture pythienne et à augmenter l'ancrage des plants. Toutefois, en substrat organique, *P. marginalis* a été le seul micro-organisme à avoir augmenté significativement la production en fruits des plants infectés. Par ailleurs, des bio-essais ont montré que *P. brevicompactum, P. solitum* souche 1, *P. fluorescens* sous-groupe G souche 2, *P. marginalis, P. putida* sous-groupe B souche 1 et *T. atroviride* stimulaient la croissance de plantules de tomate saines, suggérant que ces micro-organismes agissent comme des PGPRs (plant growth-promoting rhizobacteria) ou des PGPFs (plant growth-promoting fungi). Cette étude a mené à la sélection d'agents de lutte biologique potentiels contre la pourriture pythienne causée par *P. ultimum* chez la tomate dans les systèmes hydroponiques. Ces micro-organismes pourraient trouver des applications dans la culture commerciale de la tomate de serre en hydroponie.

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Control of greenhouse tomato root rot (*Pythium ultimum*) in hydroponic systems using plant growth-promoting microorganisms

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Abstract: Twenty-eight microorganisms showing *in vitro* antagonistic activity against *Pythium ultimum* were tested for their ability to reduce root rot (*P. ultimum*) on mature tomato plants grown in a greenhouse under hydroponic conditions. Of those, *Penicillium brevicompactum, P. solitum* strain 1, *Pseudomonas fluorescens, P. marginalis, P. putida* subgroup B strain 1, *P. syringae* strain 1 and *Trichoderma atroviride* were shown to strongly reduce root rot severity, to improve the anchorage and to increase the marketable yields of the plants grown in rockwool infested with *P. ultimum*. Experiments conducted in an organic medium containing peat, pine sawdust and compost (*v/v/v*; 60/30/10) also revealed the capability of most of these microorganisms to reduce root rot severity and to improve the anchorage of the tomato plants. However, *P. marginalis* was the only microorganism that significantly improved fruit production of infected plants grown in organic medium. Moreover, *P. brevicompactum, P. solitum* strain 1, *P. fluorescens* subgroup G strain 2, *P. marginalis, P. putida* subgroup B strain 1, and *T. atroviride* were shown to stimulate the growth of healthy tomato seedlings suggesting that they act as PGPR (plant growth-promoting rhizobacteria) or PGPF (plant growth-promoting fungi). This study led to the selection of potential biological control agents against root rot of tomato caused by *P. ultimum* in hydroponic systems. This may open the way for new alternatives for the biological control of *Pythium* diseases in hydroponic systems that not only protects the crop but also have a beneficial effect on plant growth and development in the absence of pathogens.

Key words: *Pythium ultimum*, root rot, greenhouse tomato, hydroponic, biological control

3.1 Introduction

The use of hydroponic systems has become a standard for the greenhouse tomato industry in Canada. For many years, most greenhouse tomato growers have relied on systems, such as rockwool, that are relatively sterile at the beginning of a crop. Rockwool and other soilless systems eliminate the presence of soilborne pathogen inoculum at planting and are therefore often associated with a reduction of root diseases caused by fungi (Paulitz 1997). Nevertheless, most pathogens cannot be excluded from the greenhouse environment and are often introduced through the use of contaminated plant material or equipment (Jarvis 1992). Once the pathogens are introduced, the optimal environmental conditions for plant growth can also become conducive to the rapid development of plant diseases (Menzies and Bélanger 1996). The high water content in soilless systems is especially favourable to the zoosporic pathogens *Pythium* spp. and *Phytophthora* spp. (Stanghellini and Rasmussen 1994; Zinnen 1988). Moreover, soilless substrates lack a microbial diversity that provides a biological buffer, which generally suppresses the spread and the establishment of weak competitor pathogens (Hendrix and Campbell 1973; Paulitz 1997).

Pythium ultimum Trow, a pathogen with a weak competitive ability, is the causal agent of tomato root rot. This disease, characterized by premature weakening of the root system, has become an important disease of greenhouse tomato crops grown in soilless systems. In Canada, no chemical fungicide is currently registered for greenhouse vegetable crops in hydroponic systems. The control of tomato root rot generally is based on cultural practices that are often unreliable and unsuccessful.

In this context, the biocontrol of *Pythium ultimum* in hydroponic systems through the use of a biofungicide made of one or many microorganisms as a protective measure is viewed as a potential strategy. In general, the use of biological control approaches for disease management is highly desirable where environmental conditions are controlled, such as in greenhouses, and the economic value of the crop is high (Paulitz and Bélanger

2001). In the case of Pythium root rot of greenhouse tomato, the lack of competitive microflora in rockwool hydroponic systems further facilitates the establishment of a biocontrol agent, potentially increasing the efficacy of disease control (Paulitz 1997).

In the present study, microorganisms that were shown *in vitro* to have antagonistic activity against *P. ultimum* (Gravel et al. 2005) were tested for their ability to reduce root rot in greenhouse tomato plants growing in rockwool and in organic medium under hydroponic conditions. The effect of these microorganisms on tomato plant growth and development was also investigated.

3.2 Materials and methods

3.2.1 Pathogen

A virulent strain of *Pythium ultimum* isolated from infected tomato roots was provided by the laboratory of Dr. Richard Bélanger (Université Laval, Québec, Québec, Canada). It was grown on potato dextrose agar (PDA; Difco Laboratories, Detroit, Mich., USA) at 24°C. PDA disks colonized with *P. ultimum* mycelium suspended in sterile distilled water at 24°C served as stock cultures. The propagule suspension of *P. ultimum*, which consisted of oospores and hyphae, was prepared in 1000-mL flasks containing 500 mL of potato dextrose broth (PDB; Difco Laboratories). Five PDA disks covered with actively growing mycelium of *P. ultimum* were used to inoculate the flasks which were then incubated on a rotary shaker (150 rpm) at 24°C for 1 week. The liquid culture was then homogenized using a blender and the propagule suspension was adjusted by dilution to 1 $\times 10^6$ propagules/mL based on hemacytometer counts.

3.2.2 Antagonistic microorganisms

The microorganisms tested in this study (Table 1) were isolated from rockwool and organic media where tomato plants were previously grown and were shown to have an *in vitro* antagonistic activity against *P. ultimum* (Gravel et al. 2005). Selected bacteria were

grown on tryptic soy agar (TSA; Sigma-Aldrich, Mississauga, Ontario, Canada) and stock cultures were kept in 80% glycerol at -80°C. Bacterial suspensions were prepared in 500-mL flasks containing 250 mL of tryptic soy broth (TSB; Sigma-Aldrich). The flasks were incubated on a rotary shaker (150 rpm) at 24°C for 24 h after which the bacterial cells were removed by centrifugation (2000g, 10 min), resuspended and diluted with sterile distilled water to 4×10^7 bacteria/mL. Selected fungi were grown on PDA and stock cultures were freezed-dried and kept at -20°C. The spore suspensions were prepared by scraping the surface of 2-week-old culture on PDA with a glass rod. The concentration of each spore suspension was adjusted by dilution with sterile distilled water to 1×10^6 spores/mL based on hemacytometer counts.

3.2.3 Effect of antagonistic microorganisms on Pythium root rot severity on tomato plants grown in rockwool

The effect of the microorganisms on the severity of tomato Pythium root rot was evaluated according to a modified version of the method described by Rankin and Paulitz (1994). The first greenhouse assay was performed on a summer crop (May to August). The 28 microorganisms tested were *Acremonium potronii, Acrodontium griseum, Enterobacter cloacae, Fusarium oxysporum* strain 1, *F. tumidum, Penicillium brevicompactum, P. griseofulvum, P. janthinellum* strain 1, *P. restrictum, P. simplicissimum* strains 1 and 2, *P. solitum* strains 1 and 2, *Pseudomonas corrugata* strain 1, *P. fluorescens, P. fluorescens* subgroup F, *P. fluorescens* subgroup G strain 2, *P. marginalis, P. putida* subgroup B strain 1, *P. resinovorans, P. syringae* strain 1, *P. viridiflava, Trichoderma atroviride, T. longibrachiatum* and 4 unidentified fungi (fungus 5-12-6; fungus 8-13-1; fungus 2-8-8; fungus 2-6-2).

Tomato seeds (*Lycopersicon esculentum* Mill. cv. Trust F1; De Ruiter Seeds, Columbus, Ohio, USA) were sown in multicellular blocks of rockwool and grown for 4 weeks at 25°C and 80% relative humidity. The 10 cm tall plants were transplanted into rockwool slabs and drenched with 200 ml of either a bacterial (4×10^7 bacteria/mL) or a fungal spore (1×10^6 spores/mL) suspension of each antagonistic microorganism. Controls

received 200 mL of sterile distilled water. A week later, the plants were drenched with 200 mL of a propagule suspension (1×10^6 propagules/mL) of *P. ultimum*. The plants were grown under typical greenhouse growing conditions [temperatures of 18°C (night) and 24°C (day), 80% relative humidity, natural daylight] and drip irrigated using a nutrient solution containing 0.51 g/L of 6-11-31 (N-P-K) and 0.69 g/L of 15.5-0-0 (N-P-K) (Plantprod, Brampton, Ontario). The 6-11-31 formulation contained Mg (3.0 %), S (3.5%), Fe (0.3%), Mn (0.06%), Zn (0.02%), Cu (0.004%), B (0.027%) and Mo (0.009%). As for the 15.5-0-0 formulation, it contained Ca (19%). Nutrient solution conductivity (EC) and pH were monitored daily and were constant among the different experiments. The EC was kept at 3.0 mS/cm whereas the pH was kept between 5.5 and 6.0 through the addition of phosphoric acid. After 4 months following pathogen inoculation, the severity of Pythium root rot was evaluated as the percentage of roots with at least one site of infection (infection rate) based on 100 roots for each plant. A complete randomized block design with 2 replicates was used. The experimental unit consisted of a rockwool slab in which 3 plants were grown.

A second greenhouse assay was performed during a fall crop (September to December) with 17 selected isolates in order to evaluate the ability of the microorganisms to suppress root rot under different conditions. The tomato plants were treated and inoculated as described above. For this experiment, the rockwool slabs were placed on elevated gutters instead of directly on the greenhouse floor. The natural daylight was supplemented with high-pressure sodium (HPS) lamps (100 $\mu E/m^2$ per second PAR) to maintain a photoperiod of 16 h. The experimental design was a complete randomized block design with 3 replicates. The experimental unit consisted of a rockwool slab in which 3 plants were grown.

3.2.4 Effect of antagonistic microorganisms on root rot severity, root development, plant growth and fruit yields of *P. ultimum* infected tomato plants grown in rockwool and in organic medium

Greenhouse assays were conducted in order to evaluate the effect of the antagonistic microorganisms on root rot severity, root development, plant growth and fruit yields in

both rockwool and organic medium. The rockwool experiment was performed under spring conditions (January to June) while the organic medium experiment was conducted under fall conditions (July to December). The organic medium was a mixture of peat, pine sawdust and compost (*v/v/v*; 60/30/10). Tomato plants were treated, inoculated and watered using a nutrient solution as described previously. For the organic medium experiment, the natural daylight was supplemented with HPS lamps (100 $\mu E/m^2$ per second PAR) to maintain a photoperiod of 16 h. Fruit yield was measured throughout the crop. The fruits were harvested twice a week for a period of 11 weeks and were separated into 5 categories: #1 (fresh weight of 114 g to 450 g), #2 (fresh weight of 88 g to 114 g), #3 (fresh weight of less than 88 g), #4 (blossom end rot) and #5 (misshaped fruits, fresh weight usually over 450 g). The marketable yield included the fruits from the #1 and #2 categories whereas the total yield included all 5 categories. Throughout the crops, stem diameters were measured weekly to assure that plants were developing normally. At the end of the crop (6 months), the severity of Pythium root rot, the amount of roots, the anchorage of the plants and the stem length were evaluated. Pythium root rot severity was evaluated as previously described. The anchorage of the plant in the growing media was evaluated qualitatively on a scale of 0 to 5. The evaluation scale was based on the force needed to manually remove the plants from the growing medium. The scale was as follows: 0 (plant not anchored), 1 (hardly anchored), 2 (very weakly anchored), 3 (weakly anchored), 4 (well anchored), and 5 (strongly anchored). The amount of roots at the point of anchorage was also evaluated on a scale of 0 to 5. The scale was based on the percentage of surface coverage by roots under the rockwool blocks. The scale was as follows: 0 (0% coverage), 1 (1-20% coverage), 2 (21-40% coverage), 3 (41-60% coverage), 4 (61-80% coverage) and 5 (81-100% coverage). The experimental design was a complete randomized block design with 3 (rockwool experiment) or 6 replicates (organic medium experiment). The experimental unit consisted of 3 plants in each slab and 2 plants in each 9-L plastic container for the rockwool and the organic medium experiments, respectively. The biological control efficacy for each of the microorganisms was calculated using the following formula: {[number of infected roots (*P. ultimum* infected control) – number of infected roots (treatment with the antagonistic microorganisms)]/number of infected roots (*P. ultimum* infected control)} × 100.

3.2.5 Effect of antagonistic microorganisms on the growth of healthy tomato seedlings

Tomato seeds were soaked in sterile distilled water for 6 h and were planted in pots (10 cm diameter) containing perlite (1 plant/pot). These were treated with 1 mL of sterile distilled water (control), a bacterial suspension (4×10^7 bacteria/mL) or a fungal spore suspension (1×10^6 spores/mL) of one of the antagonistic microorganisms. The seedlings were kept under typical greenhouse growing conditions [temperatures of 18°C (night) and 24°C (day), 80% relative humidity, 16 h photoperiod] for 30 days and watered daily with a nutrient solution of 20-20-20 (N-P-K) (Plantprod) at a concentration of 1%. This fertilizer contained the following micronutrients: Fe (0.1%), Mn (0.05%), Zn (0.05%), Cu (0.05%), B (0.02%) and Mo (0.0005%). The seedlings were then gently removed from the pot and the root system was washed with distilled water to remove the perlite. Roots and shoots were seperated, dried for 48 h at 75°C and weighed. A complete randomized design with 4 replicates was used. The experimental unit consisted of a pot containing 1 plant.

3.2.6 Statistical analysis

Data were analyzed with SAS (SAS Institute, Cary, NC), using the general linear models procedure. When significant ($P<0.05$), treatment means were compared using the Fisher protected LSD test.

3.3 Results

3.3.1 Effect of antagonistic microoganisms on Pythium root rot severity on tomato plants grown in rockwool

Of the 28 microorganisms tested in the first trial (summer crop), 27 significantly reduced root rot severity compared to the control (*P. ultimum* inoculated plants) (Table 1). The microorganisms allowing an infection rate less than 60% were selected for the second

trial (fall crop) with the exception of fungus 8-13-1 and *Pseudomonas corrugata* strain 1 that displayed irregular *in vitro* growth. Among the 17 microorganisms tested, only *P. restrictum* failed to significantly suppress infection compared to the control (*P. ultimum* inoculated plants) (Table 1). Eight microorganisms markedly reduced the severity of the root rot infection rate to less than 25% [*Penicillium brevicompactum* (23.8%), *P. solitum* strain 1 (15.0%), *Pseudomonas fluorescens* (18.8%), *P. fluorescens* subgroup G strain 2 (22.8%), *P. marginalis* (22.8%), *P. putida* subgroup B strain 1 (15.0%), *P. syringae* strain 1 (11.1%) and *Trichoderma atroviride* (21.9%)]. These microorganisms were selected for further study.

3.3.2 Effect of antagonistic microorganisms on root rot severity, root development, plant growth and fruit yields of *P. ultimum* infected tomato plants grown in rockwool and in organic medium

All the microorganisms tested significantly reduced the rate of infection resulting from *P. ultimum* inoculation (Table 2). *P. syringae* strain 1 (77.8%) and *P. brevicompactum* (69.9%) exhibited the highest biocontrol efficacies in rockwool and in organic medium, respectively. In the rockwool, all the microorganisms significantly improved the anchorage of the plants without significantly increasing the amount of roots under the blocks (Table 2). Plants treated with *P. brevicompactum, P. fluorescens*, *P. marginalis* and *P. syringae* strain 1 had significantly greater stem lengths than the control (*P. ultimum* inoculated plants). In the organic medium, *P. brevicompactum*, *P. solitum* strain 1, *P. fluorescens, P. putida* subgroup B strain 1, *P. syringae* strain 1 and *T. atroviride* treated plants had significantly greater amount of roots at the point of anchorage. Except for *P. solitum* strain 1 and *P. syringae* strain 1, the improvement was associated with a significant increase in the anchorage of the plants. Plants treated with any one of the eight microorganisms had significantly longer stems as compared to the control (*P. ultimum* inoculated plants). Stem diameters (average of 11 cm) were not significantly different between treatments throughout the crop.

In regard to fruit yields, *P. solitum* strain 1, *P. fluorescens*, *P. marginalis*, *P. putida* subgroup B strain 1, *P. syringae* strain 1 and *T. atroviride* treated plants had significantly

greater fruit size and both marketable and total yields compared to *P. ultimum* infected tomato plants grown in rockwool. In the organic medium, *P. marginalis* was the only microorganism out of the eight tested to significantly affect fruit production of *P. ultimum* infected plants. *P. marginalis* treated plants had significantly greater marketable fruit yield compared to the control (*P. ultimum* inoculated plants) (Table 3). This increase in fruit yield was associated with a significantly higher number of fruit per plant.

3.3.3 Effect of antagonistic microorganisms on the growth and root development of healthy tomato seedlings

The microorganisms significantly influenced the development of both the shoot and the root system of the healthy tomato seedlings. *P. brevicompactum, P. solitum* strain 1, *P. marginalis* and *T. atroviride* treated plants had significantly greater shoot and root dry weights whereas *P. fluorescens* subgroup G strain 2 and *P. putida* subgroup B strain 1 treated plants had significantly greater shoot dry weight only (Table 4).

3.4 Discussion

Tomato crops grown in hydroponic systems that use inert material as growing medium are especially susceptible to pathogens such as *P. ultimum,* the causal agent of tomato root rot (Stanghellini and Rasmussen 1994; Zinnen 1988). The disease is characterized by a premature weakening of the root system and a poor anchorage in the growing medium that affect water absorption. As a result, a gradual degeneration of the plant throughout the production period is observed leading to a decrease in plant growth and fruit yield.

In this study, several microorganisms previously shown to have *in vitro* antagonistic activity against *P. ultimum* (Gravel et al. 2005), were tested in greenhouse assays in order to evaluate their effect on tomato root rot development as well as on plant growth and fruit yield. The results showed that, except for *Penicillium solitum* strain 2, treatment with any of the 27 antagonistic microorganisms tested resulted in a significant suppression of disease severity. However, the extent of suppression depended on the

microorganisms used. Among the microorganisms tested, *P. brevicompactum*, *P. solitum* strain 1, *P. fluorescens*, *P. fluorescens* subgroup G strain 2, *P. marginalis*, *P. putida* subgroup B strain 1, *P. syringae* strain 1 and *T. atroviride* generally had the greatest effect as compared to the control during both growing seasons. These eight microorganisms were therefore further tested for their effect on anchorage, growth and yield of *P. ultimum* infected plants. Although *P. syringae* is known as the causal agent of tomato bacterial leaf spot (Richard and Boivin 1994), our strain did not cause this disease on plants throughout the experiments.

The anchorage of the plant in a *Pythium* spp. infested growing medium can be decreased either by a reduction of the development of the root system or by a degradation of the anchoring roots (Hendrix and Campbell 1973). When plants were grown in rockwool in the presence of any of the eight microorganisms, the root system was healthier (less symptoms of root rot) but the amount of roots in the rockwool slab was not affected suggesting that the improved anchorage results from a reduced degradation of the anchoring roots.

Among the microorganisms selected, the biological control potential of *P. brevicompactum*, *P. fluorescens*, *P. putida* and *T. atroviride* has been reported against numerous diseases such as *Fusarium* crown and root rot on tomato (Menzies and Ehret 1997), cucumber root rot (Rankin and Paulitz 1994), sugar beet damping-off (Shah-Smith and Burns 1996) and apple ring rot (Kexiang et al. 2002), respectively. This study reports, for the first time, the biocontrol efficacy of those microorganisms against Pythium root rot of mature greenhouse tomato crops grown in hydroponics. The possible mechanisms involved in the reduction of root rot in our experiment have not yet been identified. However, the suppressive activity of *P. fluorescens* and *P. putida* has, in the past, been associated with the production of antibiotic compounds (Thrane et al. 2000) and the induction of systemic resistance (Hoffland et al. 1996; Raupach et al. 1996; Ongena et al. 2000, 2002; Ramamoorthy et al. 2002). The biocontrol potential of *T. atroviride* has often been associated with its mycoparasitic activity (Olmedo-Monfil et al. 2002; Rocha-Ramírez et al. 2002; Lu et al. 2004) and its production of chitinases (Mach

et al. 1999; Brunner et al. 2003; Hoell et al. 2005) whereas the antagonistic activity of *P. brevicompactum* has been associated with its synthesis of active metabolites (Cantín et al. 1998; Moya et al. 1999).

Concerning their effect on tomato plant development, *P. brevicompactum*, *P. solitum* strain 1, *P. fluorescens*, *P. marginalis*, *P. putida* subgroup B strain 1, *P. syringae* strain 1 and *T. atroviride* improved the reproductive growth of infected tomato plants which resulted in an increase in fruit size and yield. In this regard, they can be considered as plant growth-promoting microorganisms with an indirect effect on plant growth (Antoun and Prévost 2005). Indeed, the beneficial effect of those microorganisms seems related, at least partially, to the suppression of *P. ultimum* resulting in a generally better health of the plant. *P. brevicompactum*, *P. fluorescens*, *P. marginalis,* and *P. syringae* strain 1 also stimulated the vegetative growth of tomato plants in rockwool infested with *P. ultimum.* Such a beneficial effect on plant growth of *P. fluorescens, P. putida* and *T. atroviride* has previously been reported in the presence of *Sclerotinia sclerotiorum* on alfalfa (Li et al. 2005), *Rhizoctonia solani* on wheat (de Freitas and Germida 1991) and *Fusarium culmorum* on rye (Kurek and Jaroszuk-Ścisel 2003), respectively.

Considering the results obtained, it is possible that the microorganisms could have a direct impact on the growth of healthy plants. In order to verify this hypothesis, the effect of these microorganisms on stem and root growth of healthy tomato seedlings was evaluated. The results obtained showed a stimulating effect of six microorganisms (*P. brevicompactum, P. solitum* strain 1, *P. fluorescens* subgroup G strain 2, *P. marginalis, P. putida* subgroup B strain 1, and *T. atroviride*) on root dry weight, shoot dry weight or both, strongly suggesting that these microorganisms act as PGPR (plant growth-promoting rhizobacteria) or PGPF (plant growth-promoting fungi). To the best of our knowledge, this is the first study to report the plant growth-promoting effect of *P. solitum*, *P. marginalis* and *P. syringae.* The PGPR effect of *P. putida* has been reported on cucumber seedlings (Utkhede et al. 1999). Other studies have shown that *P. brevicompactum*, when used in combination with other microorganisms (Menzies and Ehret 1997) and *P. fluorescens* (Gagné et al. 1993) have a stimulating effect on the

vegetative growth of greenhouse tomato. Further studies will be undertaken to evaluate the capacity of these microorganisms to improve fruit yield and plant growth of mature plants under hydroponic conditions.

P. brevicompactum, *P. solitum* strain 1, *P. fluorescens*, *P. fluorescens* subgroup G strain 2, *P. marginalis*, *P. putida* subgroup B strain 1, *P. syringae* strain 1 and *T. atroviride* were shown to suppress root rot infection on tomato plants grown in an organic medium consisting of a mixture of peat, pine sawdust and compost. The presence of an abundant microflora at planting in the organic medium did not appear to interfere with the biocontrol activity of the antagonistic microorganisms tested. The reduction in the disease severity was generally associated with a better anchorage in the growing medium, as was the case in the rockwool experiment, and probably resulted from a healthier and slightly more abundant root system. For all the microorganisms tested, the reduction in the disease resulted in a stimulation of the vegetative growth of the plant.

The greenhouse trials included in this study were done over a 2-year period and therefore were meant to test the microorganisms under a range of environmental conditions. Indeed, the development of root rot of greenhouse tomato crops is highly influenced by temperature, especially in the growing medium, and lighting conditions. The eight microorganisms selected were as effective in controlling root rot under spring (increasing temperature and natural light), summer (high temperature and optimal light), and fall (lower temperature and reduced natural light which are conducive for disease development) conditions. Whereas most work on *P. ultimum* has concentrated on damping-off of seedlings (Hultberg et al. 2000; Carisse et al. 2003; Gravel et al. 2005) or on short term crops (Caron et al. 2002), our study demonstrates the efficacy of these antagonistic microorganisms to reduce the deleterious effects of *P. ultimum* on mature tomato crops over several months.

This study led to the selection of antagonistic microorganisms that displayed biocontrol activity against tomato root rot under different environmental conditions (growing media; spring/summer/fall conditions). This may open the way for new alternatives for the

biological control of Pythium diseases in hydroponic systems that not only protect the crop but also have a beneficial effect on the plant growth and development. Further work will be conducted to develop formulations of these microorganisms in order to improve their efficacy. These will then be compared to commercially available biological control agents, including Rootshield® (*T. harzianum*) and Mycostop® (*Streptomyces griseoviridis*) that are currently registered for the control of root diseases in greenhouse vegetable crops. Further work will also be undertaken to identify the mechanisms involved in the reduction of the disease and to confirm the PGPR/PGPF effects of these microorganisms.

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Table 1. Effect of different antagonistic microorganisms on the severity of Pythium root rot of tomato plants grown in rockwool.

	Source of microorganisms[c]	Root rot severity (% infected roots) Summer crop (trial #1)	Fall crop (trial #2)
Control[a]		4.2 m	3.3 g
Control (*Pythium ultimum*)[b]		98.3 a	58.3 a
Acremonium potronii	B	80.0 b	NT
Acrodontium griseum	D	35.8 ijkl	35.0 bc
Enterobacter cloacae	D	79.2 b	NT
Fusarium oxysporum strain 1	C	68.3 bcd	NT
Fusarium tumidum	D	48.3 efghi	28.9 cde
fungus 5-12-6	B	62.5 cde	NT
fungus 8-13-1	C	51.3 efgh	NT
fungus 2-8-8	B	46.7 fghi	43.1 b
fungus 2-6-2	A	30.8 jkl	34.4 bc
Penicillium brevicompactum	B	27.5 l	23.8 cdeg
Penicillium griseofulvum	C	51.7 efgh	45.0 b
Penicillium janthinellum strain 1	A	59.2 def	28.9 cde
Penicillium restrictum	C	45.0 fghij	47.8 ab
Penicillium simplicissimum strain 1	B	57.5 defg	29.4 cd
Penicillium simplicissimum strain 2	A	79.2 b	NT
Penicillium solitum strain 1	A	43.3 ghijk	15.0 efg
Penicillium solitum strain 2	B	93.0 a	NT
Pseudomonas corrugata strain 1	R	56.7 defg	NT
Pseudomonas fluorescens	R	29.2 kl	18.8 def
Pseudomonas fluorescens F	C	73.3 bc	NT
Pseudomonas fluorescens G strain 2	R	53.3 efgh	22.8 cdef
Pseudomonas marginalis	A	35.8 ijkl	22.8 cdef
Pseudomonas putida B strain 1	C	43.3 ghijk	15.0 efg
Pseudomonas resinovorans	R	74.2 bc	NT
Pseudomonas syringae strain 1	R	34.2 ijkl	11.1 fg
Pseudomonas viridiflava	R	68.3 bcd	NT
Trichoderma atroviride	A	29.2 kl	21.9 cdef
Trichoderma longibrachiatum	B	39.0 hijkl	25.6 cde

[a] Plants were drenched with sterile distilled water (non-infected control).

[b] Plants were drenched with sterile distilled water and were inoculated with a propagule suspension of *P. ultimum*.

[c] Microorganisms were isolated from rockwool (R), a mixture of peat and compost (9:1, *v:v*) (A), a mixture of peat, pine bark and compost (6:3:1, *v:v:v*) (B), a mixture of peat, pine bark and compost (3:6:1, *v:v:v*) (C) and a mixture of pine bark and compost (9:1, *v:v*) (D).

Each value represents a mean of 2 (trial #1) or 3 (trial #2) replicates.

Within a column, values followed by the same letter are not significantly different according to Fisher protected LSD ($P<0.05$).

Note: NT, not tested

Table 2. Effect of selected antagonistic microorganisms on root rot severity, amount of roots, anchorage in the growing medium and total length of the stem of infected tomato plants grown in rockwool and in an organic medium.

	Root rot severity (% infected roots)	Amount of roots[a]	Anchorage of the plant[b]	Length of the stem (cm)
Rockwool (Spring crop)				
Control[c]	11.6 e	4.4 a	4.8 a	502 a
Control (*Pythium ultimum*)[d]	56.2 a (0.0)[e]	3.7 b	2.8 c	484 b
P. brevicompactum	37.7 b (32.9)	3.8 b	4.2 b	502 a
P. solitum strain 1	25.4 bcde (54.8)	4.0 ab	4.5 ab	499 ab
P. fluorescens	13.7 de (75.6)	3.6 b	4.0 b	505 a
P. fluorescens G strain 2	17.9 cde (68.2)	3.7 b	4.3 ab	500 ab
P. marginalis	31.6 bc(43.7)	3.9 ab	4.3 ab	506 a
P. putida B strain 1	27.5 bcd (51.1)	3.7 b	4.2 b	500 ab
P. syringae strain 1	12.5 e (77.8)	3.5 b	4.2 b	507 a
T. atroviride	27.9 bcd (50.4)	3.9 ab	4.4 ab	499 ab
Organic medium (Fall crop)				
Control	9.0 b	3.5 a	4.6 a	450 ab
Control (*Pythium ultimum*)	42.9 a (0.0)	2.4 b	3.2 c	433 a
P. brevicompactum	12.9 b (69.9)	3.5 a	3.9 ab	459 b
P. solitum strain 1	16.3 b (62.1)	3.4 a	3.8 abc	455 b
P. fluorescens	14.1 b (67.2)	3.8 a	3.9 ab	452 b
P. fluorescens G strain 2	14.6 b (66.0)	3.1 ab	3.8 abc	453 b
P. marginalis	14.2 b (67.0)	3.2 ab	3.5 bc	459 b
P. putida B strain 1	14.6 b (66.0)	3.9 a	4.2 ab	453 b
P. syringae strain 1	13.8 b (68.0)	3.8 a	3.8 abc	457 b
T. atroviride	14.6 b (66.0)	3.6 a	3.9 ab	457 b

[a] The amount of roots at the point of anchorage was also evaluated on a scale of 0 to 5 defined as follows: 0 (0% coverage), 1 (1-20% coverage), 2 (21-40% coverage), 3 (41-60% coverage), 4 (61-80% coverage) and 5 (81-100% coverage).

[b] The anchorage of the plant in the growing media was evaluated qualitatively on a scale of 0 to 5 based on the force needed to manually remove the plant from the growing medium. The scale was as follows: 0 (plant not anchored), 1 (hardly anchored), 2 (very weakly anchored), 3 (weakly anchored), 4 (well anchored), and 5 (strongly anchored).

[c] Plants were drenched with sterile distilled water (non infected control).

[d] Plants were drenched with sterile distilled water and were inoculated with a propagule suspension of *P. ultimum*.

[e] Values between brackets represent the biocontrol efficacy (%).

Each value represents a mean of 3 (rockwool) and 6 (organic medium) replicates.

For each experiment, values within a column followed by the same letter are not significantly different according to Fisher protected LSD ($P<0.05$).

Table 3. Effect of selected antagonistic microorganisms on marketable and total fruit yields of infected tomato plants grown in rockwool and in an organic medium.

	Marketable			Total		
	Yield (g/plant)	Fruits/ plant	Fruit size (g)	Yield (g/plant)	Fruits/ plant	Fruit size (g)
Rockwool (Spring crop)						
Control[a]	6003 ab	35.0 ab	172 bc	6662 ab	39.0 ab	171 ab
Control (*Pythium ultimum*)[b]	5262 c	33.3 abc	158 c	5973 b	37.7 abc	158 b
P. brevicompactum	5991 ab	32.3 bc	186 a	6557 ab	36.5 bc	180 a
P. solitum strain 1	6526 a	35.8 a	182 ab	7101 a	39.4 ab	180 a
P. fluorescens	6406 a	35.4 ab	181 ab	6855 a	38.5 ab	178 a
P. fluorescens G strain 2	5448 bc	31.3 c	174 ab	6003 b	35.1 c	171 ab
P. marginalis	6453 a	36.2 a	179 ab	6894 a	39.0 ab	177 a
P. putida B strain 1	6381 a	35.7 a	179 ab	6969 a	39.6 a	176 a
P. syringae strain 1	6345 a	36.2 a	174 ab	6771 a	38.8 ab	173 a
T. atroviride	6428 a	36.0 a	178 ab	6968 a	40.0 a	174 a
Organic medium (Fall crop)						
Control	3695 ab	24.1 ab	153 ab	3998 ab	27.4 a	146 a
Control (*Pythium ultimum*)	3359 b	20.8 b	162 ab	3877 ab	25.0 a	156 a
P. brevicompactum	3544 ab	23.3 ab	152 b	3877 ab	27.1 a	144 a
P. solitum strain 1	3833 ab	22.9 ab	166 a	4406 a	27.9 a	159 a
P. fluorescens	3749 ab	24.0 ab	156 ab	4208 ab	28.1 a	150 a
P. fluorescens G strain 2	3469 ab	20.6 b	155 ab	3654 b	24.7 a	148 a
P. marginalis	4057 a	24.7 a	163 ab	4461 a	28.4 a	156 a
P. putida B strain 1	3845 ab	23.1 ab	166 ab	4191 ab	26.3 a	160 a
P. syringae strain 1	3449 b	22.6 ab	152 ab	3944 ab	27.7 a	143 a
T. atroviride	3765 ab	22.9 ab	165 ab	4123 ab	26.3 a	157 a

[a] Plants were drenched with sterile distilled water (non-infected control).

[b] Plants were drenched with sterile distilled water and were inoculated with a propagule suspension of *P. ultimum*.

Each value represents a mean of 3 (rockwool) and 6 (organic medium) replicates.

For each experiment, values within a column followed by the same letter are not significantly different according to Fisher protected LSD ($P<0.05$).

Table 4. Effect of selected microorganisms on growth and root development of healthy tomato seedlings grown in perlite.

	Dry weight of shoot (g)	Dry weight of roots (g)
Control	0.96 c	0.09 c
P. brevicompactum	2.88 ab	0.20 ab
P. solitum strain 1	4.08 a	0.23 ab
P. fluorescens	1.75 bc	0.15 bc
P. fluorescens G strain 2	2.58 b	0.18 bc
P. marginalis	2.60 b	0.19 b
P. putida B strain 1	2.97 ab	0.18 bc
P. syringae strain 1	1.68 bc	0.15 bc
T. atroviride	2.90 ab	0.29 a

Each value represents a mean of 4 replicates.
Within a column, values followed by the same letter are not significantly different according to Fisher protected LSD ($P<0.05$).

CHAPITRE 4

STIMULATION DE LA CROISSANCE ET AUGMENTATION DU RENDEMENT EN FRUITS CHEZ LA TOMATE DE SERRE SUITE À UNE INOCULATION AVEC *PSEUDOMONAS PUTIDA* OU *TRICHODERMA ATROVIRIDE* : LE RÔLE POSSIBLE DE L'ACIDE INDOLE-ACÉTIQUE (AIA)

Sommaire : Dans le cadre des travaux présentés dans ce chapitre, cinq bactéries (*Pseudomonas fluorescens*, *P. fluorescens* sous-groupe G souche 2, *P. marginalis*, *P. putida* sous-groupe B souche 1 et *P. syringae* souche 1) et trois champignons (*Penicillium brevicompactum*, *P. solitum* souche 1 et *Trichoderma atroviride*) ont été testés afin d'évaluer leur effet sur la croissance de plants sains matures de tomate cultivés en conditions hydroponiques. Les résultats obtenus ont montré que l'inoculation avec *P. putida* ou *T. atroviride* a permis d'augmenter le rendement en fruits des plants cultivés en laine de roche et en substrat organique. Des travaux ont par la suite été entrepris afin de déterminer dans quelle mesure l'acide indole-acétique (AIA) (production ou dégradation) serait impliqué dans le mécanisme par lequel ces micro-organismes ont eu un effet stimulant sur le développement des plants. Les résultats obtenus ont démontré que *P. putida* et *T. atroviride* sont en mesure de produire l'AIA et que la production *in vitro* a été stimulée par l'ajout (200 $\mu g\ ml^{-1}$) de L-tryptophane, tryptamine ou tryptophol dans le milieu de culture. Ces micro-organismes ont également la capacité de dégrader partiellement l'auxine lorsque cultivés dans un milieu minimal supplémenté ou non avec du saccharose. Les travaux réalisés suggèrent par ailleurs que l'effet stimulant observé pourrait être attribuable, à tout le moins en partie, à une régulation de la concentration d'auxines au niveau de la rhizosphère par ces micro-organismes et à une production microbienne de 1-aminocyclopropane-1-carboxylate (ACC) désaminase. À cet effet, les travaux réalisés suggèrent qu'une synthèse microbienne d'AIA à partir de tryptophane serait impliquée dans la stimulation du développement de plantules de tomate d'une part; ils démontrent d'autre part que la présence de *P. putida* ou *T. atroviride* au niveau de la rhizosphère réduit l'effet inhibiteur de l'AIA sur l'élongation racinaire.

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Growth stimulation and fruit yields improvement of greenhouse tomato plants by inoculation with *Pseudomonas putida* or *Trichoderma atroviride*: possible role of indole-acetic acid (IAA)

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Abstract: Five bacteria (*Pseudomonas fluorescens*, *P. fluorescens* subgroup G strain 2, *P. marginalis*, *P. putida* subgroup B strain 1 and *P. syringae* strain 1) and three fungi (*Penicillium brevicompactum*, *P. solitum* strain 1 and *Trichoderma atroviride*) were evaluated to determine their promoting effect on the growth of mature healthy tomato plants grown under hydroponic conditions. *P. putida* and *T. atroviride* were shown to improve fruit yields in rockwool and in organic medium. The production or degradation of indole-acetic acid (IAA) by those two microorganisms were then investigated as possible mechanisms for plant growth stimulation. Both *P. putida* and *T. atroviride* were shown to produce IAA. The production of IAA by the two microorganisms was stimulated *in vitro* by the addition of L-tryptophan, tryptamine and tryptophol (200 µg ml^{-1}) in the culture medium. *P. putida* and *T. atroviride* also increased the fresh weight of both the shoot and the roots of tomato seedlings grown in the presence of increasing concentrations of L-tryptophan (up to 0.75 mM). Both microorganisms were also shown to partially degrade IAA *in vitro* when grown in a minimal medium supplemented or not with sucrose. In addition, the capacity of these microorganisms to reduce the deleterious effect of exogenous IAA was investigated using tomato seedlings. The results showed that the roots of tomato seedlings grown in the presence of increasing concentrations of IAA (0 to 10 µg ml^{-1}) were significantly longer when seeds were previously treated with *P. putida* or *T. atroviride*. The reduction in the detrimental effect of IAA on root elongation could be associated with a reduced ethylene production resulting from a decrease of its precursor 1-aminocyclopropane-1-carboxylic acid (ACC) caused by IAA microbial degradation in the rhizosphere and/or by ACC deaminase activity present in both microorganisms.

Key words: plant growth-promotion, greenhouse tomato, hydroponic, fruit yields, biofertilizers, indole-acetic acid

4.1 Introduction

Beneficial effects of the introduction of specific microorganisms on plant growth have been reported for numerous crops, including tomato (*Lycopersicon esculentum* Mill.) grown under field (Kokalis-Burelle et al., 2002; Guo et al., 2004) or greenhouse conditions in organic media (Gagné et al., 1993). Such beneficial microorganisms referred as PGPR (plant-growth promoting rhizobacteria) or PGPF (plant-growth promoting fungi) enhance plant growth through numerous mechanisms including the protection of roots against infection by minor and major pathogens (Whipps, 1997; 2001), the enhancement of the availability of nutrients to the host plant, the lowering of the ethylene level within the plant or the enhanced production of stimulatory compounds, such as plant growth regulators (Antoun and Prévost, 2005). Among those, indole-3-acetic acid (IAA) is the most common natural auxin found in plants and its positive effect on root growth and morphology is believed to increase the access to more nutrients in the soil (Vessey, 2003).

The involvement of IAA in the complex interaction between the rhizosphere microflora and the host plant, which relies on a constant exchange of materials and signals (Antoun and Prévost, 2005), has been the focus of numerous works (Costacurta and Vanderleyden, 1995; Patten and Glick, 1996; Persello-Cartieaux et al., 2003). Root tissues are especially sensitive to fluctuating concentrations of IAA and the development of the root system can be greatly affected by exogenous sources of this plant growth regulator, including microbial (Tanimoto, 2005). While the production of IAA by microorganisms commonly found in the rhizosphere of plants, such as *Pseudomonas* spp. and *Rhizobium* spp., is often associated with their potential to stimulate plant growth (Antoun et al., 1998; Barazani and Friedman, 1999; Patten and Glick, 2002), the synthesis of high amounts of IAA by rhizosphere bacteria has been shown to inhibit root growth rather than to promote it (Xie et al., 1996). In this context, rhizosphere microorganisms capable of degrading IAA might have a positive effect on plant growth. However, even though Leveau and Lindow (2005) reported the ecological role of such a microorganism, little is known on the specific impact of IAA-degraders on plant growth.

In the present study, eight microorganisms with a reported beneficial effect on the growth of *Pythium*-infected tomato plants (Gravel et al. 2006) were tested to evaluate their effect on the growth of mature healthy tomato plants grown in rockwool and in organic medium under hydroponic conditions, during a complete production period. The effect of selected microorganisms through the production or degradation of IAA on plant growth was also investigated.

4.2 Materials and methods

4.2.1 Microorganisms

The microorganisms tested in this study were isolated from the rhizosphere of tomato plants (Gravel et al., 2005). Bacteria tested were *Pseudomonas fluorescens* Migula, *P. fluorescens* Migula subgroup G strain 2, *P. marginalis* (Brown) Stevens, *P. putida* (Trevisan) Migula subgroup B strain 1, and *P. syringae* van Hall strain 1. Bacteria were grown on tryptic soy agar (TSA, Sigma-Aldrich, Mississauga, ON, Canada) and stock cultures were kept in 80% glycerol at -80°C. Bacterial suspensions were prepared in 500-ml flask containing 250 ml of tryptic soy broth (TSB, Sigma-Aldrich). The flasks were inoculated with 1 ml of an overnight culture of the bacteria grown in TSB. The flasks were incubated on a rotary shaker (150 rev min^{-1}) at 24°C for 24 h. Bacterial cells were removed by centrifugation (4000*g*, 10 minutes), resuspended in sterile distilled water and diluted to 4×10^7 bacteria ml^{-1} as determined by plate count on TSA medium. The fungi tested were *Penicillium brevicompactum* Dierckx, *P. solitum* Westling strain 1 and *Trichoderma atroviride* Karsten. All fungi were grown on potato dextrose agar (PDA, Difco Laboratories, Becton Dickinson, Sparks, MD, U.S.A.) and stock cultures were freezed-dried and kept at -20°C. Spore suspensions were prepared by scraping the surface of a 2-week-old mycelium grown on PDA using a glass rod. Each spore suspension was adjusted by dilution with sterile distilled water to 1×10^6 spores ml^{-1} based on hemacytometer counts.

4.2.2 Greenhouse assay on fruit yield and plant growth

Greenhouse assays were conducted to evaluate the effect of the microorganisms on plant growth and development in rockwool and in organic medium. The rockwool experiment was performed under spring conditions (January to June) while the organic medium experiment was conducted under fall conditions (July to December). The organic medium used was a mixture of peat, pine sawdust and compost (v/v/v, 60/30/10). Tomato seeds (cv. Trust F1, De Ruiter Seeds, Columbus, OH, U.S.A) were sown in multicellular blocks of rockwool and grown for 4 weeks (25°C and 80% relative humidity). The 10-cm tall plants were placed on rockwool slabs or on top of 9-L plastic containers filled with the organic medium and were drenched with 200 ml of either a bacterial (4×10^7 bacteria ml^{-1}) or a fungal spore (1×10^6 spores ml^{-1}) suspension of each microorganism. Control plants received 200 ml of sterile distilled water. Plants were grown under typical greenhouse growing conditions [temperatures of 18°C (night) and 24°C (day), 80% relative humidity] and drip irrigated using a nutrient solution containing 0.51 g l^{-1} of 6-11-31 (N-P-K; Plantprod, Brampton, ON, Canada) and 0.69 g l^{-1} of 15.5-0-0 (N-P-K; Plantprod). The 6-11-31 formulation also contained Mg (3.0 %), S (3.5%), Fe (0.3%), Mn (0.06%), Zn (0.02%), Cu (0.004%), B (0.027%) and Mo (0.009%). As for the 15.5-0-0 formulation, it contained Ca (19%). Nutrient solution conductivity (EC) was kept at 3.0 mS/cm. The pH of the nutrient solution was kept between 5.5 and 6.0 through the addition of phosphoric acid. For the organic medium experiment (fall conditions), the natural daylight was supplemented with HPS lamps (100 µE m^{-2} per second PAR) to maintain a photoperiod of 16 h. Fruit yield was measured throughout the crop. The fruits were harvested twice a week for a period of 11 weeks and were separated into marketable and total yields. The marketable yield included fruits with fresh weight between 88 and 450 g whereas the total yield included all fruits harvested including fruits with blossom end rot and misshaped fruits (usually over 450 g). Throughout the crops, stem diameter was measured weekly to assure that plants were developing normally. At the end of the crop (6 months), the length of the stem was measured. The experimental design was a randomized complete block design with 3 (rockwool experiment) and 6 replicates (organic medium experiment). The experimental unit consisted of 3 plants in

each slab and 2 plants in each container for the rockwool and the organic medium experiments, respectively. Each plant represented a sampling unit.

In each of the two experiments, the 10th leaf from the apex was collected from each plant in every experimental unit after 6 months of culture. Leaves from the same experimental unit were pooled. Digestion of the leaves collected was done according to the method described by Isaac and Johnson (1976). Briefly, leaves were dried at 75°C for 48 h, ground, and 100 mg were mixed with 2 ml of a mineralizing solution (97 g of H_2SeO_3 in 100 ml of H_2SO_4) and 2 ml of H_2O_2. The mixture was then placed on a digesting block at 400°C for 20 min. Once the samples were cooled, 4 ml of distilled water, 1 ml of lanthane solution (5%) and 94 ml of distilled water were added, in that order. The amount of nitrogen and phophorus present was evaluated colorimetrically by comparison with a standard curve of known concentrations (Tandon et al., 1968; Nkonge and Ballance, 1982). The amount of potassium was evaluated by flame atomic emission spectroscopy whereas the amount of calcium and magnesium was measured by atomic absorption spectroscopy (Atomic Absorption Spectrometer 3300, Perkin Elmer, Überlingen, Germany) (Jones and Isaac, 1969).

4.2.3 Phosphate solubilization by *P. putida* and *T. atroviride*

The phosphate solubilization ability of *P. putida* and *T. atroviride* was evaluated qualitatively. Both microorganisms were grown in the NBRI-BPB medium described by Mehta and Nautiyal (2001) containing per liter: 10 g of sucrose, 5 g of $Ca_3(PO_4)_2$, 5 g of $MgCl_2 \cdot 6H_20$, 0.25 g of $MgSO_4 \cdot 7H_2O$, 0.2 g of KCl, 0.1 g of $(NH_4)_2SO_4$ and 0.025 g of bromophenol blue (BPB). Prior to sterilization, the pH of the culture medium was adjusted to 7.0. Fifteen ml of NBRI-BPB medium in 50-ml tubes were inoculated with 100 µl of either a suspension of *P. putida* (4×10^7 bacteria ml^{-1}) or of *T. atroviride* (1×10^6 spores ml^{-1}). Controls consisted of the uninoculated culture medium. The cultures, in triplicates, were incubated for 96 h at 25°C on a rotary shaker at 150 rev min^{-1} after which they were centrifuged (4000*g*, 10 min) and the optical density of the supernatants was measured at 600 nm.

4.2.4 Production of IAA and IAA-related compounds by *P. putida* and *T. atroviride*

The production of IAA and IAA-related compounds by the microorganisms was evaluated spectrophotometrically. Liquid cultures were prepared in 250-ml flasks containing 100 ml of half-strength TSB supplemented or not (control) with 200 μg ml^{-1} of either L-tryptophan, tryptamine or tryptophol (Sigma-Aldrich). For *P. putida*, the flasks were inoculated with 100 μl of an overnight culture (OD_{600} of 0.7). For *T. atroviride*, the culture medium was inoculated with 3 PDA disks covered with actively growing mycelium. After an incubation period of 72 h (*P. putida*) or 1 week (*T. atroviride*) on a rotary shaker (150 rev min^{-1}, 24°C), bacterial cells and mycelium were removed by centrifugation (4000*g*, 10 min) and by filtration, respectively. One ml of each supernatant or each filtrate was mixed vigorously with 2 ml of Salkowski's reagent (150 ml of $HClO_4$, 250 ml of distilled water and 7.5 ml of 0.5M $FeCl_3 \cdot 6\ H_2O$) (Gordon and Weber, 1951). The mixture was incubated at room temperature for 20 min and the absorbance was measured at 535 nm. The concentration of IAA and IAA-related compounds was evaluated by comparison with a standard curve prepared using serial dilutions of a 50 μg ml^{-1} IAA (Sigma-Aldrich) solution in half-strength TSB. Four replicates were used for each treatment.

The production of IAA by *P. putida* and *T. atroviride* was confirmed with thin layer chromatography (TLC). Fifty ml of each supernatant (*P. putida*) or filtrate (*T. atroviride*) were adjusted to pH 2.0 with 1M HCl and subsequently extracted with an equal volume of ethyl acetate. The ethyl acetate layer was recovered and evaporated with a Rotavapor R-200 (Büchi Analytical inc., New Castle, DE, U.S.A.). The residue was taken up in methanol and developed on TLC plates using isopropanol-ammonia-water (10/1/1, v/v/v) as a solvent. The plates were sprayed with a reagent (3% H_2SO_4 in methanol containing 50 mg $FeCl_3$) and heated until colour development. IAA appeared as a red colour under visible light and orange colour under UV light.

4.2.5 Effect of L-tryptophan on the growth of seeds inoculated with *P. putida* or *T. atroviride*

Tomato seeds were surface sterilized by soaking in 70% ethanol for 5 min and subsequently in 2% hypochloric acid for 1 min. The seeds were then rinsed thoroughly three times with sterile distilled water. Seeds were inoculated by soaking for 1 h in a suspension of *P. putida* (4×10^7 bacteria ml^{-1}) or *T. atroviride* (1×10^6 spores ml^{-1}). Control seeds were soaked in sterile distilled water. Subsequently, two seeds were placed in individual growth pouches containing 20 ml of a sterile solution of 0, 0.25, 0.50 or 0.75 mM of L-tryptophan. Pouches, wrapped in aluminium foil, were placed in a growth chamber at 25°C for 14 d after which the following parameters were measured: length of the shoot, fresh weight of the shoot and fresh weight of the roots. The experimental design was a complete randomized design with 5 replicates. The experimental unit consisted of a pouch in which 2 seedlings were grown. Each seedling was a sampling unit.

4.2.6 *In vitro* degradation of IAA by *P. putida* and *T. atroviride*

P. putida and *T. atroviride* were grown in 125-ml flasks containing 50 ml of M9 minimal medium (per liter: 6.02 g of Na_2HPO_4, 3 g of KH_2PO_4, 0.5 g of NaCl, 1 g of NH_4Cl, 2 ml of 1M $MgSO_4 \cdot 7H_2O$, and 0.1 ml of 1M $CaCl_2$) amended with 25 $\mu g\ ml^{-1}$ of IAA with or without sucrose (2 mg ml^{-1}). For *P. putida* subgroup B strain 1, the flasks were inoculated with 50 µl of an overnight culture (OD_{600} of 0.7) whereas for *T. atroviride*, the flasks were inoculated with 2 PDA disks covered with actively growing mycelium. Control flasks were not inoculated (sterile cultures). Flasks were incubated on a rotary shaker (150 rev min^{-1}) for 72 h at 24°C. Bacterial cells and mycelium were removed by centrifugation (4000*g*, 10 min) and filtration, respectively. Bacterial and fungal biomasses were determined by measuring the optical density (OD_{600}) or the fresh weight of the mycelium in the culture medium, respectively. The amount of IAA remaining in the medium was evaluated as previously described. The rate of IAA degradation was calculated as follows: 100 – [(final concentration of IAA remaining in the culture medium expressed in $\mu g\ ml^{-1}$ / 25 $\mu g\ ml^{-1}$ IAA) × 100].

4.2.7 Effect of exogenous IAA on root development of seeds inoculated with *P. putida* or *T. atroviride*

Tomato seeds were surface sterilized and inoculated with either *P. putida* or *T. atroviride* as described previously. Control consisted of uninoculated seeds. Subsequently, two seeds were placed in individual growth pouches containing 20 ml of a sterile solution of 0, 0.01, 0.1, 1 or 10 μg ml^{-1} of IAA. Pouches, wrapped in aluminium foil, were placed in a growth chamber at 25°C for 7 d after which the length of the initial root was measured for each seedling. The experimental design was a complete randomized design with 5 replicates. The experimental unit consisted of a pouch in which 2 seedlings were grown. Each seedling was a sampling unit.

4.2.8 Evaluation of 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity in *P. putida* and *T. atroviride*

Lysates of *P. putida* and *T. atroviride* cultures were prepared as described by Saleh and Glick (2001). Briefly, 25 μl of toluene was added to a 200 μl aliquot of the washed suspensions of *P. putida* or *T. atroviride* and was vortexed vigorously for 30 s. Then, 20 μl of 0.5M ACC was added and after an incubation period of 15 min at 30°C, 1 ml of 0.56N HCl was added. The microorganisms lysates were centrifugated (10000 rev min^{-1}, 10 min) after which 1 ml of the supernatant was mixed with 800 μl of 0.56N HCl and 300 μl of 2,4-dinitrophenylhydrazine (0.2 g in 100 ml of 2N HCl). The mixtures were incubated for 30 min at 30°C after which 2 ml of 2N NaOH were added. The absorbance was measured at 540 nm. The ACC deaminase activity of *P. putida* and *T. atroviride* was evaluated quantitatively by measuring the amount of α-ketobutyrate produced by the deamination of ACC. ACC deaminase activity was expressed in μmol of α-ketobutyrate mg $protein^{-1}$ h^{-1}. Four replicates were used for each treatment.

4.2.9 Statistical analysis

When appropriate, analysis of variance (ANOVA) was performed with SAS (SAS Institute, Cary, NC), using the general linear models procedure. When significant ($P<0.05$), treatment means were compared using the Fisher protected LSD test.

4.3 Results

4.3.1 Greenhouse assay on fruit yield and stem growth

Four of the microorganisms (*P. brevicompactum, T. atroviride, P. marginalis,* and *P. putida*) significantly increased the marketable fruit yield compared to the control in rockwool (Table 1). The total yield was also increased by the microorganisms as compared to the control, however not significantly. In the organic medium, the inoculation of the plants with *T. atroviride* or with *P. putida* lead to a significant increase in the marketable and total yields as compared to the control (Table 1). The length of the stems was not markedly affected by the inoculation of the microorganisms and ranged from 482 to 509 cm (rockwool) and 447 to 471 cm (organic medium).

The mineral content in the leaves of plants grown in rockwool was not affected by the presence of any of the microorganisms (data not shown). However, in the organic medium, the amount of P was significantly higher when plants were grown in the presence of any of the eight microorganisms tested (Table 2). The N, K, Ca and Mg contents were, however, not affected significantly by the presence of the microorganisms, except in the case of *T. atroviride* which reduced significantly the amount of Ca found in the leaves as compared to the control (Table 2).

4.3.2 Phosphate solubilization by *P. putida* and *T. atroviride*

The results showed that neither *P. putida* nor *T. atroviride* were able to solubilize the sparingly soluble source of P in liquid cultures.

4.3.3 Production of IAA and IAA-related compounds by *P. putida* and *T. atroviride*

The capacity of *P. putida* and *T. atroviride* to produce IAA and IAA-related compounds was evaluated. In the absence of an indole derivative as a precursor in the culture medium (control), *P. putida* and *T. atroviride* produced low levels of IAA and IAA-related compounds (Table 3). The production of IAA by both microorganisms was

stimulated by the addition (200 μg ml^{-1}) of L-tryptophan, tryptamine and tryptophol. *P. putida* produced the highest level of IAA and IAA-related compounds (23.4 μg ml^{-1} DO_{600}^{-1}) when grown in the presence of tryptamine whereas the highest level of IAA and IAA-related compounds (38.5 μg ml^{-1}) produced by *T. atroviride* were obtained in the presence of tryptophol (Table 3). A constant increase in the production of IAA and IAA-related compounds by these two microorganisms was also observed as the concentration of L-tryptophan in the growth medium was increased from 0 to 800 μg ml^{-1} (data not shown). In all cases, the production of IAA was confirmed by TLC analysis. Compounds with R_f values close to 0.56 (pure IAA) were considered as IAA (Table 3). Other unidentified IAA-related compounds but with different R_f values were also observed on the TLC plates indicating that they were also produced by *P. putida* and *T. atroviride*.

4.3.4 Effect of L-tryptophan on the growth of seeds inoculated with *P. putida* or *T. atroviride*

The length of the shoot and the fresh weight of both the shoot and the roots of control seedlings were not significantly stimulated by the increasing concentrations of L-tryptophan (Fig. 1). For seedlings inoculated with either *P. putida* or *T. atroviride*, the fresh weight of the shoot and of the roots increased as the concentration of L-tryptophan increased to 0.75 mM (Fig. 1). A similar effect was also observed for the length of the shoot (Fig. 1).

4.3.5 *In vitro* degradation of IAA by *P. putida* and *T. atroviride*

Both microorganisms partially degraded IAA in M9 minimal medium and in M9 minimal medium supplemented with sucrose. The degradation of IAA by *P. putida* in the medium containing no sucrose (38.3%) was significantly higher than that observed in the medium amended with sucrose (22.6%). The same tendency was observed with *T. atroviride*. Bacterial and fungal biomasses obtained in media amended with sucrose were not significantly different from those obtained in media unamended.

4.3.6 Effect of exogenous IAA on root development of seeds inoculated with *P. putida* or *T. atroviride*

Root development of non-inoculated (control) seedlings was significantly affected by the addition of IAA. The length of the root was reduced significantly as the concentration of IAA increased from 0 to 10 μg ml^{-1} (Fig. 3). As compared to the control, seedlings inoculated with *P. putida* and *T. atroviride* developed a significantly longer root in the presence of 1 and 10 μg ml^{-1} of IAA (Fig. 3). The increase in the IAA concentration from 0.1 to 10 μg ml^{-1} did not affect root elongation in seedlings treated with *P. putida* and *T. atroviride* (Fig. 3).

4.3.7 Evaluation of ACC deaminase activity in *P. putida* and *T. atroviride*

The ACC deaminase activity of the two microorganisms that were shown to grow normally on medium amended with ACC as the only source of nitrogen was evaluated quantitatively. The ACC deaminase activities of *P. putida* and of *T. atroviride* were 23.7±4.3 and 13.5±2.2 μmol α-ketobutyrate mg $protein^{-1}$ h^{-1}, respectively.

4.4 Discussion

In this study, the effect of eight microorganisms on the growth and development of healthy greenhouse tomato plants was investigated under hydroponic conditions using rockwool and organic medium as substrates. Greenhouse assays allowed the evaluation of the long term effect of the microorganisms on the reproductive as well as on the vegetative growth of the tomato plants.

The results showed the stimulating effect of *P. putida* subgroup B strain 1 and *T. atroviride* on the reproductive growth of tomato plants in both growing media. Even though the organic medium experiment was performed under fall conditions (lower temperature and decreased natural light) which were less favourable to plant growth and fruit ripening, *P. putida* subgroup B strain 1 and *T. atroviride* increased fruit production, just as they did in the rockwool experiment performed under more favourable conditions

(spring crop). Previous works have already reported the stimulating effect of *P. putida* on cucumber plants (Amer and Utkhede, 2000) and on canola seedlings (Xie et al., 1996). *Trichoderma* spp. have been also shown to exhibit plant growth-promoting activity on numerous cultivated plants (Kleifeld and Chet, 1992; Ousley et al., 1994; Altomare et al., 1999; Harman, 2000; Yedidia et al., 2001). However, this study reports for the first time the plant growth-promoting activity of *P. putida* and *T. atroviride* on the fruit yield of greenhouse tomato plants in hydroponic systems. *Penicillium brevicompactum* and *P. marginalis* were also shown to have a stimulating effect on the marketable fruit yield, but in plants grown in rockwool only.

The mechanisms by which *P. putida* subgroup B strain 1 and *T. atroviride* influenced plant growth were further investigated. In this study, the inoculation with *P. putida* subgroup B strain 1 and *T. atroviride* in the organic medium increased the P accumulation in the leaves as did the inoculation of the other tested microorganisms. Even though the solubilization of P is often associated with plant growth promotion (Richardson, 2001), in this particular experiment, the P in the nutrient solution was readily available for the plant, reducing the possibility that this mechanism played an important role in the stimulation observed. Furthermore, an *in vitro* assay showed that *P. putida* subgroup B strain 1 and *T. atroviride* were not P solubilizers under the conditions tested.

The production of plant growth regulators by the microorganisms is another important mechanism often associated with growth stimulation (Vessey, 2003). The balance between vegetative and reproductive growth is controlled by hormone signalling within the plant and can therefore be highly influenced by it (Taiz and Zeiger, 1991). At relatively high concentrations, natural auxins, such as indole-3-acetic acid (IAA), stimulate shoot elongation and root induction while reducing root elongation (Tanimoto, 2005). IAA is also involved in tomato fruit development, especially during fruit setting and in the final phase of development (Srivastava and Handa, 2005). Previous works have reported that the synthesis of IAA is often associated with plant growth stimulation by microorganisms, including *P. putida* (Xie et al., 1996; Patten and Glick, 2002). In this

study, results showed that *P. putida* subgroup B strain 1 and *T. atroviride* are able to synthesize IAA from different precursors *in vitro* which supports the theory that microbial IAA could be involved in the growth stimulation observed in our greenhouse assay. Of particular interest, the results showed that the growth of seedlings inoculated with *P. putida* subgroup B strain 1 or *T. atroviride* increased as the concentration of L-tryptophan increased in the pouches. This suggests that the synthesis of IAA through tryptophan-dependent pathways by *P. putida* subgroup B strain 1 or *T. atroviride,* affected the growth of the tomato seedlings. Tryptophan is naturally secreted in root exudates of tomato plants and most of the auxin found in the rhizosphere is believed to come from the biosynthesis by microorganisms (Kamilova et al., 2006). Exogenous sources of IAA, such as the one produced by microorganisms, is known to cause changes in the morphology of the root system which influence the uptake of nutrients by the plant (Arteca, 1996). In this regard, San-Francisco et al. (2005) showed that exogenous applications of IAA increased the amount of P in roots of pepper plants grown under hydroponic conditions. It is therefore possible that the increase in the level of P in the leaves of tomato plants grown in the organic medium might also be related, at least partially, to the production of IAA by *P. putida* subgroup B strain 1 and *T. atroviride*.

In addition to having a stimulating effect on plant growth, exogenous IAA in the rhizosphere can also have a detrimental effect on the elongation of roots over a wide range of concentrations. Such an effect has been associated with an increase in the level of ethylene in the plant (Glick et al., 1997; Glick et al., 1998). IAA can increase the activity of ACC synthase, which catalyses the conversion of *S*-adenosylmethionine to 1-aminocyclopropane-1-carboxylate (ACC), the precursor of ethylene in the plant (Kende, 1993). In this study, *P. putida* subgroup B strain 1 and *T. atroviride* were shown to reduce the inhibiting effect of relatively high concentrations of IAA on root elongation of tomato seedlings. The results from this study suggest the involvement of two possible mechanisms. First, both *P. putida* and *T. atroviride* were able to partially degrade IAA *in vitro*. The degradation of IAA by *P. putida* has been reported by Leveau and Lindow (2005) however, to the best of our knowledge, this is the first report of IAA degradation by *T. atroviride*. Such degradation could have reduced the concentration of IAA in the

vicinity of the roots to a level which was not detrimental to the elongation. Also, previous studies have shown that ACC deaminase activity in PGPR, which hydrolyzes ACC into ammonia and α-ketobutyrate, prevents the synthesis of inhibiting levels of ethylene (Penrose et al., 2001). This reduction in the level of ACC in the rhizosphere increases the exudation of ACC by the plant to maintain equilibrium, reducing the potential synthesis of ethylene (Glick et al., 1998). This study demonstrated that both *P. putida* subgroup B strain 1 and *T. atroviride* possess ACC deaminase activity when grown *in vitro*, suggesting that these microorganisms could also regulate the concentration of ethylene within the plant by reducing the amount of its precursor present. ACC deaminase has previously been reported for *Pseudomonas* spp. and its activity has been associated with an increase in root elongation due to the reduced inhibition caused by ethylene (Glick et al. 1997; Wang et al., 2000; Safronova et al., 2006). The synthesis of this enzyme has also been reported in fungi such as *Penicillium citrium* (Jia et al., 2000). However, to the best of our knowledge, this is the first report of an ACC deaminase activity in *Trichoderma atroviride*.

The effect of microbial production or degradation of IAA in the rhizosphere on fruit setting and tomato yield is most likely indirect, through an effect on the overall growth of the plant. Indeed, IAA, including microbial, can greatly influence the growth of the root system depending on the amount found in the rhizosphere, through root elongation and the formation of lateral or adventitious roots (Scott, 1972; Patten and Glick, 2002). In the case of *P. putida* and *T. atroviride*, their ability to produce and degrade IAA, combined with their ACC deaminase activity, may have promoted an optimal development of the root system and of the tomato plant in general, which could have resulted in the stimulation of the reproductive growth observed in this study.

Microbial production of IAA is known to result from different pathways (Persello-Cartieaux et al., 2003). Although tryptophan independent biosynthesis pathways have been identified in numerous microorganisms, tryptophan remains the most common precursor of microbial IAA (Patten and Glick, 1996). The 4 main tryptophan dependent metabolic pathways are the followings: tryptophol, tryptamine, indole-3-pyruvic acid and

indole-3-acetamide pathways (Bartel, 1997). In regard to the specific biosynthesis pathways of IAA in *P. putida* and *T. atroviride*, this study demonstrated that the enzymes responsible for the synthesis of IAA from both tryptophol and tryptamine are active. This suggests the existence of the tryptophol and tryptamine pathways in *P. putida* and *T. atroviride,* while previous works have reported the existence of the indole-3-pyruvic acid pathway in *P. putida* (Mordukhova et al., 2000; Patten and Glick, 2002).

This research demonstrated the capacity of two microorganisms, *P. putida* subgroup B strain 1 and *T. atroviride,* to promote the reproductive growth of tomato plants under typical hydroponic growing conditions. The plant growth stimulation reported in this study is, most likely, the synergic result of numerous modes of action exhibited by each microorganism tested, including a regulation in the concentration of IAA in the rhizosphere and a regulation of the concentration of ethylene within the roots. This study showed that *P. putida* subgroup B strain 1 and *T. atroviride* could be used as biofertilizers to improve the productivity of greenhouse tomato crops under hydroponic conditions in inert or organic media. More specific works are, however, needed to further study the specific mechanisms involved in the growth stimulation by *P. putida* subgroup B strain 1 and *T. atroviride,* as well as to better understand the close interaction between the host plant and these two microorganisms.

4.5 Acknowledgements

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Table 1
Effect of microorganisms on marketable and total fruit yields of mature tomato plants grown in rockwool and in organic medium for 6 months.

Microorganisms	Fruit yields (g plant^{-1}) Marketable		Total	
Rockwool (Spring crop)				
Control[a]	6003	b	6662	ab
Penicillium brevicompactum	6760	a	7304	a
Penicillium solitum strain 1	6542	ab	7023	ab
Trichoderma atroviride	6679	a	7111	ab
Pseudomonas fluorescens	6556	ab	7047	ab
Pseudomonas fluorescens G strain 2	5978	b	6431	b
Pseudomonas marginalis	6707	a	7345	a
Pseudomonas putida B strain 1	6881	a	7350	a
Pseudomonas syringae strain 1	6607	ab	7167	ab
Organic medium (Fall crop)				
Control	3695	c	3998	c
Penicillium brevicompactum	3889	abc	4289	bc
Penicillium solitum strain 1	3720	bc	4069	bc
Trichoderma atroviride	4218	ab	4608	ab
Pseudomonas fluorescens	4020	abc	4301	bc
Pseudomonas fluorescens G strain 2	3791	bc	4238	bc
Pseudomonas marginalis	3830	abc	4234	bc
Pseudomonas putida B strain 1	4329	a	4931	a
Pseudomonas syringae strain 1	4142	abc	4504	abc

[a] Plants were drenched with sterile distilled water.
Each value represents the mean of 3 (rockwool) or 6 (organic medium) replicates.
For each experiment, values within a column followed by a same letter are not significantly different according to Fisher protected LSD test ($P<0.05$).

Table 2
Effect of microorganisms on mineral content of leaves of tomato plants grown for 6 months in organic medium (Fall crop).

	N (%)	P (%)	K (%)	Ca (%)	Mg (%)
Control[a]	3.32 a	0.55 d	7.44 a	3.05 a	0.40 a
Penicillium brevicompactum	3.53 a	0.73 abc	8.13 a	2.59 ab	0.34 a
Penicillium solitum strain 1	3.58 a	0.75 ab	7.93 a	2.45 ab	0.36 a
Trichoderma atroviride	3.53 a	0.73 abc	8.11 a	2.32 b	0.33 a
Pseudomonas fluorescens	3.65 a	0.67 bc	8.07 a	2.92 ab	0.37 a
Pseudomonas fluorescens G strain 2	3.65 a	0.67 bc	7.99 a	3.06 a	0.33 a
Pseudomonas marginalis	2.81 a	0.81 a	7.92 a	2.63 ab	0.36 a
Pseudomonas putida B strain 1	3.51 a	0.66 c	7.57 a	3.05 a	0.37 a
Pseudomonas syringae strain 1	3.57 a	0.73 abc	7.99 a	2.50 ab	0.34 a

[a] Plants were drenched with sterile distilled water.
Values within a column followed by a same letter are not significantly different according to Fisher protected LSD test ($P<0.05$).

Table 3
Production of indole-acetic acid (IAA) and IAA-related compounds by *P. putida* subgroup B strain 1 and *T. atroviride* in liquid cultures containing either L-tryptophan, tryptamine or tryptophol.

	IAA and IAA related compounds[a]			
	Control	Tryptophan	Tryptamine	Tryptophol
P. putida B strain 1	1.5±0.3[b] (0.55)[c]	3.3±0.6 (0.54)	23.4±2.2 (0.59)	11.8±0.9 (0.60)
T. atroviride	1.2±0.1[d] (0.54)	6.2±0.1 (0.54)	9.8±0.3 (0.60)	38.5±9.2 (0.53)

Each value represents a mean of 4 replicates.
[a]The production of IAA and IAA-related compounds by *P. putida* and *T. atroviride* was evaluated in culture medium amended with 200 µg ml^{-1} of tryptophan, tryptamine or tryptophol or without any IAA precursor (control).
[b] Expressed in µg of IAA equivalent ml^{-1} DO_{600}^{-1}.
[c] Values between brackets represent the Rf values for IAA present in the culture supernatant or filtrate.
[d] Expressed in µg of IAA equivalent ml^{-1}.

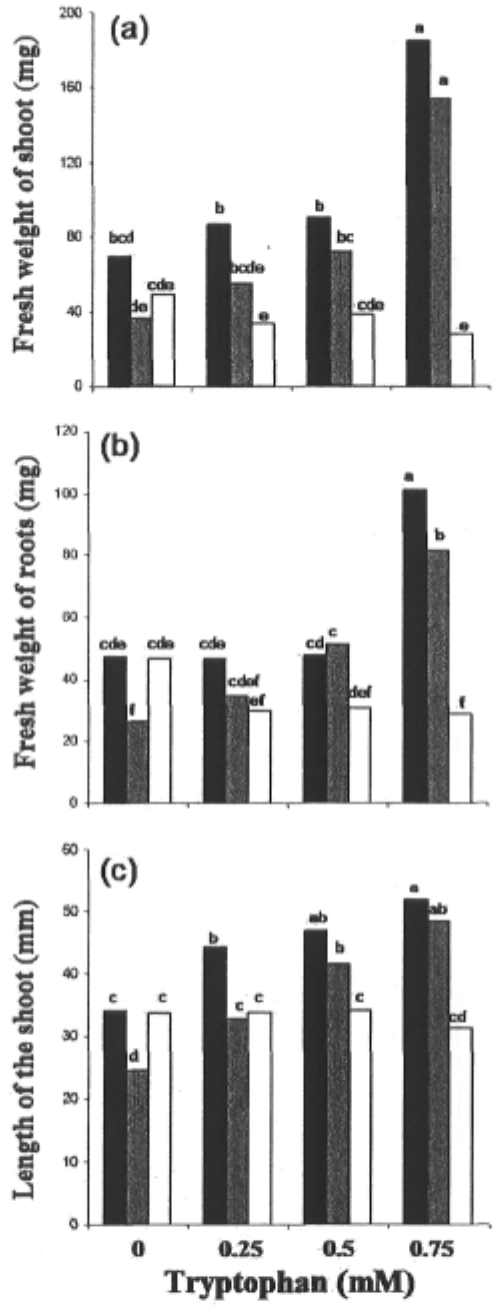

Fig. 1. Effect of tryptophan on fresh weight of shoot (a) and roots (b) and on shoot length (c) of tomato seedlings grown in pouches for 14 days in the presence of *P. putida* subgroup B strain 1 (4×10^7 bacteria ml^{-1}, ■) or *T. atroviride* (1×10^6 spores ml^{-1}, ■). Control seedlings were grown in absence of *P. putida* and *T. atroviride* (□). Each value represents a mean of 5 replicates. Bars with a same letter are not significantly different according to Fisher protected LSD test ($P<0.05$).

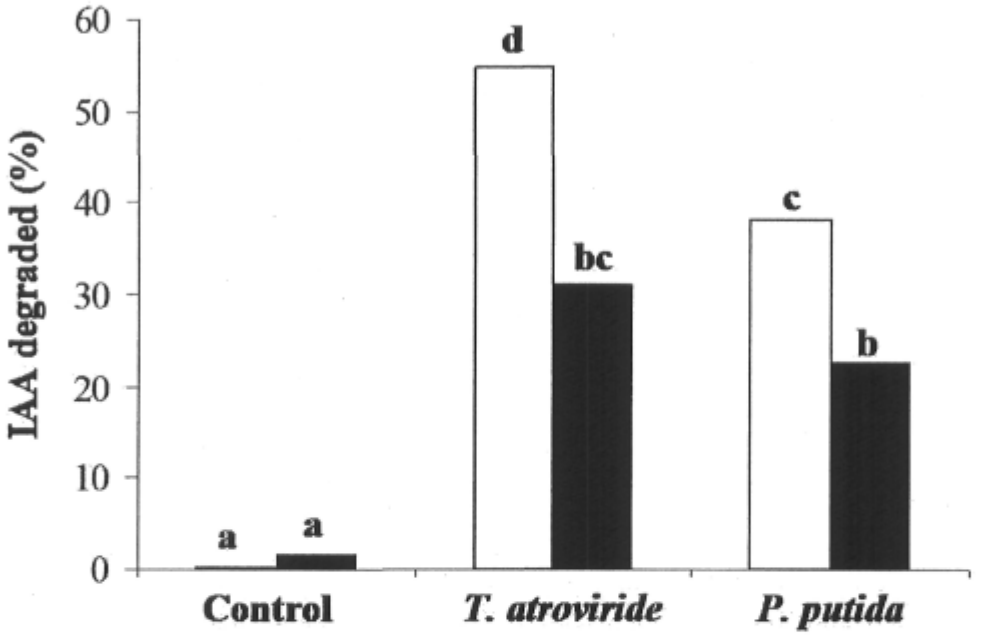

Fig. 2. *In vitro* degradation of indole-acetic acid (IAA) by *Trichoderma atroviride* and *Pseudomonas putida* subgroup B strain 1 in culture medium containing IAA (25 µg ml^{-1}) amended (2 mg ml^{-1}, ■) or not (□) with sucrose. Control flasks were not inoculated. Bars with a same letter are not significantly different according to Fisher protected LSD test ($P<0.05$).

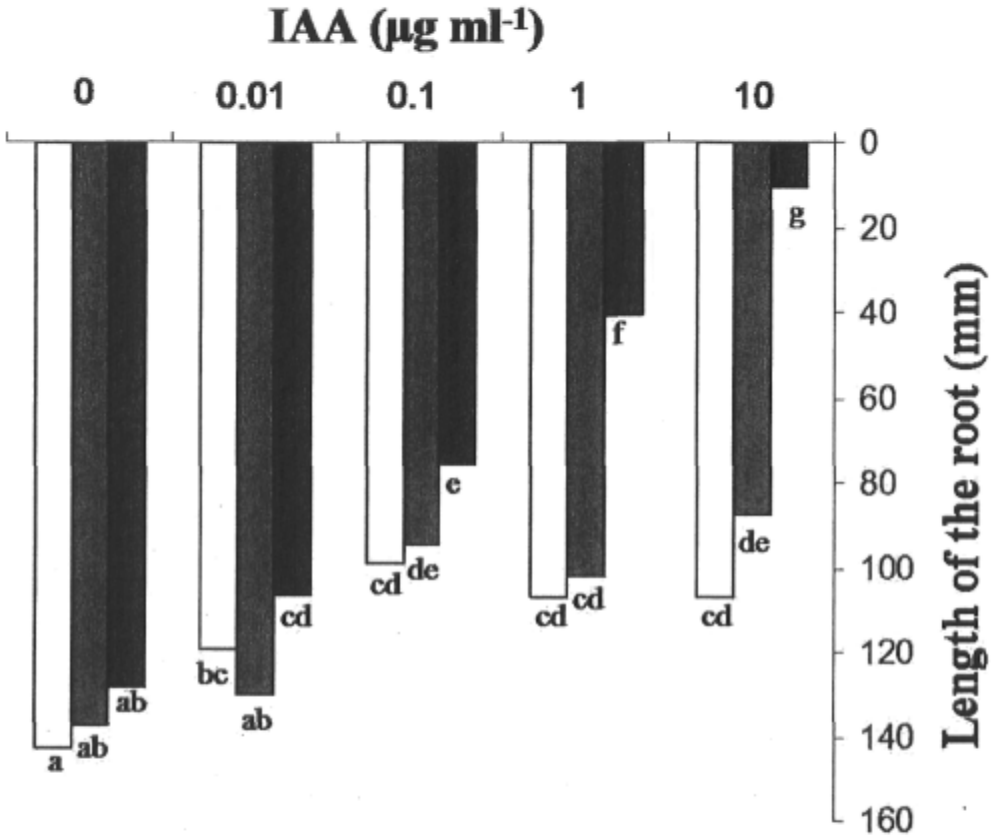

Fig. 3. Effect of different concentrations of exogenous indole-acetic acid (IAA) on the elongation of tomato roots grown in pouches in the absence (control, ■) and in the presence of *P. putida* subgroup B strain 1 (1×10^8 bacteria ml^{-1}, ■) or *T. atroviride* (1×10^6 spores ml^{-1}, □). Bars with a same letter are not significantly different according to Fisher protected LSD test ($P<0.05$).

CHAPITRE 5

EFFET DE L'ACIDE INDOLE-ACÉTIQUE (AIA) SUR LE DÉVELOPPEMENT DE SYMPTÔMES CAUSÉS PAR *PYTHIUM ULTIMUM* CHEZ LA TOMATE

Sommaire : Les résultats présentés au chapitre 4 suggèrent que la bactérie *Pseudomonas putida* sous-groupe B souche 1 et le champignon *Trichoderma atroviride* aient un effet stimulant sur le développement des plants de tomate sains via leur influence sur la concentration d'acide indole-acétique (AIA) au niveau de la rhizosphère. Les travaux exposés dans ce chapitre avaient pour principal objectif d'évaluer, au moyen de différents bio-essais, l'effet de l'AIA sur le développement de symptômes causés par *Pythium ultimum* chez les plants de tomate. Les résultats obtenus ont montré que l'application de ce régulateur de croissance au niveau des racines ou des parties aériennes influence le développement des symptômes attribuables à cet agent pathogène chez la tomate. Ainsi, de faibles (0 à 0,1 µg/ml) concentrations d'AIA au niveau de la rhizosphère ont augmenté la sévérité des symptômes, réduisant de façon significative la croissance de plantules de tomate en conditions axéniques; tandis que de fortes concentrations (10 µg/ml), appliquées soit au niveau du substrat ou au niveau des parties aériennes de plants de tomate, ont réduit significativement les dommages causés par cet agent pathogène. Le taux de fonte des semis (damping-off) des graines de tomate placées dans un substrat inoculé avec *P. ultimum* n'a toutefois pas été affecté par la présence d'AIA (5 µg/ml). Les travaux réalisés ont par ailleurs montré que cet agent pathogène est en mesure de produire de l'AIA lorsque cultivé en milieu liquide supplémenté (200 µg/ml) ou non avec du L-tryptophane, du tryptamine ou du tryptophol.

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Effect of indole-acetic acid (IAA) on the development of symptoms caused by *Pythium ultimum* on tomato plants

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Abstract: The effect of indole-acetic acid (IAA) on the development of symptoms caused by *Pythium ultimum* on tomato plants was investigated using different bioassays. Application of IAA (5 μg ml^{-1}) on tomato seedlings inoculated with *P. ultimum* did not affect their emergence suggesting that IAA did not affect the severity of Pythium damping-off. However, IAA was shown to influence the development of *P. ultimum* symptoms on tomato plantlets. Indeed, it was shown that low concentrations of IAA (0 to 0.1 μg ml^{-1}) within the rhizosphere of plantlets increased the severity of the symptoms caused by *P. ultimum,* while higher concentrations (10 μg ml^{-1}), applied either by drenching to the growing medium or by spraying on the shoot, reduced the damages caused by this pathogen. In addition, the study demonstrated that *P. ultimum* produces IAA in liquid culture amended with L-tryptophan, tryptamine or tryptophol (200 μg ml^{-1}) or not amended.

Key words: damping-off, plant growth regulator, *Pythium ultimum*, root rot, systemic response, tomato

5.1 Introduction

Over the last few years, diseases caused by *Pythium ultimum* Trow have become problematic in greenhouse tomato (*Lycopersicon esculentum* Mill.) production, especially under hydroponic conditions. Pythium damping-off, affecting young seedlings, and pythium root rot, causing an early degeneration (reduction in the development and the elongation) of the root system of mature tomato plants, results in important losses. In order to have a better understanding of the development of those diseases, previous studies have focused on the infection process of roots by *P. ultimum* (Chérif et al., 1991; Désilets and Bélanger, 1991). Typical symptoms of *P. ultimum* infection have been associated with the production of toxins, which cause cell death prior to the penetration by the pathogen, and hydrolytic enzymes (Désilets et al., 1994; Rey et al., 2001).

Indole-acetic acid (IAA) is the most common natural auxin found in plants. IAA is involved in physiological processes such as cell elongation and tissue differenciation (Taiz and Zeiger, 1991) and has also been associated with the plant growth-promoting effect of numerous rhizospheric microorganisms (Patten and Glick, 2002; Persello-Cartieaux et al., 2003; Vessey, 2003). In addition to its key role in the growth of plants, IAA plays an important role in numerous plant-pathogen interactions (Yamada, 1993; Jameson, 2000). Symptoms caused by tumorigenic bacteria and the expression of genes involved in the virulence of these bacteria have been associated with the effect of IAA (Yamada, 1993). Of particular interest, auxins seem to be involved in the infection process of root pathogens, such as *Pythium* spp. (Shimada et al., 1999; Rey et al., 2001; Le Floch et al., 2003). In the case of *P. ultimum*, IAA through the alteration of the morphology of the root tissues, could facilitate the infection process by this pathogen (Désilets and Bélanger, 1991; Rey et al., 2001).

In the present study, the objective was to evaluate the effect of IAA on the development of the symptoms caused by *P. ultimum*. The capacity of *P. ultimum* to produce IAA was also investigated.

5.2 Materials and methods

5.2.1 *Pythium ultimum*

A virulent strain of *P. ultimum* isolated from infected tomato roots was provided by the Laboratory of Dr. Richard Bélanger (Université Laval, Québec, Québec, Canada). The pathogen was grown on potato dextrose agar (PDA; Difco Laboratories, Becton Dickinson, Sparks, MD, USA) at 24°C. PDA disks colonized with *P. ultimum* mycelium suspended in sterile distilled water at 24°C served as stock cultures. The propagule suspension of *P. ultimum*, which consisted of oospores and hyphae, was prepared in 1000-ml flasks containing 500 ml of potato dextrose broth (PDB; Difco Laboratories). Five PDA disks colonized with actively growing mycelium of *P. ultimum* were used to inoculate the flasks which were then incubated on a rotary shaker (150 rev min^{-1}, 24°C) for 1 week. The liquid culture was then homogenized using a blender and the propagule suspension was adjusted by dilution to 1×10^6 propagules ml^{-1} with sterile distilled water based on hemacytometer counts.

5.2.2 Production of IAA and IAA-related compounds by *P. ultimum*

The production of IAA and IAA-related compounds by *P. ultimum* was evaluated spectrophotometrically. Briefly, liquid cultures were prepared in 250-ml flasks containing 100 ml of half-strength tryptic soy broth (TSB; Sigma-Aldrich, Mississauga, ON, Canada) supplemented with 200 μg ml^{-1} of either L-tryptophan, tryptamine or tryptophol (Sigma-Aldrich) or not supplemented (control). The culture medium was inoculated with 3 PDA disks colonized with actively growing mycelium of *P. ultimum*. Blanks consisted of uninoculated culture media amended or not with the three precursors. Flasks in eight replicates were incubated on a rotary shaker (150 rev min^{-1}, 24°C) for 1 week after which the mycelium was removed by filtration. One ml of each culture filtrate was mixed vigorously with 2 ml of Salkowski's reagent (150 ml of H_3PO_4, 250 ml of distilled water and 7.5 ml of 0.5M $FeCl_3 \cdot 6\ H_2O$) (Gordon and Weber, 1951). The mixture was incubated at room temperature for 20 min and the absorbance was measured at 535 nm. The concentration of IAA and IAA-related compounds was evaluated by comparison

with a standard curve prepared using serial dilutions of a 50 μg ml^{-1} IAA (Sigma-Aldrich) solution in half-strength TSB.

The production of IAA was confirmed with thin layer chromatography (TLC) using the method described by Hasan (2002). Fifty ml of each culture filtrate were adjusted to pH 2.0 with 1M HCl and subsequently extracted with an equal volume of ethyl acetate. The ethyl acetate layer was recovered and evaporated with a Rotavapor R-200 (Büchi Analytical inc., New Castle, DE, U.S.A.). The residue was taken up in methanol and developed on TLC plates using isopropanol-ammonia-water (10/1/1; v/v/v) as a solvent. The plates were sprayed with a reagent (3% H_2SO_4 in methanol containing 50 mg $FeCl_3$) and heated until colour development. IAA appeared as a red colour under visible light and orange colour under UV light.

5.2.3 Effect of IAA on Pythium damping-off of tomato seedlings

The effect of IAA on damping-off was evaluated in rockwool. Briefly, tomato seeds (cv. Vita Gold; Pike Seeds, Brandon, MB, Canada) were surface sterilized by soaking in 70% ethanol for 5 min and subsequently in 2% hypochloric acid for 1 min. The seeds were then rinsed thoroughly three times with sterile distilled water and soaked for 15 min in a 5 μg ml^{-1} solution of IAA. Control seeds were soaked in sterile distilled water. Seeds were subsequently sown in multicellular blocks of rockwool and received 1 ml of a suspension (1×10^6 propagules ml^{-1}) of *P. ultimum* or sterile distilled water (control). Seeds were covered with vermiculite and placed in a growth chamber (24°C, 80% relative humidity and 16 hrs photoperiod). Every three days, germinating seeds received 1 ml of either sterile distilled water (control) or a 5 μg ml^{-1} solution of IAA. Damping-off was evaluated as the lack of emergence of the seedlings after 2 weeks of incubation in a growth chamber. A complete randomized design with five replicates was used. The experimental unit consisted of 20 rockwool plugs each containing one seed.

5.2.4 Effect of low concentrations of IAA on tomato seedlings development in the presence or absence of *P. ultimum*

Tomato seeds (cv. Vita Gold; Pike seeds) were surface sterilized as described previously. Seeds were then placed in individual growth pouches containing 20 ml of a sterile solution of 0, 0.0001, 0.001, 0.01 or 0.1 µg ml^{-1} of IAA. Pouches, wrapped in aluminium foil, were placed in a growth chamber at 25°C for 5 days after which the seedlings were inoculated with 5 ml of a suspension (1×10^6 propagules ml^{-1}) of *P. ultimum* or sterile distilled water (control). The seedlings were grown for two weeks following inoculation with the pathogen after which the fresh weight of the roots, fresh weight of the shoot and length of the shoot were measured. The experimental design was a complete randomized design with 5 replicates. The experimental unit consisted of a growth pouch in which two seedlings were grown. Each seedling was a sampling unit.

5.2.5 Effect of IAA on symptoms of tomato plants inoculated with *P. ultimum*

Tomato seeds (cv. Vita Gold; Pike Seeds) were surface sterilized as described previously. The seeds were pre-germinated in Petri dishes on a sterile filter paper soaked with sterile distilled water for 48 hr. Germinating seeds were then sown in 10-cm pots containing industrial quartz (0.65 mm; Unimin Canada ltd, St-Canut, Québec, Canada). The pots were placed in a growth chamber [temperatures of 20°C (day) and 24°C (night), 80% relative humidity and 16 hrs photoperiod]. For the first experiment, once the plants had reached the second leaf stage, they were drenched with 10 ml of a 10 µg ml^{-1} solution of IAA. Five hours later, they were inoculated with 10 ml of a suspension (1×10^6 propagules ml^{-1}) of *P. ultimum*. Plants were treated with IAA a second time seven days later in the same way. For the second experiment, plants (stem and leaves) at the second leaf stage were sprayed with 10 ml of a 10 µg ml^{-1} solution of IAA and were then inoculated with *P. ultimum* as described previously. Plants were treated a second time with IAA after seven days. For both experiments, plants were grown for a total of 30 days after which *Pythium* root rot severity was evaluated. For both experiment, controls were not inoculated with *P. ultimum* and were not treated with IAA. The infection was evaluated through binocular microscope observations as the number of infection points

on 5 roots, including root hairs, sampled randomly from each plant. The development of the symptoms on root surface was also rated according to a disease index of 0 to 4 (0: no symptom, 1: 1-25% of root surface showing symptoms, 2: 26-50%, 3: 51-75%, and 4: 76-100%). The length of the stem as well as the fresh weight of the shoot and the roots were measured. For each experiment, a complete randomized design with 6 replicates was used.

5.2.6 Statistical analysis

When appropriate, analysis of variance (ANOVA) was performed with SAS (SAS Institute, Cary, NC), using the general linear models procedure. When significant, treatment means were compared using the Fisher protected LSD test.

5.3 Results

5.3.1 Production of IAA and IAA-related compounds by *P. ultimum*

P. ultimum produced IAA in all the culture media tested (Table 1). The highest amount of IAA (7.6 µg of IAA equivalent ml^{-1}) was produced when L-tryptophan was added to the culture medium whereas the smallest amount of IAA (3.1 µg of IAA equivalent ml^{-1}) was produced when tryptophol was added (Table 1). In addition, the production of IAA was not further stimulated by an increase up to 800 µg ml^{-1} in the concentration of L-tryptophan in the growth medium (data not shown). In all cases, the production of IAA was confirmed by TLC analysis. Compounds with R_f values close to 0.60 (pure IAA) were considered as IAA (Table 1). Other unidentified IAA-related compounds but with different R_f values were also observed on the TLC plates indicating that such compounds were also produced by *P. ultimum.*

5.3.2 Effect of IAA on *P. ultimum* damping-off of tomato seedlings

Pythium ultimum-inoculated seeds treated (rate of emergence of 18%) or not with IAA (rate of emergence of 16%) showed a significantly lower rate of emergence as compared

to the control (rate of emergence of 99%) (Table 2). The application of IAA on non-inoculated seeds did not affect their emergence (rate of emergence of 97%) as compared to the control.

5.3.3 Effect of low concentrations of IAA on the development of non-inoculated and *P. ultimum*- inoculated tomato seedlings

The length of the shoot and the fresh weight of the roots of non-inoculated seedlings (control) were not affected significantly by application of IAA. Concentrations of 0.001, 0.01, and 0.1 μg ml^{-1} of IAA significantly reduced the fresh weight of the shoot (Fig. 1). Application of increasing concentrations of IAA on *P. ultimum*-inoculated seedlings generally resulted in a significant decrease of the fresh weight of both the roots and the shoot (Fig. 1). The results obtained also showed that, for each concentration of IAA tested, fresh weight of the shoot and of the roots were significantly lower when seedlings were inoculated with *P. ultimum* (Fig. 1). This was not observed for the length of the shoot.

5.3.4 Effect of IAA on Pythium root rot severity of tomato plants

Application of 10 μg ml^{-1} IAA by drenching on non-inoculated plants significantly reduced its overall growth. Indeed the length of the stem and the fresh weight of both shoot and roots of non-inoculated plants treated with IAA were significantly lower as compared to the control (Table 3). As for *P. ultimum*-inoculated plants, the length of the stem and the fresh weight of the roots were significantly increased when treated with IAA.

Application of IAA on the shoot did not affect significantly the growth of both non-inoculated and *P. ultimum*-inoculated plants as compared to the control (Table 3). The application of IAA on the stem however significantly increased the length of the stem and the fresh weight of both shoot and roots of *P. ultimum*-inoculated plants. Application of IAA, either by drenching or by spraying the stem, on *P. ultimum*-

inoculated plants reduced significantly the disease index and the number of infection points (Table 3).

5.4 Discussion

The genus *Pythium* includes numerous plant pathogens. Most of them are not host-specific, causing important infections on a great number of host plants. Common symptoms caused by *Pythium* spp. infection include seedling damping-off, a reduction in root development (root stunting), watery brown lesions on roots, and a reduced overall growth of the host plant (Hendrix and Campbell, 1973). In some species of *Pythium*, the production of IAA seems implicated in the development of symptoms resulting from the infection. *Pythium* group F, a minor pathogen of tomato, causes symptoms that suggest the implication of auxinic compounds, such as a reduction in the elongation of the root and a proliferation of root hairs (Rey et al., 2001; Le Floch et al., 2003). Pythium red blight symptoms on bentgrass (*Agrostis* L. spp.) caused by *Pythium aphanidermatum* (Edson) Fitzp. has also been associated with IAA produced by the pathogen (Shimada et al., 1999). However, the symptoms observed on roots infected by *P. ultimum* has been associated with the production of toxins and hydrolytic enzymes rather than auxin compounds produced by the pathogen (Désilets and Bélanger, 1991). This study was intended to further investigate the possible impact of IAA on the development of *P. ultimum* symptoms on tomato plant.

This study showed that, applied at low concentrations (0 – 0.1 µg/ml), IAA did not have a marked detrimental effect on the development of the root system of healthy seedlings grown under axenic conditions but increased the damages caused by *P. ultimum*. Considering that *Pythium* spp. mainly infect juvenile or succulent tissues, such as feeder roots, root tips and root hairs (Hendrix and Campbell, 1973) and that IAA stimulates, among other things, the proliferation of root hairs (Scott, 1972), it could be hypothesized that IAA increased damages caused by *P. ultimum* by increasing areas susceptible to the pathogen.

Applied by drenching on the root of young tomato plants inoculated with *P. ultimum*, IAA at relatively high concentration (10 µg/ml) caused a significant reduction in the disease severity and a significant improvement in the growth of the plant. However, the development of the tomato plant was severely affected by the treatment with IAA regardless of the presence of the pathogen. Foliar application of IAA on young *P. ultimum*-inoculated tomato plants was also shown to reduce the infection by *P. ultimum* and improved the overall development of the plant. Moreover, it did not affect adversely the growth of non-inoculated plants. The repressive effect of IAA application on disease development was previously reported. Indeed, Fernández-Falcón et al. (2003) reported on the reduction of the infection of banana rhizomes by *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *cubense* (E.F. Smith) Snyder & Hansen following foliar application of IAA. The repressive effect of IAA on post-harvest diseases, such as dry rot (*Gibberella pulicaris* (Fr.:Fr.) Sacc.) of potato (Slininger et al., 2004), or foliar pathogens, such as *Magnaporthe grisea* (T.T. Hebert) Yaegashi & Udagawa on rice (Ueno et al., 2004) has also been reported. Of particular interest, the repressive effect of IAA has been reported against *Phytophthora infestans* (Mont.) de Bary, an oomycete which causes late blight on potato (Martínez Noël et al., 2001). However, to the best of our knowledge, this is the first report of the repressive activity of IAA on the development of *P. ultimum* symptoms. The mechanism involved in the disease repression observed in this study have not yet been identified. However, the results suggest that the repressive effect of IAA could be related to the induction of plant defence mechanisms rather than through a direct impact on the pathogen. In this regard, previous studies have reported that IAA is involved in the induction of plant defence reactions. Martínez Noël et al. (2001) showed that exogenous IAA regulates β-1,3-glucanase and chitinase accumulation in potato leaves following inoculation with *P. infestans*. Furthermore, the regulation of the enzymatic activity of a glutathione *S*-transferase in potato by IAA and the possible effect of such a regulation on the induction of plant defence mechanisms have also been reported (Hahn and Strittmatter, 1994). Ueno et al. (2004) also describe IAA as an activator of plant resistance causing an enhanced activity of enzymes such as phenylalanine ammonia-lyase and peroxidase. Although IAA was shown to affect the

development of *P. ultimum* symptoms on young tomato plantlets, IAA at the concentration tested had no effect on the emergence of tomato seedlings inoculated with *P. ultimum* suggesting that its application did not repress damping-off.

In regard to IAA production by *P. ultimum*, the results of this study are in agreement with a previous report showing the production of this plant growth regulator by *P. ultimum* (Rey et al., 2001). Indeed, IAA and IAA-related compounds were produced by *P. ultimum* in all the media tested. Moreover, the TLC analysis detected the presence of IAA in all the media tested, although previous studies reported that *P. ultimum* is unable to produce IAA in tryptophan-amended medium (Furukawa et al., 1996; Rey et al., 2001). In addition, it was shown that the amount of IAA and IAA-related compounds produced in the culture medium amended with tryptophol or tryptamine was lower than the amount produced in the unamended culture medium, suggesting that the pathways involving those two precursors were not active under the conditions tested. However, the existence of the tryptamine pathway in *Pythium* spp. and their capacity to synthesize tryptophol have previously been reported (Rey et al., 2001; Le Floch et al., 2003). As it is the case with most microorganisms, more than one pathways are most likely involved in the synthesis of IAA by *P. ultimum*.

This study suggests that IAA influences the development of the symptoms caused by *P. ultimum* on tomato seedlings. It also suggests that the effect (repressive or stimulating) of IAA on the development of the symptoms varies according to the concentration used. Although this study showed that *P. ultimum* is able to secrete IAA, further work is needed to investigate the impact of IAA produced by the pathogen in the rhizosphere on symptom severity and plant development.

5.5 Acknowledgements

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Table 1. Production of indole-acetic acid (IAA) and IAA-related compounds by *Pythium ultimum* in liquid culture amended with either L-tryptophan, tryptophol or tryptamine.

Precursors ($200\ \mu g\ ml^{-1}$)	IAA (μg of IAA equivalent ml^{-1})
Control[a]	5.5±0.4 (0.60)[b]
L-tryptophan	7.6±1.2 (0.60)
Tryptophol	3.1±0.9 (0.61)
Tryptamine	4.9±1.0 (0.59)

Values are a mean of 8 replicates ±standard deviation.

[a] Control medium consisted of half-strength TSB not amended with any precursor.

[b] Values between brackets represent the TLC Rf values for IAA extracted from each culture.

Table 2. Effect of indole-acetic acid (IAA) on *Pythium* damping-off of tomato seedlings in rockwool.

Treatments	Emergence (%)
Control[a]	99 a
IAA[b]	97 a
IAA-*P. ultimum*[c]	18 b
P. ultimum[d]	16 b

[a] Seeds were treated with sterile distilled water.
[b] Seeds were treated with 5 μg ml^{-1} IAA.
[c] Seeds were inoculated with *P. ultimum* and treated with 5 μg ml^{-1} IAA.
[d] Seeds were inoculated with *P. ultimum*.
Each value represents the mean of 5 replicates. Values followed by the same letter are not significantly different according to Fisher protected LSD test (alpha=0.05).

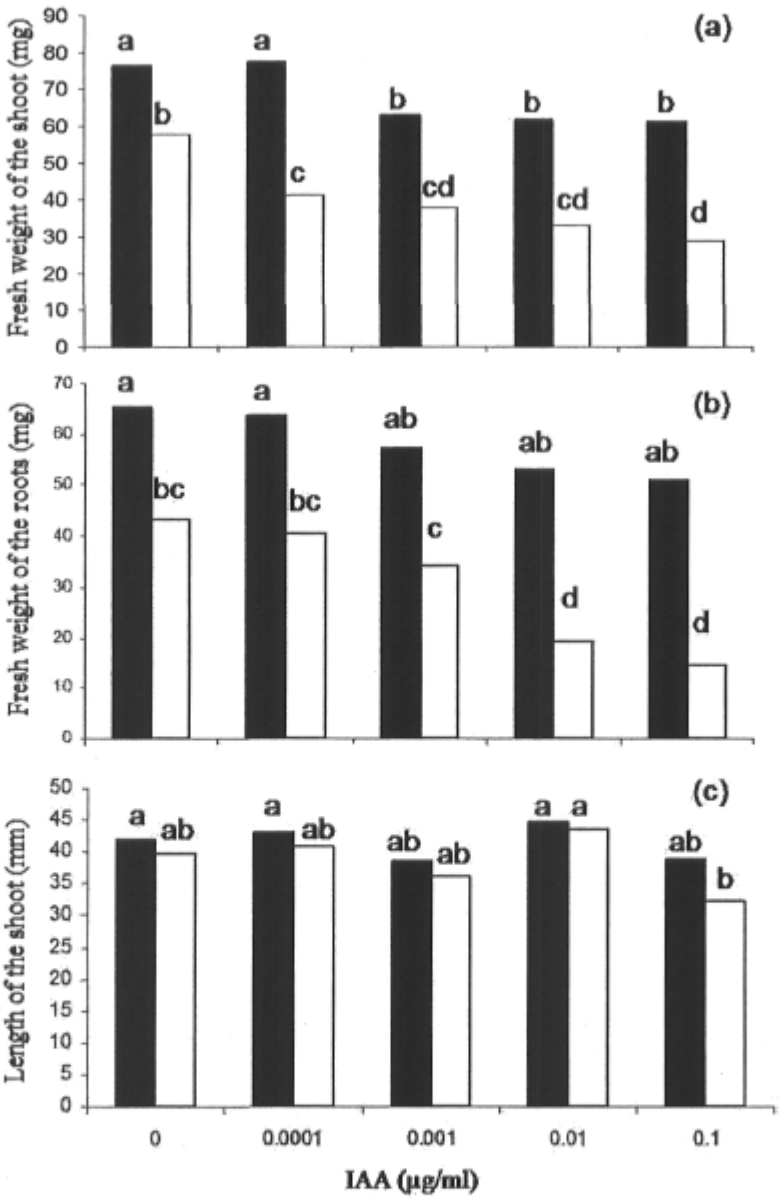

Figure 1. Effect of low concentrations of indole-acetic acid (IAA) on the fresh weight of the shoot (a) and of the roots (b) as well as on the length of the shoot (c) of *Pythium ultimum*-inoculated (□) and non-inoculated (control; ■) tomato seedlings grown in pouches. Each value represents the mean of 5 replicates. Bars with the same letter are not significantly different according to Fisher protected LSD test (alpha=0.05).

Table 3. Effect of indole-acetic acid (IAA) on symptoms severity caused by *Pythium ultimum* on tomato plants.

	Length of stem (mm)	Fresh weight: Shoot (g)	Fresh weight: Roots (g)	Disease index[a]	Number of infection points[b]
IAA on root system[c]					
Control[d]	84.0 a	3.65 a	3.76 a	0 a	0 a
IAA[e]	53.0 b	1.58 b	1.82 b	0 a	0 a
IAA-*P. ultimum*[f]	40.4 c	0.87 bc	1.60 b	2.0 b	8.6 b
P. ultimum[g]	22.4 d	0.56 c	0.34 c	4.0 c	46.0 c
IAA on shoot[h]					
Control	100.6 a	5.26 ab	5.40 a	0 a	0 a
IAA	100.4 a	5.56 a	5.24 a	0 a	0 a
IAA-*P. ultimum*	94.4 a	4.67 b	4.88 a	1.4 b	11.1 b
P. ultimum	75.4 b	3.54 c	3.61 b	3.6 c	47.9 c

[a]Disease index: 0: no symptoms; 1: 1-25% of root surface showing symptoms; 2: 26-50%; 3: 51-75%; 4: 76-100%.
[b]Number of infection points on roots (including root hairs)
[c]Roots were treated (drenching) twice with 10 ml of 10 µg ml^{-1} IAA.
[d]Plants were treated with sterile distilled water.
[e]Plants were treated with 10 µg ml^{-1} IAA.
[f]Plants were inoculated with *P. ultimum* and treated with 10 µg ml^{-1} IAA.
[g]Plants were inoculated with *P. ultimum*.
[h]Stem and leaves were treated (spraying) twice with 10 ml of 10 µg ml^{-1} IAA.
Each value represents the mean of 6 replicates. For each experiment, values within a column followed by the same letter are not significantly different according to Fisher protected LSD test (alpha=0.05).

CONCLUSION GÉNÉRALE

Les travaux présentés dans cette thèse visaient l'élaboration d'une stratégie de lutte envers *P. ultimum*, l'agent pathogène responsable de la pourriture pythienne chez la tomate de serre cultivée en conditions hydroponiques, par une approche exploitant l'effet bénéfique de certains micro-organismes. Les objectifs de ce projet étaient les suivants : (1) sélectionner des micro-organismes démontrant à la fois un effet répressif envers la pourriture pythienne et un effet stimulant sur la croissance des plants de tomate cultivés en conditions hydroponiques, (2) étudier les mécanismes possiblement impliqués dans la stimulation de la croissance et notamment l'implication de l'AIA, (3) évaluer l'effet de l'AIA sur le développement de la pourriture pythienne chez la tomate.

P. ultimum est un agent pathogène peu compétitif dont le potentiel de colonisation et d'infection dépend de la disponibilité de la niche écologique (Hendrix et Campbell, 1973). L'établissement de cet agent pathogène est donc facilité dans les systèmes hydroponiques relativement stériles en début de culture (Zinnen, 1988). Dans le cadre de cette étude, la majorité des micro-organismes testés lors des essais en serre ont démontré un effet répressif significatif sur le développement de la pourriture pythienne. Ces résultats suggèrent que la colonisation des racines par les micro-organismes antagonistes en début de culture et particulièrement avant le début de l'infection par *P. ultimum,* augmente la compétition pour la niche écologique et résulte en l'effet répressif observé.

Bien que de nombreuses études aient été effectuées sur *P. ultimum* (Hultberg et al., 2000; Caron et al., 2002; Carisse et al., 2003), peu d'entre elles ont porté sur les dommages causés par cet agent pathogène chez des plants de tomate de serre à maturité cultivés en hydroponie. Les travaux présentés dans cette thèse montrent que *Penicillium brevicompactum, Penicillium solitum* souche 1, *Trichoderma atroviride, Pseudomonas fluorescens, Pseudomonas fluorescens* sous-groupe G souche 2, *Pseudomonas*

marginalis, Pseudomonas putida sous-groupe B souche 1 et *Pseudomonas syringae* souche 1 présentent un fort effet répressif à long terme sur le développement de la pourriture pythienne de la tomate cultivée en hydroponie. Ces huit micro-organismes ont, par ailleurs, démontré un effet comparable au niveau de la répression de la maladie sous différentes conditions de culture (cultures de printemps vs cultures d'automne). De plus, les résultats obtenus en substrat organique montrent que, même en présence d'une microflore établie, l'effet répressif de ces micro-organismes demeure suggérant ainsi une certaine capacité à compétitionner avec les micro-organismes déjà établis dans le substrat. Les mécanismes d'action impliqués dans l'antagonisme observé n'ont pas été étudiés plus spécifiquement dans le cadre de ces travaux. Cependant, les essais *in vitro* ont démontré que des composés antifongiques sont produits et pourraient être impliqués dans la répression de la maladie. Des travaux devront toutefois être entrepris afin d'identifier les modes d'action précis. De plus, l'efficacité de ces derniers à réduire la pourriture pythienne devrait être comparée aux biofongicides Rootshield® (*Trichoderma harzianum*) et Mycostop® (*Streptomyces griseoviridis*), qui sont maintenant disponibles sur le marché pour lutter contre les maladies racinaires. Il serait également important de développer des formulations à base de l'un ou l'autre de ces micro-organismes afin de faciliter leur utilisation.

Les essais effectués ont de plus permis de démontrer l'effet stimulant indirect, tel que décrit par Whipps (2001), de ces huit micro-organismes sur la croissance des plants de tomate. En effet, l'effet répressif de *P. brevicompactum, P. solitum* souche 1, *T. atroviride, P. fluorescens, P. fluorescens* sous-groupe G souche 2, *P. marginalis, P. putida* sous-groupe B souche 1 et *P. syringae* souche 1 envers *P. ultimum* a résulté en une augmentation de la croissance végétative et/ou reproductive des plants inoculés avec cet agent pathogène. Cet effet bénéfique des micro-organismes semble lié à un meilleur développement du système racinaire (dégénérescence attribuable à *P. ultimum* moins importante), améliorant vraisemblablement l'absorption de l'eau et des éléments minéraux.

Dans le cadre de cette étude, les travaux ont été concentrés principalement sur l'effet bénéfique de *P. putida* et de *T. atroviride* sur le développement de plants de tomate. Outre leur activité antagoniste, ces micro-organismes ont démontré un effet stimulant sur la croissance reproductive des plants de tomate sains, augmentant ainsi le rendement en fruits de façon significative chez des plants cultivés en laine de roche et en substrat organique. Ces travaux suggèrent que l'effet bénéfique serait lié à un meilleur développement du système racinaire résultant d'un phénomène de régulation de la concentration de certains régulateurs de croissance. Les travaux ont premièrement montré que *P. putida* et *T. atroviride* sont en mesure de produire de l'AIA, un régulateur de croissance important chez la plante qui, selon sa concentration, stimule ou inhibe son développement (Tanimoto, 2005). La production de ce régulateur de croissance est d'ailleurs souvent associée à la stimulation de la croissance par les micro-organismes bénéfiques (Patten et Glick, 1996 ; 2002 ; Persello-Cartieaux et al., 2001 ; Vessey, 2003). Dans le cas du système étudié, l'AIA d'origine microbienne synthétisé à partir du tryptophane semble jouer un rôle important dans le développement général de plantules de tomate, en influençant principalement la croissance et la morphologie du système racinaire.

Le rôle de l'AIA produit par certains micro-organismes dans le développement de certaines maladies (Yamada, 1993) et dans la stimulation de la croissance des plantes (Costacurta et Vanderleyden, 1995 ; Persello-Cartieaux et al., 2001) a été grandement étudié. Toutefois, jusqu'à présent, l'effet de la dégradation microbienne de ce régulateur de croissance sur le développement de la plante n'a reçu que peu d'attention, bien que ce phénomène ait été rapporté depuis longtemps (Proctor, 1958; Libbert et Risch, 1969). Les présents travaux ont montré que *P. putida* et *T. atroviride* sont en mesure de dégrader partiellement l'AIA et de l'utiliser pour leur propre développement. Il est possible que leur présence au niveau de la rhizosphère crée un puit pour ce régulateur de croissance, affectant ainsi la concentration endogène de ce dernier dans la plante. Certains auteurs ont de plus avancé l'hypothèse que la sécrétion d'AIA dans les exsudats racinaires de la plante permettrait à cette dernière d'exercer un contrôle sur la diversité de la microflore présente dans la rhizosphère (Leveau et Lindow, 2005). Cette sélection de

populations microbiennes spécifiques pourrait créer une compétition entre agents microbiens, favorisant ainsi les micro-organismes bénéfiques capables de dégrader l'AIA au détriment de certains agents pathogènes. Considérant la capacité qu'ont *P. putida* et *T. atroviride* à dégrader l'AIA, il est possible qu'une telle compétition soit impliquée, du moins en partie, dans l'effet répressif observé chez ces deux micro-organismes envers *P. ultimum* au niveau des racines de tomate. Des travaux supplémentaires seront toutefois nécessaires afin de vérifier cette hypothèse.

De plus, selon les résultats obtenus, l'effet bénéfique de *P. putida* et *T. atroviride* sur le développement du système racinaire pourrait impliquer une régulation de la concentration en éthylène au niveau des racines. L'AIA présent au niveau de la rhizosphère stimule l'activité de la 1-aminocyclopropane-1-carboxylate (ACC) synthase, l'enzyme responsable de la formation du précurseur de l'éthylène, l'ACC (Kende, 1993). Les résultats obtenus suggèrent donc que *P. putida* et *T. atroviride* pourraient affecter le développement du système racinaire via un effet sur la synthèse de l'ACC, soit par une production accrue d'AIA ou par la dégradation de ce dernier. De plus, les travaux ont montré que *P. putida* et *T. atroviride* possèdent une activité ACC désaminase. Cette enzyme réduit la concentration d'ACC par son hydrolyse et diminue la synthèse possible d'éthylène, évitant ainsi des concentrations inhibitrices de ce régulateur de croissance et favorisant du même coup l'élongation du système racinaire (Glick et al., 1998 ; Penrose et al., 2001). Il serait intéressant, lors de travaux futurs, d'étudier l'interaction entre ces deux micro-organismes et la plante hôte afin de confirmer que l'activité ACC désaminase ainsi que la production et la dégradation microbienne de l'AIA sont bien impliquées dans l'effet observé.

Considérant l'intérêt que revêt l'AIA dans l'interaction entre la plante hôte et les micro-organismes présents au niveau de la rhizosphère, des travaux ont été effectués afin de vérifier l'implication possible de ce régulateur de croissance dans le développement de la pourriture pythienne chez la tomate. Les résultats montrent que l'effet de l'AIA sur le développement de cette maladie dépend principalement de sa concentration. Ainsi, de faibles concentrations d'AIA (0 à 0,01 µg/ml) semblent augmenter le développement des

symptômes causés par *P. ultimum*. Toutefois, les travaux ont également démontré l'effet répressif de ce régulateur de croissance envers la pourriture pythienne lorsqu'appliqué à plus forte concentration (10 µg/ml). Un tel effet répressif a d'ailleurs été rapporté pour d'autres agents pathogènes (Martínez Noël et al., 2001 ; Fernández-Falcón et al., 2003 ; Slininger et al., 2004 ; Ueno et al., 2004). De plus, les travaux ont montré qu'une application foliaire d'AIA réduit le développement de la pourriture pythienne au niveau des racines, suggérant une réponse systémique chez les plants de tomate traités. Les mécanismes impliqués n'ont pas été identifiés dans le cadre de ces travaux. Toutefois, l'effet observé pourrait être lié à une stimulation des réactions de défense de la plante, incluant une activité accrue de la phénylalanine ammonia-lyase et des peroxidases (Hahn et Strittmatter, 1994 ; Martínez Noël et al., 2001 ; Ueno et al., 2004).

Les résultats montrent également que *P. ultimum* est en mesure de synthétiser l'AIA comme le rapportent plusieurs travaux (Désilets and Bélanger, 1991 ; Furukawa et al., 1996 ; Rey et al., 2001). Des travaux additionnels seront toutefois nécessaires afin d'évaluer dans quelle mesure l'AIA synthétisé par cet agent pathogène peut influencer le développement et la morphologie du système racinaire.

Dans le cadre de ces travaux, *P. putida* et *T. atroviride*, deux micro-organismes produisant de l'AIA, ont réduit les dommages causés par *P. ultimum* chez la tomate de serre. Il est possible que l'AIA d'origine microbienne soit impliqué dans l'effet répressif de *P. putida* et de *T. atroviride* via une stimulation des réactions de défense de la plante. Des études ont toutefois démontré que la production d'AIA par *P. fluorescens* ne semble pas impliquée directement dans l'effet répressif de ce dernier envers certaines maladies (Beyeler et al., 1999 ; Suzuki et al., 2003). Considérant que plusieurs mécanismes sont souvent associés à l'activité antagoniste des micro-organismes, il est possible que l'AIA produit par *P. putida* et *T. atroviride* joue un rôle dans l'interaction entre la plante hôte et l'agent pathogène, sans toutefois être essentiel à la répression de la maladie. Des travaux plus spécifiques devront être entrepris afin de vérifier cette hypothèse.

Ces travaux ont montré l'effet bénéfique de plusieurs micro-organismes, dont la bactérie *P. putida* et le champignon *T. atroviride*, sur le développement de plants de tomate sains ou infectés par *P. ultimum*. Ces micro-organismes bénéfiques pourraient éventuellement trouver des applications dans la culture commerciale de la tomate de serre.

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