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Brief Introduction of Research Progresses in Control and Biocontrol of Clubroot Disease in China

Yueqiu He*, Yixin Wu, Pengfei He and Xinyu Li

Yunnan Agricultural University, Kunming 650201, China

*Email: ynfh2007@163.com

Clubroot disease of crucifers has occurred since 1957. It has spread to the whole China, especially in the southwest and northeast where it causes 30-80% loss in some fields. The disease has been expanded in the recent years as seeds are imported and the floating seedling system practices. For its effective control, the Ministry of Agriculture of China set up a program in 2010 and a research team led by Dr. Yueqiu HE, Yunnan Agricultural University. The team includes 20 main researchers of 11 universities and 5 institutions.

After 5 years, the team has made a lot of progresses in disease occurrence regulation, resources collection, resistance identification and breeding, biological agent exploration, formulation, chemicals evaluation, and control strategy. About 1200 collections of local and commercial crucifers were identified in the field and by artificial inoculation in the laboratories, 10 resistant cultivars were bred including 7 Chinese cabbages and 3 cabbages. More than 800 antagonistic strains were isolated including bacteria, streptomyces and fungi. Around 100 chemicals were evaluated in the field and greenhouse based on its control effect, among them, 6 showed high control effect, especially fluzinam and cyazofamid could control about 80% the disease. However, fluzinam has negative effect on soil microbes.

Clubroot disease could not be controlled by bioagents and chemicals once when the pathogen *Plasmodiophora brassicae* infected its hosts and set up the parasitic relationship. We found the earlier the pathogen infected its host, the severer the disease was. Therefore, early control was the most effective. For Chinese cabbage, all controlling measures should be taken in the early 30 days because the new infection could not cause severe symptom after 30 days of seeding. For example, a biocontrol agent, *Bacillus subtilis* Strain XF-1 could control the disease 70%-85% averagely when it mixed with seedling substrate and was drenching 3 times after transplanting, i.e. immediately, 7 days, 14 days.

XF-1 has been deeply researched in control mechanisms, its genome, and development and application of biocontrol formulate. It could produce antagonistic protein, enzyme, antibiotics and IAA, which promoted rhizogenesis and growth. Its genome was sequenced by Illumina/Solexa Genome Analyzer to assembled into 20 scaffolds then the gaps between scaffolds were filled by long fragment PCR amplification to obtain complete genome with 4,061,186 bp in size. The whole genome was found to have 43.8% GC, 108 tandem repeats with an average of 2.65 copies and 84 transposons. The CDSs were predicted as 3,853 in which 112 CDSs were predicted to secondary metabolite biosynthesis, transport and catabolism. Among those, five NRPS/PKS giant gene clusters being responsible for the biosynthesis of polyketide (pksABCDEFHJLMNRS in size 72.9 kb), surfactin (srfABCD, 26.148 kb), bacilysin (bacABCDE 5.903 kb), bacillibactin (dhhABCEF, 11.774 kb) and fengycin (ppsABCDE, 37.799 kb) have high homology to function confirmed biosynthesis gene in other strain. Moreover, there are many of key regulatory genes for secondary metabolites from XF-1, such as comABPQKX Z, degQ, sfp, yczE, degU, ycxABCD and ywfG. were also predicted. Therefore, XF-1 has potential of biosynthesis for secondary metabolites surfactin, fengycin, bacillibactin, bacilysin and Bacillaene.

Thirty two compounds were detected from cell extracts of XF-1 by MALDI-TOF-MS, including one Macrolactin (m/z 441.06), two fusaricidin (m/z 850.493 and 968.515), one circulocin (m/z 852.509), nine surfactin (m/z 1044.656~1102.652), five iturin (m/z 1096.631~1150.57) and forty fengycin (m/z 1449.79~1543.805). The top three compositions types (containing 56.67% of total extract) are surfactin, iturin and fengycin, in which the most abundant is the surfactin type composition 30.37% of total extract and in second place is the fengycin with 23.28% content with rich diversity of chemical structure, and the smallest one is the iturin with 3.02% content. Moreover, the same main compositions were detected in *Bacillus* sp.355 which is also a good effects biocontrol bacterial for controlling the clubroot of crucifer. Wherefore those compounds surfactin, iturin and fengycin may be the main active compositions of

XF-1 against *P. brassicae*.

Twenty one fengycin type compounds were evaluate by LC-ESI-MS/MS with antifungal activities, including fengycin A C_{16-C19}, fengycin B C_{14-C17}, fengycin C C_{15-C18}, fengycin D C_{15-C18} and fengycin S C_{15-C18}. Furthermore, one novel compound was identified as Dehydroxyfengycin C₁₇ according its MS, 1D and 2D NMR spectral data, which molecular weight is 1488.8480 Da and formula C₇₅H₁₁₆N₁₂O₁₉.

The fengycin type compounds (FTCPs 250 µg/mL) were used to treat the resting spores of *P. brassicae* (10⁷/mL) by detecting leakage of the cytoplasm components and cell destruction. After 12 h treatment, the absorbencies at 260 nm (A₂₆₀) and at 280 nm (A₂₈₀) increased gradually to approaching the maximum of absorbance, accompanying the collapse of *P. brassicae* resting spores, and nearly no complete cells were observed at 24 h treatment. The results suggested that the cells could be lyzed by the FTCPs of XF-1, and the diversity of FTCPs was mainly attributed to a mechanism of clubroot disease biocontrol.

In the five selected medium MOLP, PSA, LB, Landy and LD, the most suitable for growth of strain medium is MOLP, and the least for strains longevity is the Landy sucrose medium. However, the lipopeptide highest yield is in Landy sucrose medium. The lipopeptides in five medium were analyzed with HPLC, and the results showed that lipopeptides component were same, while their contents from *B. subtilis* XF-1 fermented in five medium were different. We found that it is the lipopeptides content but ingredients of XF-1 could be impacted by medium and lacking of nutrition seems promoting lipopeptides secretion from XF-1.

The volatile components with inhibition fungal *Cylindrocarpon* spp. activity which were collect in sealed vesel were detected with methods of HS-SPME-GC-MS in eight biocontrol *Bacillus* species and four positive mutant strains of XF-1 mutagenized with chemical mutagens, respectively. They have same main volatile components including pyrazine, aldehydes, oxazolidinone and sulfide which are composed of 91.62% in XF-1, in which, the most abundant is the pyrazine type composition with 47.03%, and in second place is the aldehydes with 23.84%, and the third place is oxazolidinone with 15.68%, and the smallest ones is the sulfide with 5.07%.

*For communication, please contact the authers through ynfh2007@163.com.