Isolation and Screening of Endophytic Microorganisms from Sweetpotato for the Production of Antimicrobial Compounds

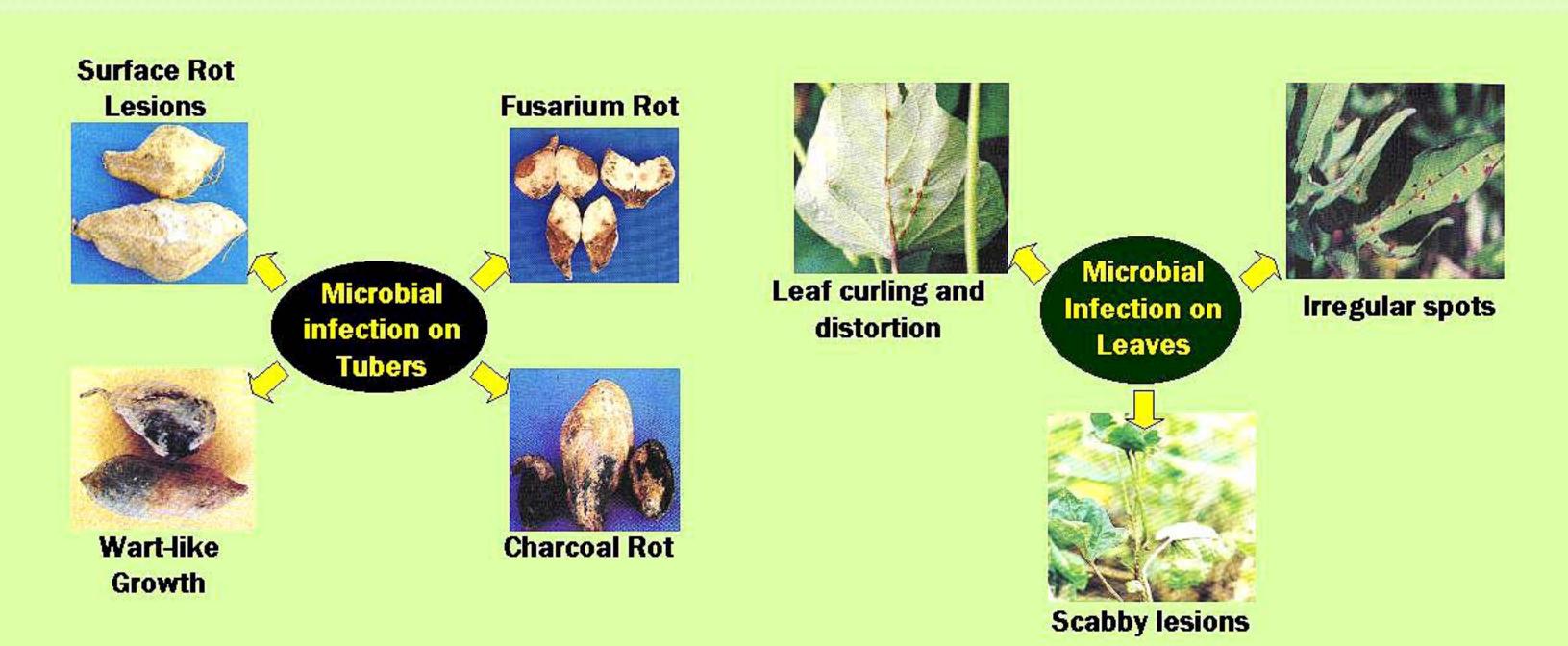
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ABSTRACT

Endophytes are naturally occurring microorganisms within the living plant tissues which do not cause any pathogenic symptoms. Most of the novel compound-producing endophytes were isolated from tropical plants. This study was an attempt to isolate endophytic microorganisms from sweetpotato, being a tropical plant in a hot and humid environment like the Philippines. The growth and proliferation of endophytes in rootcrops is an important index in the incidence of pests and diseases in many varieties of rootcrops. The isolation of endophytes was also an attempt to discover novel antimicrobial compounds for food, feed and pharmaceutical industries and other biotechnological works. In this study, endophytic microorganisms were isolated from surface-sterilized stem cuttings of selected sweetpotato varieties such as BSP-SP-17, BSP-SP-22, and NSIC-25. The isolates were purified and tested for antimicrobial activities using spot inoculation method against Lasiodiplodia theobromae (sweetpotato rot-causing mold), Collectorichum gloeosporioides (yam anthracnose-causing mold), Diplodia natalensis (watermelon and citrus stem end rot-causing mold), and Bacillus subtilis (potato soft rot-causing bacteria). Of the 96 isolates, 10 were tested positive against L. theobromae, 8 against C. gloeosporioides, 2 against D. natalensis, and 14 against B. subtilis. Very promising isolate coded as 12531, showed positive antimicrobial activities against all indicator microorganisms tested.

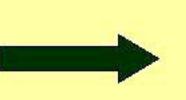
INTRODUCTION

Endophytes are naturally occurring microorganisms within the living plant tissues which do not cause any pathogenic symptoms. Various researches have been conducted on the production of antimicrobial compounds by endophytes which prevent the growth and proliferation of disease-causing microorganisms in the host plants. Most of the novel compound-producing endophytes were isolated from tropical plants. This study was an attempt to isolate endophytic microorganisms from sweetpotato, being a tropical plant in a hot and humid environment like the Philippines. The growth of endophytes in sweetpotato is an important index in the incidence of pests and diseases in many sweetpotato varieties. The isolation of endophytes was also an attempt to discover novel antimicrobial compounds for food, feed and pharmaceutical industries and other biotechnological works.

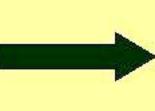


MATERIALS AND METHODS

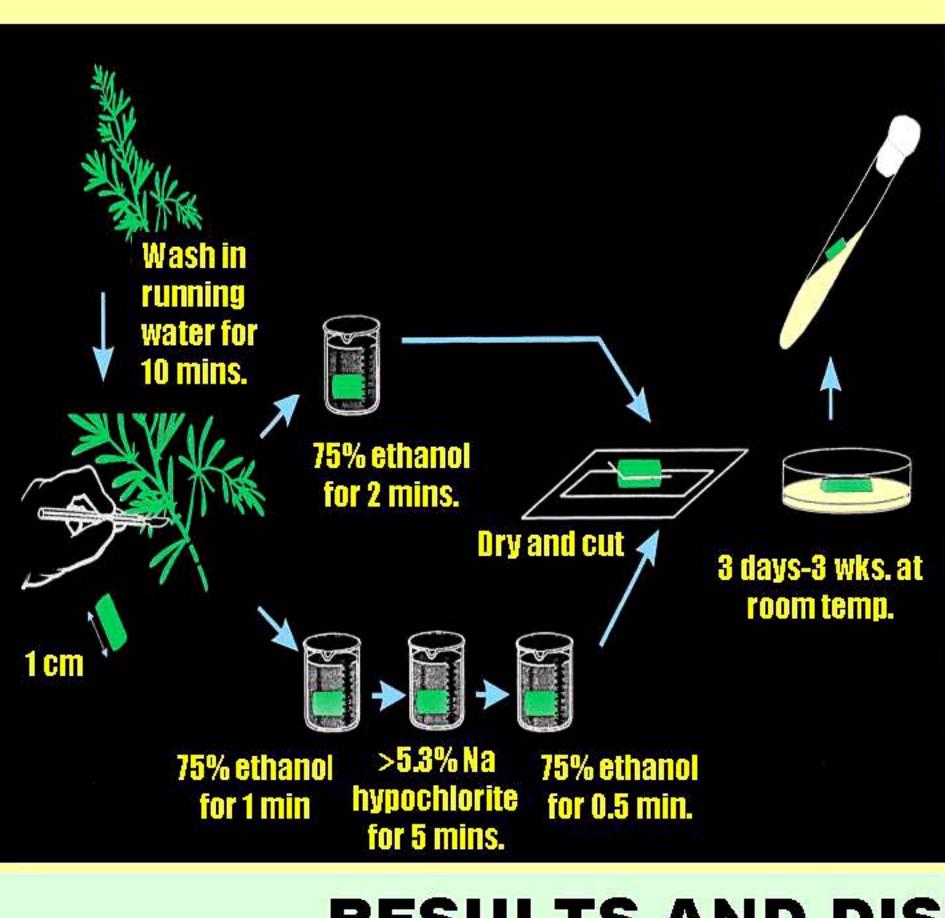
Collection of Sample and Isolation of Endophytes

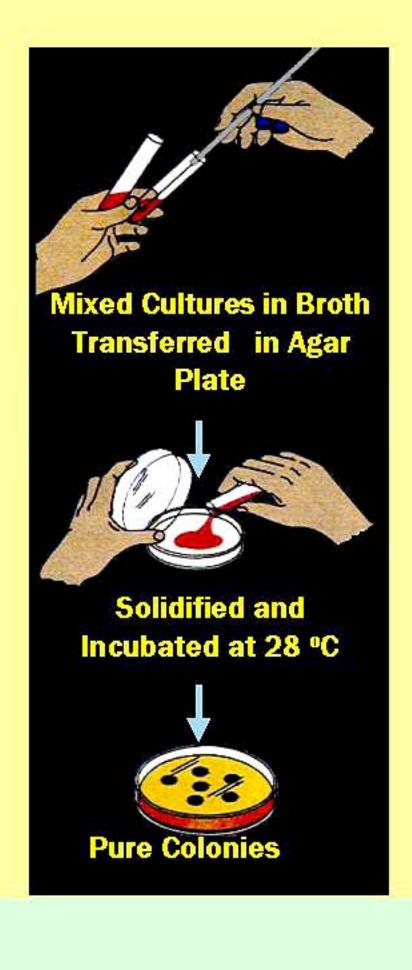


Purification of isolates



Screening of isolates for antimicrobial production











RESULTS AND DISCUSSION

A total number of 96 endophytic microorganisms were isolated from sweetpotato plants (Var. BSP-SP-17, BSP-SP-22 and NSIC 25). Of the 96 isolates, 67 were bacteria and 29 were molds. Most of these isolates with antimicrobial activities were bacteria. There were 14 (6 bacteria and 8 molds) isolates which showed positive activity against B. subtilis, 8 (5 bacteria and 3 molds) against C. gloeosporioides, 10 (6 bacteria and 4 molds) against L. theobromae and 2 (both bacteria) against D. natalensis (Table 1). Two bacterial isolates were found to possess antimicrobial activities against most of the indicator tested. Isolates coded as I2531 and I2532 showed inhibitory activity against C. gloeosporioides, L. theobromae and D. natalensis. Isolate 12531 was found to be a very promising isolate with antimicrobial activities against all the four indicator microorganisms tested. The inhibitory behavior of this isolate is demonstrated by a nonoverlapping and non-contact display of growth between the isolates and the indicator microorganisms in agar plates (Figure 1). The halo or area of growth inhibition of the indicator microorganisms is suggestive of the production of active compounds by the isolates which will be characterized and identified.

The endophytic microorganisms normally enter and colonize the inner portion of the plants via the vascular tissues. Other host entry points include stomata, lenticels germinating radicles, tissue wounds or foliar damage. It is also believed that seedlings or planting materials have been embedded with these microorganisms which are resistant to stresses that growing plants undergo before maturity. In the process of growth and proliferation within the plant, active compounds are elaborated which help the plant to fight against pests and diseases and other related stresses.

Activity	B. subtilis	C. gloeosporioides	L. theobromae	D. natalensis	
Positive	14	8	10	2	
		2			
	1				
Neutral	7	28	26	7	
		3			
	0				
Negative	75	60	60	87	
		50			
	42				

Table 1. Microbial activities of the isolates against some selected indicator microorganisms.





Figure 1. Positive antimicrobial activity of Isolate I2531 against Colletotrichum gloeosporioides (a) and Diplodia natalensis (b).

SUMMARY AND CONCLUSION

This study is a report of a very potential endophytic microorganism coded as I2531 that was isolated from sweetpotato plants. This isolate possessed inhibitory activity against disease-causing and pathogenic theobromae microorganisms such as Lasiodiplodia (sweetpotato rot-causing mold), Colletotrichum gloeosporioides (yam anthracnose-causing mold), Diplodia natalensis (watermelon and citrus stem end rot-causing mold), and Bacillus subtilis (potato soft rot-causing bacteria). Isolate I2532 were also observed to inhibit three indicator microorganisms tested.

Further studies will involve testing of the isolates against other disease-causing microorganisms. The isolates and the compound(s) produced by promising microorganisms will be characterized. Other endophytic microorganisms from other rootcrop plants such as cassava, yam and taro will also be isolated and screened for antimicrobial compound production. The incidence of diseases in rootcrops will also be correlated with the growth and proliferation of these endophytes in the host plants.