Studdy of the biological control of bilharzian snails and soil nematodes using a genetically cxonstructed microbial strain

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Azotobacter Chroococcum, Azotobacter vinelandii andEscherichia coli. were subjected to genetic transformation in order toaquiresaponin for producing ability. The transforming materials in caseof Azotobacter sp. were Saponaria high molecular weight DNA,Saponaria officinalis fresh roots DNA, Poinciana regia chromatinDNA and Asparagus officinalis fresh roots DNA, each ofconcentration 22 ug/ml, In case of E.coli, the transforming material wasSaponaria fresh roots DNA of concentrations 66, 50 and 30 J.1g/ml.Some biolgocial activites of saponin in each ofliquid culture oftransfonnant and untransfonnant Azotobcter, culture supernatant, cellsuspensions and extracted (TLC) saponin bands of culture supernatantsof isolate (1) of transfonnant, untransfonnant and isolate ”2” oftransfonnat’ltE. coli were tested with comparison to aqueous extracts of3 plants (Saponaria, Poinciana: and Asparagus) of different concentrationsas follow:. I. Haemolytic activity:About 0.5 ml from each saponin solution mentioned above wasadded to 5 ml of blood corpuscles suspension, Kept at room temp. for 5minutes, centrifuged at 3000 rpm for 15 minutes. The content ofH.b insupernatant was measured in coloremeter at 540 G.D. The saponincontent of unknown samples were caleculated from the linear portion--_.of the standrd curve which was constructed for white saponin ofMerck.n Moluscicidal activity:Ten snails (Biomphalaria alexandrina) were transferred toabout 10 ml from each tested saponin solutionand dechlorinated H2Oas control for 24 hrs in duplicate then transferred to dechlorinatedwater for 24 hr. After recovery, mortality was calculated.m. Nematicidal activity:About 1000 2-nd stage juveniles were transferred to 10 ml ofeach tested saponin soultion and distilled water as control in triplicatesfor 24 hrs then were transferred to aerated water for 24 hrs. Mortalitypercentages of nematodes were calculated after recovery.- Type of saponin in plants was determined by the colour produced byapplication of the liebennann- Burchard reaction.- Various transformation frequencies in case of Azotobacter wereobserved. The highest frequency was obtained from the two types ofSaponaria DNA.- Transfonnant E.Coli were obtained with the concentration 66 ~glmland the transformation frequency was 0.00096.- Untransfonnant bacterial strain don’t produce saponin and don’texhibit any of its examined biological activities.- Transfonnant strains exhibited various degrees of biological activitesof saponin as follows:-- The Azotobacter chroococcum transformed by Saponaria highmolecular wieght DNA gave the highest (60%) molluscicidal activity.This

mortality value was equal to that obtained from standard saponin(Merck) cone, of 0.015mglml). While the highestmolluscicidalactivity of cell suspensions of E.coli was that of the isolate (2) oftransformed strain (30%, equivalent to 0.014 mglml of Mercksaponin) which cause decrease in DNA content of snails to the lowestvalue (5.2 mglg of snail tissue) with comparison to control which have(23.1 mglg of snail tissue).- The purified saponin extracted from culture supernatant oftransfonnant E.coli mortalized snails at the maximum percentage(100%), which was equivalent to the mortality caused by 0.0175mglml of Merck saponin, the DNA content of dead snail tissue wasreduced to about 4.1 mglg of snail tissue. Cell suspension and thespots dervied from the wild E.Coli showed no molluscicidal activity.- The best potent saponin examined in 3 plants was that of Saponariaand Asparagus (LCso were 35, 40 mglml) respectively for snails,which equivalent ot the mortality caused by 0.015 mglml of Mercksaponm.Nematicidal activity:- A. chroococcum transformed by Saponaria high molecular weightDNA gave the highest nematodes mortality (50%) which correspondsto 80 mglml concentation of Merck saponin.-Cells suspension of isolate (1) oftransfonnant E.Coli gave the highestnematodes mortality percentage (60%) in comparison to the cellsuspension of wild type and isolate (2) oftransfonnant E.Coli-Purified saponin extracted from culture supernatant of isolate (1) oftransformant and isolate (2) of transformant E.coli. caused 100%nematodes mortality, equivalent to 160 mglml of Merck saponin.- The best potent saponin examined in 3 plants was that of Saponariaand Asparagus LCso for nematodes were 100 mg plant! ml from eachplant.Haemolytic activity:Culture of A. chroococcum transformed by fresh Saponaria.DNA could hemolyze red blood cells to an extend equal to the obtainedby 40.8 mgl ml of standard saponin.- Haemolytic activity of isolate(l) of transformant and isolate (2) oftransformant E.coli cell suspensions was greater than that of thesupernatants. Isolate (1) of transformed strain gave strongerhemolytic action than isolate (2) of transformed strain in both cases.- The hemolytic activity of purified saponin extracted from culturesupernatant of isolate (1) oftransfonnant E.coli was lower than thatof isolate (2) oftransfonnant one (0.5 and 0.736) respectively.- Aqueous extracts of Saponaria roots and Poinciana seeds at cone. of100 mg plant! ml gave stronger hemolytic action than aqueousextract of the same cone, from Asparagus, this may be due to thedifferene in.raponin type (triterpenoid in Saponaria and Poinciana,but it its steroid in Asparagus).- Aqueous extract of Saponaria could hemolyze red blood cells to anextend equal to that obtained by 0.DI5 mglml of standard saponin, soSaponaria is considered to be the most producer for saponin thanAsparagus and Poinciana.