REFERENCE


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ANTIMICROBIAL ACTIVITY
OF
POPLAR CALLUS
Introduction

Plants have evolved a number of defence responses to deter potential microbial pathogens from initiating successful infections (Bell, 1981). One well studied defence mechanism is the synthesis and accumulation of the low molecular weight antimicrobial compounds called phytoalexins (Cruikshank, 1977; Albershein and Valent, 1978; Bailey 1982; Dixon et al., 1983). Antimicrobial compounds are normally absent in healthy plant tissues, accumulates at sites of infection where they are thought to play a role in inhibiting further invasion of the microorganism (Bailey et al., 1982; Mansfield, 1982; Smith, 1982; Darvill and Albershein, 1984; Halm et al., 1985).

Difficulties encountered in studying these antimicrobial substances include production of amount sufficient for chemical and biological experiments and quantification of activity levels. The axenic culture technique is useful in such studies (Mansfield, 1982). In addition, in vitro tissue growth provides a mechanism of control as well as variation of both nutritional and environmental conditions (Mathes, 1967) which assists in determining the exact potential of the cells.

The occurrence of antimicrobial substances in higher plants has been investigated extensively (Nickell, 1960). Antimicrobial activity has been demonstrated in callus tissue cultures from higher plants (Carew and Staba, 1956;
Mathes, 1967; Khanna and Staba, 1968; Mathes et al., 1971; Tabata et al., 1982).

The present investigation reports the antimicrobial activity observed in the petiole callus of a hybrid poplar, cl. 65/27 against a pathogenic strain of Staphylococcus aureus. This preliminary study was performed to address whether the antimicrobial activity remained persistent at all the stages of tissue growth, if not when the maximum production is realized.

Materials and Methods

Establishment of Callus:

Plant materials were collected from the WIMCO nursery, Alambazar, and sterilized using 0.1% HgCl₂. Petioles were cut into 1 cm pieces and inoculated in Linsmaier and Skoog's nutrient agar medium (Linsmaier and Skoog, 1965) supplemented with 1.5 mg L⁻¹ of 2,4-dichlorophenoxyacetic acid. Isolated calluses (approx 400 mg) were transferred in petriplates containing the same medium.

Preparation of Bacterial Broth:

The pathogenic strain of Staphylococcus aureus was collected from the Microbiology Department, Bose Institute and maintained in the culture tubes containing nutrient agar medium (Swarup et al., 1986) (Table 1). Bacteria were inoculated in the nutrient broth and incubated at 37°C in an gyrotary shaker till the exponential growth of the organism was reached.
Co-Cultivation of Callus With Bacteria:
The petriplates containing static callus cultures were flooded with the above prepared broth every alternate day, in order to address whether the age of the callus has any influence on the production of the antimicrobial compound(s) to inhibit the growth of the bacteria. Ten replicate cultures were maintained for each experiments. All infected petriplates were incubated at ambient temperature (30°C) for 24 hrs and the zones of antimicrobial activity were measured.

Growth Measurement of Callus:
Initially culture vessels with medium were weighed and these were reweighed after inoculation with callus pieces. The difference between the final and the initial weight gave the fresh weight of the callus. Callus pieces of similar fresh weight were dried under vacuum at 56° - 60 °C till a constant dry weight was attained. They were weighed to determine initial dry weight of the callus. Cultured calluses were harvested from the growing medium every alternate day and dried as above to obtain the final dry weight. Each set of harvest contained 10 replicates. Growth Index (G.I.) was calculated by dividing the increased dry weight of the callus by the initial dry weight of the callus.

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G.I. = \frac{\text{Increase in callus dry weight}}{\text{Initial dry weight of the callus}}
\]
Growth curve was plotted on a graph paper. The days were plotted along the abcissa and G.I. values as the ordinate.

Results and Discussion

Growth analysis revealed that calli passed through a brief lag phase which persisted till 4th day of incubation and entered the phase of active growth called logarithmic or exponential phase from 6th day (Fig. 1). This phase persisted up to 20th day. Calluses entered the stationary phase thereafter when the biomass remained constant (Fig. 1). The petriplates containing the callus pieces when flooded with *S. aureus* broth after the 2nd day of callus growth, a zone of bacterial stimulation was obtained near the immediate vicinity of callus while a diffused bacterial growth was observed on the rest part of the petriplate (Fig. 2A). The stimulatory zone was more prominent after the 4th day of callus growth (Fig. 2B) and then gradually decreased. A narrow zone of bacterial inhibition was visible adjacent to the callus in the 6th day (Fig. 2C). This inhibitory zone gradually increased in diameter (Figs. 2D and E) which was proportional to the tissue growth (Fig. 1). The inhibitory zone was maximum on the 18th day of callus growth (Fig. 2F) and remained constant thereafter. Such inhibition may be due to secretion of some antimicrobial compound (s) during the exponential phase of the tissue growth, i.e. from the 6th to 18th day.
The results from a number of studies have established that phytoalexin accumulation is an important defence mechanism in plants (Mathes, 1967; Mathes et al., 1971; Bailey, 1982; Darvill and Albershein, 1984; Scaysbrook et al., 1992). Studies on the induction of phytoalexin accumulation have demonstrated that various types of molecules can trigger plant cells to synthesize phytoalexins (Mathes, 1967; Mathes et al., 1971; Bell, 1981; Darvill and Albershein, 1984). The accumulation of bacterial growth near the tissue at the initial stage of infection may be due to the increased availability of nutrient pool within the immediate vicinity of callus and as the tissue started producing antimicrobial compounds during the exponential phase, this stimulatory zone disappeared with concomitant appearance of zone of bacterial inhibition. Thus, the present study revealed that the antimicrobial compound production by poplar callus commenced in presence of pathogen at the exponential phase of the growth cycle, which remained active in the stationary phase of growth as well.

Chemical characterization and analysis of this antimicrobial compound, isolated during the present investigation is underway. The composition of such compounds may be used to establish the chemotaxonomic relationships of poplar.
References


Mathes MC, Helton ED and Fisher KD, 1971. The production of


Explanation of Figures

Fig. 1: The progressive increase in the diameter of antimicrobial zone as related to growth index of the Poplar callus tissue.
Fig. 1

- inhibition zone
- growth index
Explanation of Figures

Figs. 2 A - B: The influence of the growth periods of isolated callus tissue on the growth of Staphylococcus aureus. The tissues were grown on the medium for 2 (A), 4 (B), 6 (C), 10 (D), 14 (E) and 18 (F) days before co-cultivation with the microorganism.