ATTEMPTS FOR GENETIC IMPROVEMENT OF AN AZOTOBACTER SPECIES THROUGH AQUIRING RHIZOBIA GENES

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بسم الله الرحمن الرحيم

قالوا

سبحانك لا علم لنا إلا ما علمتنا
إنك أنت العليم الحكيم

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بسم الله الرحمن الرحيم

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Preface

Aerobic, nitrogen fixing - free - living soil micro-organisms such as *Azotobacter* may with the aid of genetic manipulation, provide an additional source of renewable nitrogen for enhancing crop yields.

Azotobacter has some biological properties that could be utilized in the development of an effective bacterial fertilizer. It is found in temperate zones, its sticky outer coat enable it to be used as a seed dressing and it is quite hardly and not soil specific. Therefore, the initial target in our investigation is to increase the nitrogen fixation efficiency of *Azotobacter* strains through isolation of different *Azotobacter* strains with different genotypes, and through transformation by transferring the genetic materials of *Rhizobia* and studying the symbiotic relations of such transformants with different plants.

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PART I

A- INTRODUCTION

B- MATERIALS AND METHODS

INTRODUCTION

I.a Gene transfer systems in *Azotobacter*

Genetic information can be transferred in *Azotobacter* by transduction (Wyss, 1962) and by transformation (David et al., 1981, and Kennedy & Drummond, 1985).

Although transformation originally reported for *A. Vinelandii* and *A. chroococcum*, Sen et al. (1969) studied and used only *A. vinelandii*.
Page and Sadoff (1976) described the first reliable procedure for inducing competence and for the transformation of *A. vinelandii* culture. They also studied the different environmental factors influencing transformation. They examined each of the major constituents of the Burk nitrogen free media and its effect on transformation, they also concluded that crude lysate DNA was the best donor for the transformation of *A. vinelandii* and that purification of the DNA was very detrimental to the transformation frequency. This conclusion was probably due to that crude lysate DNA containing some chemical or cell fractions which are required for transformation.

Page (1978) found that the frequency of nif^+ transformants using *Rhizobium* DNA was always less than the frequency using *Azotobacter* wild type DNA but was greater than the spontaneous reversion frequency. *Azotobacter* nif$^+$ recombinants also were stable, some of them had a lower nitrogenase activity a delayed nitrogenase depression time. He also found that mutants defective in nitrogenase component I were transformed to nif$^+$ by asymbiotic nitrogen fixing *Rhizobium* sp. but not the symbiotic nitrogen fixing sp.

Maier, et al. (1978) transformed *Azotobacter vinelandii* that is unable to fix N_2 nif$^-$ to nif$^+$ with DNA from *Rhizobium Japonicum*. They also found that of 50 nif$^+$ transformants 3 contained the O antigen-related polysaccharide that is present on the cell surface of nodulating *R. Japonicum* strain but is absent from a non nodulating mutant strain. They used wild type *R. Japonicum* 6 [A76] as donor and *A. vinelandii* nif$^-$ mutant uw_{10} as recipient. The transformation frequency was 9.7×10^{-7} nif$^+$ transormants per cell plated. They examined fifty of these nif$^+$transformants for agglutination by rabbit antiserum prepared against whole cell of wild type *R. Japonicum*. They found that four of these transformants strains gave a positive reaction.

Page and Von Tigerstrom (1978) induced transformation competence in *A. vinelandii* iron-limited cultures.

They induced *A. vinelandii* strains (uw_1) to form competent cells by growing culture in Burk medium without added iron. They found that the competent culture was highly colored by the characteristic fluorescent green pigment of *A. vinelandii* but all pigmented cultures were not competent. In liquid culture competent cells required a fixed nitrogen source ammonia, nitrate or urea that was also a nitrogenase repressor. Glucose, sucrose, glycerol and mannitol (1% w/v) were excellent C. sources for competence induction while acetate (1% w/v) and βhydroxybutyrate (0.2 w/v) were ineffective. Cells induced in liquid culture remained competent for 12 h period before the end of the exponential growth phase. Then competence decreased rapidly upon the initiation of stationary phase of growth.

Page and Von-Tigestrom (1979) reported that the optimal trasformation of *Azotobacter vinelandii* required 20 min incubation of the competent cells with DNA at 30°C in Burk buffer pH = 7.2 containing 8 mM $MgSO_4$. Nitrogen fixing transformants of nitrogen fixation deficient recipient could be plated immediately on selective medium, but transformants requiring rifampin and streptomycin resistant required preincubation in non selective medium. The three phenotypes achieved an approximately equal and stable frequency after 17 h of growth in non selective medium.

Page and James (1981) studied the effect of calcium on transformation, they found that cells were grown in calcium-limited medium appeared to have leaky cell envelopes and released a diverse array of protein into the culture supernatant. They also concluded that, calcium may form specific protein and LPS complexes which effect envelope permeability. These conditions greatly increase the transformation percentages.

Doran and Page (1983) reported that heating competent *A. vinelandii* at 37°C or 42°C resulted in a total loss of competence with no loss of viability. This investigation indicate that competent *A. vinelandii* heated at 24°C for 20 min. is unable to transport donor DNA across the all evnelope but is fully capable of binding DNA in a DNase - resistant state. The survival and expression of newly transported donor DNA marker are insensitive to heating at 42°C.

Page (1985) investigated that *A. vinelandii* <u>nif</u>$^-$ mutants uw_3, uw_{45} and uw_{91} that did not become competent for genetic transformation after growth in Fe-limited competent induction medium and did not become competent when Mo starvation also was imposed. Mo starvation of other Fe-limited competent strains increased their frequency of transformation. Mo starvation also increased frequency of transformation of strain uw_1 with heterologous

DNA from *Azotobacter*, *Rhizobium* and *Bajerinckia* Mo starvation did not increase the competence of the encapsulated *A. vinelandii strain*.

Click et al. (1986 b) developed a cellulolytic *A. vinelandii* by transformation of *A. vinelandii* in liquid media with broad host range plasmid PKK 2501, RSF 1010 amd PG 5515.

Abd-El Salam et al. (1987 a) used purified *Azotobacter chroococcum*, *A. vinelandii* and *R. leguminosarum* DNA to produce E. Coli K_{12}. N_2-fixing transformants with the frequencies of 0.319 x 10^{-6}, 0.332 x 10^{-6} and 0.391 x 10^{-6} respectively. *R. leguminosarum* nif^- plasmid gave a transformation frequency of 0.338 x 10^{-6}. The results proved homology between nif genes regions of both *A. chroococcum* and *A. vinelandii*. *R. leguminosarum* nif genes were found to be closer than those of *A. chroococcum* or *A. vinelandii* The nodulation experiments indicated that nod genes were apart from nif genes in *R. leguminosarum* and that nodulation may require many different genes.

Abd-El Salam et al. (1987 b) found that the equal DNA concentrations of either *Azotobacter chroococcum* & *A. vinelandii* were able to produce about the same frequencies of nitrogen fixing *B. subtilis* transformants.

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The ability of *R. leguminosarum* DNA and plasmids to transform *B. subtilis* was very low.

Nieveen (1987) showed transformants had different levels of nitrogen fixation efficiency ranged from 4.0-40 mg N_2/l MM in both intra and interspecific transformation using *A. chroococcum* and *A. vinelandii*. Great differences in nitrogen fixation were obtained among the single transformants of each recipient strain. Some of them were more efficient to fix nitrogen than their recipient or donor strains and others were less efficient.

I.B Gene transfer systems in *Rhizobium leguminosarum*

Dunican and Tierney (1974) transferred an F-like R-factor into *Rhizobium trifolii* which has been able to transfer the nitrogen-fixing genes from *R. trifolii* to *klebsiella aerogenes* 418, a strain incapable of fixing nitrogen. This intergeneric transfer has occurred at a frequency of 10^{-7} or higher. The *K. aerogenes* hybrids reduced acetylene at rates similar to a naturally occurring nitrogen-fixing strain, *K. pneumoniae* M5A1.

Johnston et al. (1978) conjugated *Rhizobium leguminosarum* (nodulates pea) to *R. leg.* bv. *trifolii* or *R. leg.* bv. *phaseoli* and yielded *R. leg.* bv. *trifolii* or *R. phaseoli* transconjugants with the ability to nodulate pea.