BIOTECHNOLOGY: WORKING WITH NATURE TO IMPROVE FOREST RESOURCES AND PRODUCTS

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ABSTRACT

The role of biotechnology in the forest products industry is expanding beyond the pulp and paper industry. Applications that can contribute to forest sustainability include genetic engineering and phytoremediation. Genetic engineering makes it possible to modify forest trees for desirable traits, such as disease or pest resistance, and to replicate these improved trees by cloning. An example of genetic modification that has direct environmental implications is phytoremediation, the use of genetically modified trees for soil remediation. Phytoremediation works by decontaminating toxic pollutants in the soil or by stabilizing and sequestering toxins. This paper provides an overview of recent publications in these new areas of biotechnology.

INTRODUCTION AND BACKGROUND

The United States is both the largest producer of wood pulp in the world (U.S. mill output equals that of the next four top countries combined) and the largest per capita consumer of paper and paper products. An anticipated rapid growth in world population will place increased pressures on land and wood resources, with an estimated 50% increase in global demand for paper products alone (1). Trees, the raw material of the industry, are a renewable resource; however, we are faced with meeting the increased demand for forest products at a time of increased restrictions on land use and environmental controls.

Environmental issues are intertwined with the whole fabric of society and have many facets—social, political, economic, scientific, and ethical. Biologically based processes can be used in the pulp and paper industry to reduce some negative environmental impacts. Similarly, forest biotechnology can solve some problems faced by forest managers. Effective management of forested lands is central to our quality of life and the sustainability and health of the planet (2). Policies, or a lack of policies, in one part of the world cannot be isolated from their impact on the global community. We need to be concerned with tree improvement as it relates to forest health, biodiversity, sustainability, resiliency, and other conditions linked to the global forest resource (3).

While new breeding techniques, fertilizers. pesticides, and improved cultural methods are conventional ways to improve productivity, genetic engineering is a more controversial alternative. However, biotechnology has the potential for generating forest tree cultivars that cannot be produced by conventional breeding alone. Biotechnological approaches are being investigated for integrating conventional forest tree breeding with forest resource productivity. This paper reviews some possibilities for improving forest trees through hybridization and genetic engineering. An overview of a unique application of genetically altered trees for ameliorating toxins, phytoremediation, is also presented.

BIOTECHNOLOGY APPLICATIONS IN PULP AND PAPER INDUSTRY

Biotechnology has provided environmentally sound solutions to problems faced by the papermaking industry. Kuhad et al. (4) attribute the importance of biotechnology to its potential for more specific reactions, less environmentally deleterious processes, energy savings, and capacity to be used in place of nonbiological processes. Biological products have long played a critical role in paper mill sludge treatment and effluent clarification. Newer biological applications include targeting organic contaminants in thermomechanical pulp (TMP) mill white water for

fungal treatment (5) and dechlorinating kraft bleach effluents with *Rhizopus oryzae* (6). Enzyme preparations are effective in curbing bacterial growth in mill process water and inhibiting the build-up of biofilms (7).

The idea that microorganisms and their enzymes can successfully replace or supplement conventional chemical processes in the pulp and paper industry is gaining acceptance in the United States, following the lead of Scandinavia and Canada. Enzyme preparations have moved from laboratory exploration to mill settings for various industrial applications. Moran (8) views biotechnology as providing new and exciting cost-effective alternatives to complement rather than totally replace traditional technologies. Extensive reviews (9.10) of the use of enzymes for bleaching (11-13), deinking (14,15), pitch removal and drainage enhancement (16,17) illustrate the broad interest in and potential of biotechnology. Relining energy can be significantly reduced if wood chips are pretreated with fungal inocula in the biopulping process (18) or with a bacterial inocula to reduce pitch (19). All of these technologies have advanced to mill-scale trials. Applications that have been successfully transferred to commercial use include xylanases for bleach boosting, cellulases for improved drainage, lipases for pitch removal, and cellulase-hemicellulase mixture for deinking (20,21).

FOREST TREES IN THE ERA OF MODERN GENETICS

The American Forest and Paper Association, through *Agenda 2020,* and the National Research Council of. the National Academy of Sciences, through its work *Forestry Research a Mandate for Change, have recognized the* potential of biotechnology through establishing common research priorities for industry:

Sustainable forestry—Sustain productivity of intensively managed forests for present and future generations.

Selection and hybridization—Develop optimum natural species and hybrids for use in local conditions.

Genetic engineering and tree breeding—Modify gene structure and improve genetic selection programs to increase growth rates, improve insect and disease resistance, improve fiber quality, and enhance enviromnental adaptability.

In keeping with these research priorities, we will discuss two emerging applications of biotechnology for forest trees. These interrelated technologies include

1. genetically altering trees for improved forest health through a higher tolerance for pest infestation, disease resistance, and severe climatic conditions, as well as for enhanced properties such as accelerated growth and unproved papermaking qualities (i.e., reduced pitch or lignin content), and

2. phytoremediation of contaminated soil, air, and water.

Genetic Altering of Trees

Recent advances in gene mapping techniques permit researchers to identify trees with desired traits (e.g., fast growth, resistance to disease or cold temperatures) that can be used to breed improved species by conventional means. Mapping permits researchers to focus on specific genes and their components on the molecular level. Identification of gene function permits gene manipulation and the introduction of new and desirable traits not available in the breeding population. Ultimately, such mapping should permit isolation of desired tree genes that could be engineered directly into target tree species. New techniques for identifying gene ‘markers” facilitate the location of desired genes useful for tree breeding Once potentially valuable genes are located, they can be cloned and improved strains of the same or other tree species can be created. Using this technique, Finnish researchers have identified gene markers for cold hardiness in Scots pine and are using these markers to identify trees that could thrive near the Arctic Circle (22).

This approach has also been used by researchers at North Carolina State University, the USDA Forest Service in Athens, Georgia, and the New Zealand Forest Research Institute at Rotorua to locate a gene that imparts resistance to a major fungal pathogen in loblolly pine (23,24). At the University of Wisconsin-Madison and Ohio State University—Corvallis, researchers introduced genes with desirable traits from non-tree species to poplars and white spruce to make these wood species resistant to insect pests or herbicides and further improve their qualities by

genetic manipulations. The resultant trees were protected against defoliating insects and, in some cases, a high percentage of the insects feeding on their leaves were killed (25).

Cloning permits replication of genetically engineered trees and enables mass production of embryos of identical trees that contain one or more value-added traits. Embryos are inserted into manufactured seed and the seeds are sown following conventional culture in a nursery. Identical trees are advantageous in ensuring a uniform raw material that is relatively predictable in its requirements for conversion to pulp and paper (26).

Another approach to genetic altering of trees, which utilizes "antisense constructs" (nucleic acids that bind to the genes themselves or messenger RNAs) to inhibit enzyme production needed for lignin synthesis, has been used by Belgian researchers in their work to minimize the lignin content of trees (27). Obviously, successful development of this work would revolutionize delignification as it is now known.

Some of the tree species selected for genetic engineering include loblolly pine, eucalyptus, poplar, sweet gum, and spruce. These species are either fast growing or especially valuable in the pulp and paper industry. Most tree genetics research is presently conducted at universities or government agencies, often in cooperation with paper companies.

Phytoremediation

One of the most intriguing possibilities of biotechnology identified in this review is that of genetically improving trees to remediate soil contaminated by toxic wastes. Trees are already used for wastewater clean-up, for site stabilization, and as barriers to subsurface flow of contaminated groundwater. Trees are ideal remediators because they are fast-growing perennial plants. with extensive root systems and high transpirational rates (28). Their large biomass is advantageous because it permits higher tolerance for toxic materials and has the capacity for accumulating contaminants. Because plant remediation is done *in situ,* it has the potential to be substantially less expensive than alternative technologies used for detoxification.

The main methods of phytoremediation discussed in the literature are

1. decontamination, in which the amount of toxic pollutants in the soil is significantly reduced or eliminated, and

2. stabilization and containment in which plants and their associated microflora do not remove contaminants but rather alter the soil chemistry and sequester, reduce, or eliminate the environmental risk of the toxin (29).

Work is underway to screen tree species for their ability to tolerate, take up, translocate, sequester, and degrade organic compounds and heavy metal ions. Clonal propagation and genetic engineering techniques already exist for a number of species, which opens the door to the creation of tree "remediation" cultivars (30). This *in situ* use of plants to stabilize, remediate, and restore a comaminated site is referred to as phytoremediation (31).

All plants have the ability to accumulate metals essential for their growth and development; these metals include iron, manganese, zinc, copper, magnesium, molybdenum, and possibly nickel (32). Certain plants accumulate heavy metals that have no known biological function: these metals include cadmium, chromium, lead, cobalt, silver, selenium, and mercury. However, significant accumulation of heavy metals is usually toxic to most plants. For some time, botanists have been aware that certain tree species are endemic to soils containing high metal content (33). Identification of heavy metal tolerance by some plants has led to research that exploits this characteristic for removing metal contaminants by establishing selected vegetation on contaminated soil; these plants are called "accumulating" plants. A specific example of this technology is the development of a transgenic yellow poplar developed for remediating mercury-contaminated soil (34).

Hyperaccumulating plants promise effective, inexpensive remediation of soil, sediment, and groundwater. Whereas metal-tolerant plants exclude toxic metal ions from uptake, hyperaccumulating plants take up high amounts of toxic metals and other ions. An exciting possibility of applying biotechnology lies in identifying a tree species with the ability to tolerate or accumulate toxic substances such as heavy metals or organic compounds. Once identified, this tree species could be introduced in contaminated areas. Furthermore, genetic modification could accelerate

remediation by making the tree a hyperaccumulator, by adapting its growth to diverse climatic conditions, or by enabling faster growth (35).

Phytoremediation is based on root uptake of contaminants and storage in the plant or partial/complete degradation to less toxic compounds. This type of remediation could promote degradation of organic pollutants by increasing soil organic carbon content or by releasing enzymes that promote microbial activity through the plant roots. Phytoremediation could be useful in ameliorating heavy metals and organic compounds such as 2,4,6-trinitrotoluene (TNT), trichloroethylene (TCE), benzene, toluene, xylene, and ethylbenzene (36).

The benefits of phytoremediation include the fact that it is done *in situ* and that it is a passive, solar-driven "green" technology. Roots are exploratory, liquid-phase extractors that can find, alter, and/or translocate elements and compounds against large chemical gradients (37). This technology is most effective on sites containing a low level of contamination that are widely dispersed over a large area in the upper surface of the soil. Phytoremediation can work side by side with site restoration with minimum site disruption. Additionally, plant biomass can be harvested to remove contaminants From the site and trees will re-sprout without disturbing the site. In sites where a valuable heavy metal has accumulated, it may be possible to reclaim the metal from the harvested tree.

Phytoremediation techniques are less expensive than *ex situ* methods, but they require a long time to work. Long-term site remediation and stabilization using trees makes remediation and restoration synonymous, which lowers costs and is compatible with public objectives. The most appropriate type of remediation for a specific site depends on the degree of pollution and the type of toxic material. More intensive remedies are required for localized, highly contaminated sites. Conventional soil remediation methods are more suitable for these sites. These methods typically involve excavation of contaminated soil followed by extraction of the toxin. This *ex situ* technique is usually extremely expensive.

Toxic metal contamination of soil and groundwater is a major environmental and human health problem for which affordable, effective solutions are urgently needed. In agricultural areas, sites are fequently contaminated by a build-up of residual herbicides Atrazine, a commonly used agricultural herbicide, has been the focus of bioremediation researchers (38). Research with hybrid poplars has resulted in somaclonal variants that tolerate lethal dosages of herbicides. Another aspect of engineered tolerance, pesticide and herbicide resistance, is especially interesting. If trees could be engineered to be more tolerant of the ubiquitous chemicals in soils, substantially higher yields of forest trees could be realized. Such an application was reported by Meilan and others (39) in their work on an engineered resistance to the herbicide Roundup @.

Other possibilities for phytoremediation range from removing concentrations of naturally occurring selenium solubilized in irrigation water and accumulated in surrounding groundwater (40) to using genetically altered eucalyptus trees for absorbing and metabolizing air pollutants (41). The possibilities seem to be limited only by the imagination of researchers and the toxic material present.

CONCLUDING REMARKS

Forest sustainability depends on global interdisciplinary cooperation in silvicultural techniques and continued research to meet projected needs while maintaining a healthy ecosystem. Biotechnology is becoming an increasingly important component of the forest industry. Application of enzyme technology in pulp and paper manufacture has demonstrated environmental advantages. Tree genetics offers the possibility to resolve the increased demands on forest resources through the development of trees more tolerant to diseases, pests, and chemicals, which have a detrimental impact on forest health. Phytoremediation technology has the potential to provide a cost-effective solution to contaminated soil. However, for phytoremediation to become a viable technology, a more complete understanding of the process is essential. End products must be identified and the mechanism by which toxic materials are converted needs to be elucidated. Biotechnology, as applied to forest trees, is still a new science that requires answers to the numerous questions it continuously unveils. This overview of recent publications in the area is to be used as a starting point for understanding some new possibilities that biotechnology can offer the forest products industry.

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