

REPORT DOCUMENTATION PAGE			Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204 Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188) Washington, DC 20503.				
1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE 8/26/94	3. REPORT TYPE AND DATES COVERED Final: 5/1/91 - 4/30/94		
4. TITLE AND SUBTITLE Biochemically vulnerable sites for antifungal intercession in the control of fungal growth		5. FUNDING NUMBERS  DAAL03-89-D-0003		
6. AUTHOR(S)  L. W. Parks		8. PERFORMING ORGANIZATION REPORT NUMBER		
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)  North Carolina State University Box 7003 Raleigh, NC 27695-7003		10. SPONSORING / MONITORING AGENCY REPORT NUMBER  ARO 29097.3 -LS		
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)  U.S. Army Research Office P. O. Box 12211 Research Triangle Park, NC 27709-2211		11. SUPPLEMENTARY NOTES The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy, or decision, unless so designated by other documentation.		
12a. DISTRIBUTION / AVAILABILITY STATEMENT  Approved for public release; distribution unlimited.		12b. DISTRIBUTION CODE		
13. ABSTRACT (Maximum 200 words)  Virtually every antifungal agent in use intercedes some aspect of sterol synthesis or function. Ergosterol is the principal sterol in fungi, while cholesterol is the most abundant sterol in vertebrates. Research in our laboratory has shown that the structural differences in ergosterol, in comparison to cholesterol, have distinctive biochemical and physiological effects in fungi.  Under the completed contract we have shown that as the sterol composition of sterol auxotrophic strains of <i>Saccharomyces cerevisiae</i> is altered there is disturbance of the mating efficiency of the strains. The normal sterol, ergosterol, mediates a 30-fold higher productive mating efficiency in the auxotrophs than when the cells are supplied with stigmasterol. Using electron and visible microscopy, we have shown that the mated pairs in stigmasterol remained adherent but prezygotic even after 12 hours incubation. Ergosterol rescued the cells and permitted zygote formation.  Based on those experiments it was clear that membrane fusion was perturbed by sterol alterations. Continuing work with the renewal grant is focusing on membrane fusion in various normal cell biological processes.  DTIC QUALITY INSPECTED 8				
14. SUBJECT TERMS  Yeast, antifungal agents, membranes, lipids, ergosterol		15. NUMBER OF PAGES		
		16. PRICE CODE		
17. SECURITY CLASSIFICATION OF REPORT UNCLASSIFIED	18. SECURITY CLASSIFICATION OF THIS PAGE UNCLASSIFIED	19. SECURITY CLASSIFICATION OF ABSTRACT UNCLASSIFIED	20. LIMITATION OF ABSTRACT UL	

## FINAL REPORT

Title: Biochemically vulnerable sites for antifungal intercession in the control of fungal growth.

Contract: DAAL03-89-D-0003-07

Period of contract: 01-05-91 to 30-04-94

Report: Fungal biodeterioration accounts for millions of dollars in damage to stored material and food-stuffs annually. In addition, the production by fungi of secondary metabolites such as aflatoxins, tricothecenes, ergot and clavine alkaloids, destroy or make unacceptable large quantities of stored materials such as various grains. Yet, there are no effective controlling agents for fungi under those conditions. In addition to the deterioration of supplies, fungal infections in field personnel remain the most recalcitrant to therapy, seriously eroding readiness and availability. Thus, effective control of fungi under a wide variety of conditions is a high priority concern. However, all antifungal agents that are currently in use are either highly toxic, poorly soluble, or both. More suitable agents are sorely needed.

Virtually every antifungal agent in use intercedes some aspect of sterol synthesis or function. Ergosterol is the principal sterol of fungi, while cholesterol is the most abundant animal sterol. Research in our group has demonstrated that the structural differences in ergosterol, in comparison to cholesterol, have distinctive biochemical and physiological effect in the fungi. Since an ideal fungal controlling agent would perturb some essential metabolic function in the fungus without affecting animal physiology, the cited structural differences are attractive starting points for probing the unique metabolic and physiological roles for sterols in fungi. With that information in hand, it is logical that more effective and specifically targeted antifungal agents could be developed through biorational design.

Sterol auxotrophic strains of *Saccharomyces cerevisiae* were grown and allowed to conjugate on media supplemented with various sterols. The mating efficiency of the auxotrophs is perturbed by the replacement of the normal yeast sterol, ergosterol, with other sterols. After 4 h of mating, cells grown on ergosterol exhibited a 30-fold higher productive mating efficiency than those cells grown in stigmasterol. Aberrant budding by the conjugants was enhanced following incubation on stigmasterol and other non-ergosterol sterols.

Using light and electron microscopy, we demonstrated that there is a reduced ability for stigmasterol-grown cells to undergo cytoplasmic fusion during conjugation. Many of the mated pairs remained adherent but prezygotic even after 12 h of incubation. The addition of ergosterol to cells previously grown on stigmasterol rescued the organisms, allowing for zygote formation and normal budding.

Insertion of the unsaturation at C22 of the side-chain of ergosterol is one of the final reactions in the synthesis of the sterol. The reaction is important because it introduces rigidity into the side-chain, making the sterol much more refractory to intercalation with the long acyl groups of the phospholipids in membranes. Such physical effects on the membranes are

19941128 063

important in maintaining structural integrity and regulating the activity of various membrane functions. The desaturase reaction is therefore viewed as critical in normal sterol function and should be an additional site for biochemical intercession.

Our research was extremely frustrated because of an inability to obtain mutants totally lacking C22 desaturase activity. The structural gene for that enzyme, *ERG5*, could not be cloned by complementation until we had a reliable mutant, *erg5*, for selection of the wild-type allele. After pursuing many separate strategies we were finally successful in defining conditions that reliably gave us *erg5* mutants. With those in hand it was necessary to devise an appropriate selection criterion for isolating the prototrophs following transformation with a clone bank containing the *ERG5* allele. Very recently, we have succeeded in developing a highly reliable selective medium, and we are currently screening the suspected clones.

Substantial progress was made in pursuing the objectives of the original grant. Under the continuation of our work, we shall make additional effort in resolving the physiological roles for sterols in fungi and in defining biochemically vulnerable sites for antifungal intercession.

#### Publications:

Tomeo, M. E., G. Fenner, S. R. Tove, and L. W. Parks. 1992. Effect of sterol alterations on conjugation in *Saccharomyces cerevisiae*. *Yeast* **8**, 1015-1024 (1992).

Tomeo, M. E., G. Fenner, S. R. Tove, and L. W. Parks. 1992. Effect of sterol alterations on the efficiency of conjugation in sterol auxotrophs of the yeast, *Saccharomyces cerevisiae*. (Abstr) American Society for Microbiology.

Casey, W. M., M. E. Tomeo, C. E. Rolf, and L. W. Parks. 1993. Regulatory effects of palmitoleic acid on membrane lipid biosynthesis. (Abstr) XVI International Symposium on Yeast **16**, 110.

Tomeo, M. E., S. R. Tove, and L. W. Parks. 1993. Examination of esterification defect in the *Saccharomyces cerevisiae* secretory mutant HMSF 134. (Abstr) NC American Society for Microbiology.

Parks, L. W., W. Casey, S. Smith, M. Tomeo, N. Sabnis, and J. Crowley. 1994. Biochemical and physiological effects of sterol alterations in yeast. *Inform* **5**, 495 (1994).

Tomeo, M. E., W. M. Casey, S. R. Tove, and L. W. Parks. 1994. Characterization of sterol esterification defect in the *Saccharomyces cerevisiae* secretory mutant HMSF 134 (sec 5-24). (Abstr) American Society for Microbiology.

Parks, L. W., S. J. Smith, M. E. Tomeo, and J. H. Crowley. 1994. Regulation and functions of sterols in fungi. 11th Internat. Congr. Plant Lipids. (Abstr).

Participating personnel in this project:

L. W. Parks  
M. E. Tomeo  
S. R. Tove

Degrees earned: None

Degree candidates with degree: M. E. Tomeo, for Ph.D.

Reportable inventions: None

Accession For	
NTIS CRA&I	<input checked="" type="checkbox"/>
DTIC TAB	<input type="checkbox"/>
Unannounced	<input type="checkbox"/>
Justification	
By	
Distribution/	
Availability Codes	
Dist	Avail and/or Special
A-1	