2005

The effect of farnesol on amino acid incorporation by a wild-type and cell-wall variant strain of Candida albicans

Phyllis C. Braun
Fairfield University, pcbraun@fairfield.edu

Published version available at:
http://nrcresearchpress.com/doi/pdf/10.1139/w05-050

Peer Reviewed

Published Citation

Braun PC. 2005. The effect of farnesol on amino acid incorporation by a wild-type and cell-wall variant strain of Candida albicans. Canadian Journal of Microbiology 51(8): 715-718

The Effect of Farnesol on Amino Acid Incorporation by Wild-Type and Cell-Wall Variant Strain of *Candida albicans*

Phyllis C. Braun, PhD

Address Correspondence to:

Phyllis C. Braun, PhD
Biology Department
Fairfield University
1073 North Benson Road
Fairfield, CT 06824
U.S.A.
pcbraun@mail.fairfield.edu

ABSTRACT

Cell-surface hydrophobicity has been shown to be an important factor in the ability of *C. albicans* to adhere to host tissue and in determining its resistance to phagocytic killing. Farnesol was recently identified as a quorum-sensing molecule that inhibits mycelial development in this organism. In this study, *C. albicans* A9 (wild type) and V10 (cell-wall–variant strain) were used. Previous experiments have indicated that A9-WT organisms have hydrophilic cell walls, while A9-V10 exhibits hydrophobic cell surfaces when both are grown in Yeast Nitrogen Broth (without amino acids) supplemented with 2% glucose at 37° C. The effect of farnesol on the incorporation of arginine, aspartic acid, glycine, histidine, leucine, and serine was studied in wild-type and cell-wall variant strains under these conditions. It was determined that both strains incorporated significantly greater amounts of all amino acids tested, except for glycine, as the concentration of farnesol increased to 10 ug/mL. Farnesol concentrations greater than 10 ug/mL abolished the enhanced effects of amino acid transport in both strains of *C. albicans*. In addition, it was determined that farnesol-treated A9-V10 hydrophobic cell surface organisms had increased amino acid uptake compared with the hydrophilic A9-WT strain although the magnitude of the effect (% increase) varied depending on the amino acid studied.

(Key Words: *Candida albicans*, farnesol, amino acid incorporation)

Candida species are ubiquitous fungi and the most common fungal pathogens affecting humans. *C. albicans* is the primary etiologic agent of candidiasis, a disease that can vary from superficial mucosal lesions in the immunocompetent host to systemic or disseminated infection in the immunocompromised individual (Odds 1988; Ahearn 1978). The cell wall of the organism is important to its success as a pathogen (Odds 1985) because it is required for growth; provides rigidity and protection against osmotic insult; and is the site of contact between the organism and its surroundings. Survival of *C. albicans* in its environment is dependent on the transport of nutrients into the organism. For molecules to enter, they must first pass through the cell wall and then the plasma membrane, a limiting structure between the cell wall and the cytoplasm that acts as a permeability barrier to many solutes.

The molecular complexity of the *C. albicans* cell wall is well known. The cell wall is a heterogeneous structure composed predominantly of polysaccharides (mainly mannan, glucan, and chitin), proteins, and lipids (Calderone and Braun 1991; Odds 1985). The proteins located on the external surface of *C. albicans* are composed of high–molecular-weight mannoproteins, and constitute the outer fibrillar layer of the cell wall. These mannoproteins are regarded as important sites that influence amino acid transport by this organism (Braun 1999). Investigators have observed that the presence of fibrillar mannoproteins determines the hydrophilic nature of *C. albicans* cells (Glee et al. 1995; Hazen and Hazen 1992). Any changes in the length or concentration of mannoprotein fibrils, or decrease in the amount of phosphodiester-linked, acid-labile mannosyl-groups results in a hydrophobic cell surface (Masuoka and Hazen 1997).

Farnesol ($C_{15}H_{26}O$), a recently discovered extracellular quorum-sensing molecule produced by *C. albicans*, blocks yeast-to-mycelium conversion when it accumulates above threshold levels. Farnesol is a hydrophobic molecule that alters cell morphology but does not alter growth rate. It is produced at all growth temperatures, and is produced continuously during growth of *C. albicans* (Hornby et al. 2001). Inhibition of *C. albicans* biofilm formation by farnesol also has been reported (Ramage et al. 2002).

The purpose of this study was to determine how amino acid incorporation was influenced by exogenous supplements of farnesol. Any changes in nutrient transport due to farnesol presence would affect the growth and presumably the pathogenesis of this organism. Two strains of *C. albicans* were used. When grown in amino-acid–deficient Yeast Nitrogen Broth (YNB) supplemented with 2% glucose, the wild-type strain of *C. albicans* (A9-WT) exhibits hydrophobic cell surfaces at 23°C and the typical hydrophilic morphology at 37°C. The cell wall variant (A9-V10) however, displays cell-surface hydrophobicity under all growth conditions (Masuoka and Hazen 1997).

C. albicans A9-WT and A9-V10 were maintained on brain-heart infusion slants at 4°C. The two strains were grown to mid-stationary phase in YNB buffered with 0.05-M sodium phosphate (pH 7.2) and supplemented with 2% (w/v) glucose (YNB+G) at either 23°C or 37°C to induce hydrophobic or hydrophilic cell wall conditions. Cells were washed and resuspended in PB (sodium phosphate buffer, 0.05 M, pH 7.2) and kept on ice.

Cell-surface hydrophobicity (CSH) of the culture was determined by using the polystyrene microsphere assay (Hazen and Hazen 1988). A working 10% solution was prepared by placing 6 mL of stock microspheres (diameter, 0.825 um; Sigma Chemical Co.). into 2.0 mL of PB, and kept on ice. The assay was performed by mixing equal volumes (l00 mL) of washed yeast cells (5 x 10^7 cells per mL) and microspheres in polypropylene test tubes and incubating the mixture for 2 min at room temperature. The mixtures were vortexed subsequently for 30 s, and microscope slides were prepared. Cultures were determined to exhibit CSH when 95% of the population had more than three attached microspheres.

Yeast cells were resuspended in 1 mL of sterile YNB+G at a concentration of 5 x 10^7 cells per mL. Triplicate samples were incubated with 0, 1, or 10 micrograms of free farnesol (Sigma; trans, trans-Farnesol) for 10 minutes at 37°C. Subsequently, [^{3}H] L-amino acids ([2,3-^{3}H]arginine, specific activity 36.8 Ci/mmol; [2,3-^{3}H]aspartic acid, specific activity 15.5 Ci mmol^{-1}; [2-^{3}H]glycine, specific activity 43.8 Ci mmol^{-1}; [2,5-^{3}H]histidine, specific activity 50.4 Ci mmol^{-1}; [4,5-^{3}H]leucine, specific activity 52 Ci mmol^{-1}; [3-^{3}H]serine, specific activity 21.7 Ci mmol^{-1}; New England Nuclear), were individually assayed by adding the radioactively labelled substance, 1 uCi ml^{-1} to the cultures These cultures were further incubated for 1 h at 37°C (100 rpm). Incorporation of radiolabel was stopped by the immediate addition of 1 mL of 15% TCA. The suspensions were filtered through Whatman GF/A glass fiber filters, and the precipitate remaining on each filter was washed twice with 15% TCA and once with 95% ethanol. The filters were dried and transferred to scintillation vials, and the radioactivity was counted in ScintiSafe-LSC Cocktail.

Dry weights were determined by filtering 1-mL (5 x 10^7 cells) samples on pretared Whatman filters. These filters were washed with distilled water, dried, and weighed. Uptake of the radiolabeled nutrient is expressed as the number of picomoles incorporated per milligram of dry weight.

Statistical variables used to analyze the data included mean value, standard error of the mean, and two-tailed Student's t-test results. When two groups were compared, a *P*-value of less than 0.05 was considered to indicate a significant difference between the groups.

To determine whether A9-WT and A9-V10 strains had different growth patterns in YNB, 24-hour growth curves were performed. Calculations of the mean growth rate constant (k) indicated insignificant differences between wild-type and cell-wall variant at 23°C or 37°C, respectively. The mean generation time (g) also indicated very little growth difference between these strains at the experimental temperatures. There was, however, a significant difference in the growth rate constant and generation time for each strain at the two different temperatures **(Table 1)**.

Incubation at 37°C of stationary-phase, farnesol-untreated cultures with the [^{3}H] amino acid demonstrated that A9-V10, possessing CSH, preferentially incorporated more of the individually tested nutrient than A9-WT with a hydrophilic surface **(Figures 1 and 2)**. Amino acid incorporation was linear in both strains up to 60 minutes (data not shown). However, the percentage of increase varied according to the amino acid, with A9-V10 incorporating 5% more aspartic acid and leucine, 11% more arginine, and 30% more histidine and serine than A9-WT. Experimental

results indicated A9-WT and A9-V10 cultures were unable to transport glycine under the described conditions (data not shown). Incorporation of arginine, histidine, leucine, and serine by both A9-WT and A9-V10 was further enhanced by the presence of 1 or 10 ug of farnesol compared to untreated. However, the magnitude of the effect of farnesol on transport by A9-WT and A9-V10 was dependent on the amino acid studied **(Table 2)**. Aspartic acid incorporation also was enhanced by farnesol treatment; however, 10 μg of farnesol increased A9-WT incorporation to 1.1 picomoles per mg dry weight, and A9-V10 incorporation to 1.8 picomoles per mg dry weight from the untreated baseline of 0.76 and 0.8 picomoles per mg dry weight, respectively. Farnesol concentrations greater than 10 ug/mL reduced the incorporation and presumably the transport of all amino acids tested. Increased farnesol concentrations (up to 100 ug) abolished the augmented effects of the lower concentrations. Amino acid incorpoation at these higher concentrations was reduced to base-line (0 ug farnesol) in both strains of *C. albicans* (data not shown).

The role of the microenvironment apparently has a very important role in determining the pathogenicity of *C. albicans*. Farnesol, which is produced in situ by *C. albicans,* affects nutrient transport. This discovery may be relevant during in vivo infections. The results of these experiments indicate that very limited micromolar amounts of farnesol increased amino acid incorporation by this organism. The exact nature of the interaction of farnesol with *C. albicans* is not understood; however, the hydrophobic A9-V10 appears to exhibit greater nutrient transport than the A9-WT strain. This is likely a result of the lipophilic properties of farnesol, which probably enables more effective binding of this compound to a receptor site on the cell

membrane. Presumably, an increase in nutrient transport affects the growth, metabolism, and virulence of *C. albicans* and the morphological transformation from yeast to mycelia (a crucial step in the pathogenesis of *C. albicans*).

This study demonstrates that as farnesol concentrations increased, the initial incorporation enhancement was diminished to control levels (0 μg farnesol). These results indicate a gradient effect produced by farnesol that influences metabolic activities of this organism. These results, as well as previous investigations that have shown that farnesol prevents biofilm formation (Ramage et al. 2002) and inhibits mycelial development in a quorum-sensing manner (Hornby et al. 2001) suggests that farnesol might be the first of a new class of antifungal compounds.

In addition, Hornby et al. (2003) recently reported that compounds that block sterol biosynthesis in *C. albicans* caused an increase in intracellular and extracellular farnesol levels. Farnesol would not kill this organism and upset the balance of the normal flora in an individual. Instead, the metabolism of *C. albicans* would be affected which ultimately controls morphogenesis. Farnesol would limit *C. albicans* growth to the yeast form and in so doing restrict pathogenesis in its host. Further studies on the effect of farnesol on *C. albicans* are needed to strengthen its role as a therapeutic agent.

REFERENCES

Ahearn, D.G. 1978. Medically important yeasts. Annu. Rev. Microbiol. **32**:59-68.

Braun, P.C. 1999. Nutrient uptake by *Candida albicans*: the influence of cell surface mannoproteins. Can. J. Microbiol. **45**:353-359.

Calderone, R.A., Braun, P.C. 1991. Adherence and receptor relationships of *Candida albicans.* Microbiol. Rev. **55**:1-20.

Glee, P.M., Sundstrom, P., Hazen, K.C. 1995. Expression of surface hydrophobic proteins by *Candida albicans* in vivo. Infect. Immun. **63**:1373-1379.

Hazen, B.W. and Hazen, K.C. 1988. Dynamic expression of cell surface hydrophobicity during initial yeast cell growth and before germ tube formation of *Candida albicans*. Infect. Immun. **56**:2521-2525.

Hazen, K.C. and Hazen, B.W. 1992. Hydrophobic surface protein masking by the opportunistic fungal pathogen *Candida albicans*. Infect. Immun.**60**:1499-1508.

Hornby, J.M., Jensen, E.C., Lisec, A.D., Testo, J.J., Jahnke, B., Shoemaker, R., Dussault, P.,Nickerson, K.W. 2001. Quorum sensing in the dimorphic fungus *Candida albicans* is mediated by farnesol. Appl. Environ. Microbiol. **67**:2982-2992.

Hornby, J.M., Kebaara, B.W., Nickerson, K.W. 2003. Farnesol biosynthesis in *C. albicans*: Cellular response to sterol inhibition by zaragozic acid B. Antimicrob. Agents Chemother. **47**:2366-2369.

Masuoka, J., Hazen, K.C. 1997. Cell wall protein mannosylation determines *Candida albicans* cell surface hydrophobicity. Microbiology.**143**:3015-3021.

Odds, F.C. 1988. *Candida* and Candidosis. W.B. Sanders, London.

Odds, F.C. 1985. Morphogenesis in *Candida albicans*. Crit. Rev. Microbiol.**12**:45-93.

Ramage, G., Saville, S.P., Wickes, B.L., Lopez-Ribot, J.L. 2002. Inhibition of *Candida albicans* biofilm formation by farnesol, a quorum-sensing molecule. Appl. Environ. Microbiol. **68**:5459-5463.

Acknowledgement

P.C.B. is grateful for the lively discussions and editorial assistance of John Zoidis and the excellent technical assistance of Elizabeth Vancza and Lisa Runco.

Table 1. Mean Growth Rate Constant (k) and Mean Generation Time (g) for *C. albicans* Strains A9-WT and A9-V10 Grown at 23°C and 37°C.

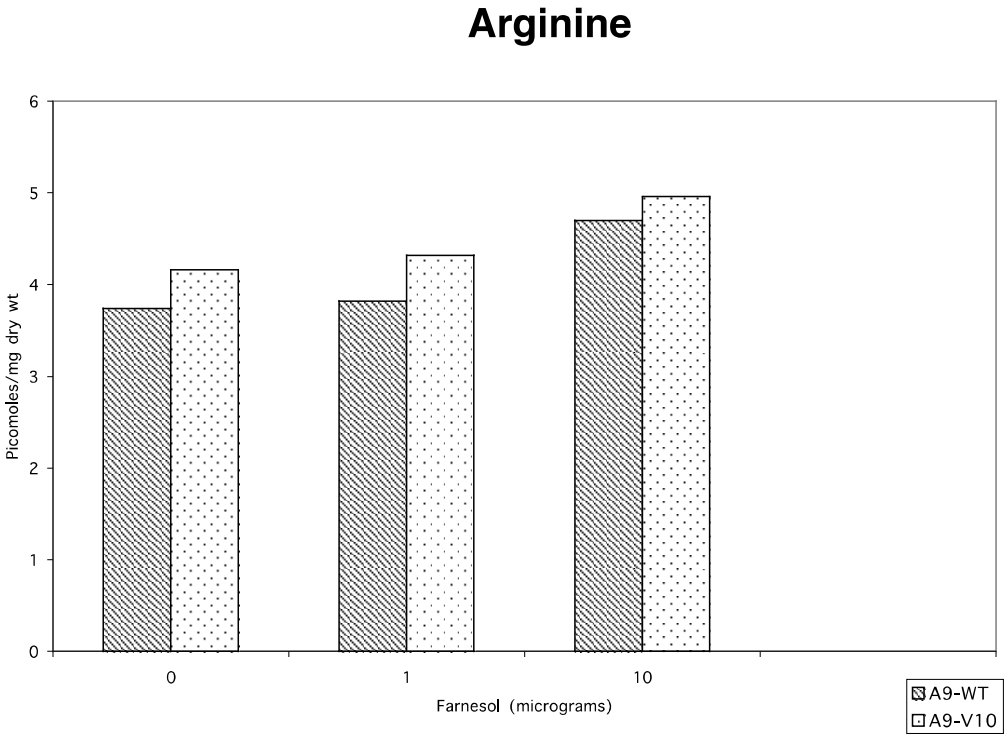
	A9-WT		A9-V10	
	23°C	**37°C**	**23°C**	**37°C**
k	0.3479	0.7262	0.4592	0.7573
g (hours)	2.874	1.377	2.177	1.320

A9-WT and A9-V10 cultures were grown for 24 hours in YNB plus 2% glucose broth. Values represent the average of 5 experimental growth curves. Data were analyzed using Student's t-test. When growth comparisons were determined, a *P*-value less than 0.05 was considered to indicate a significant difference between the groups.

Table 2. Magnitude of Effect (% Increase) of Farnesol 1 µg and 10 µg on Amino Acid Transport by *C. albicans* Strains A9-WT and A9-V10.

	A9-WT		A9-V10	
	Farnesol 1 µg	**Farnesol 10 µg**	**Farnesol 1 µg**	**Farnesol 10 µg**
Arginine	2	26	4	19
Histidine	14	23	10	21
Leucine	21	42	22	55
Serine	44	54	33	90

Figure 1


Arginine
Picomoles/mg dry wt
6
5
4
3
2
1
0
0
1
10
Farnesol (micrograms)
A9-WT
A9-V10

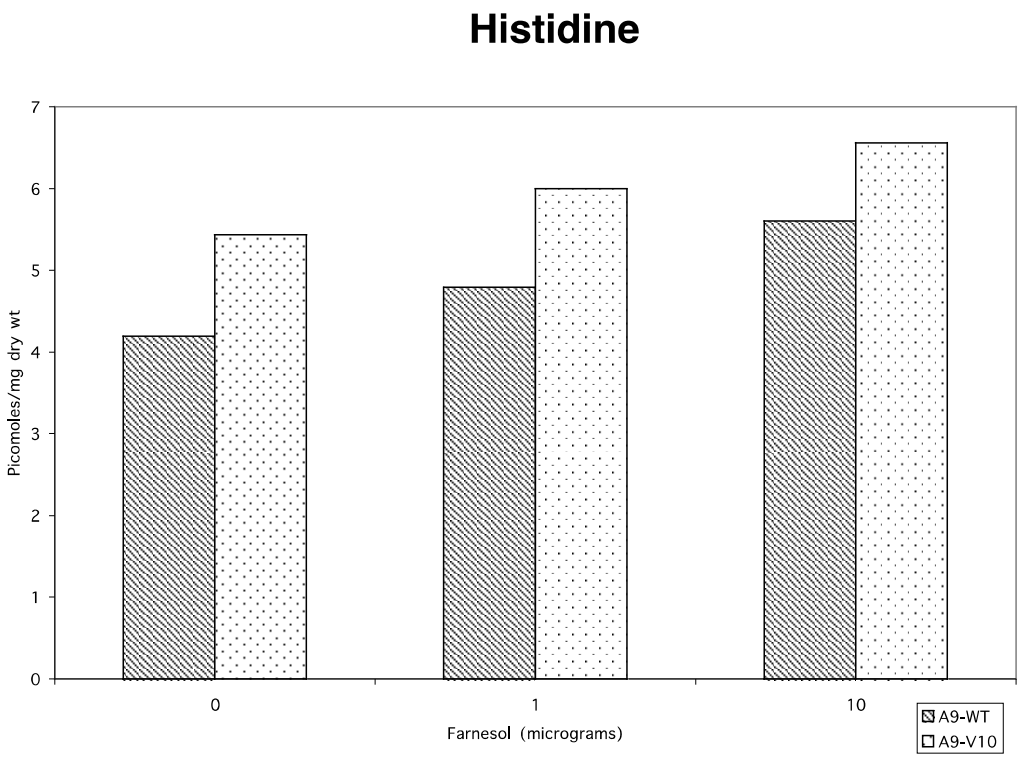
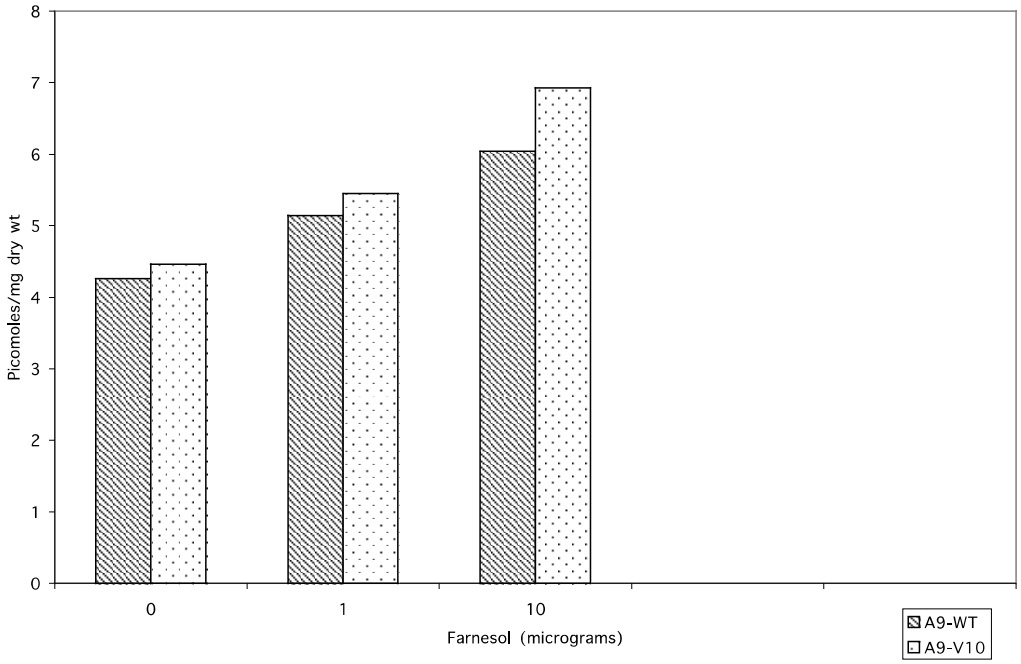
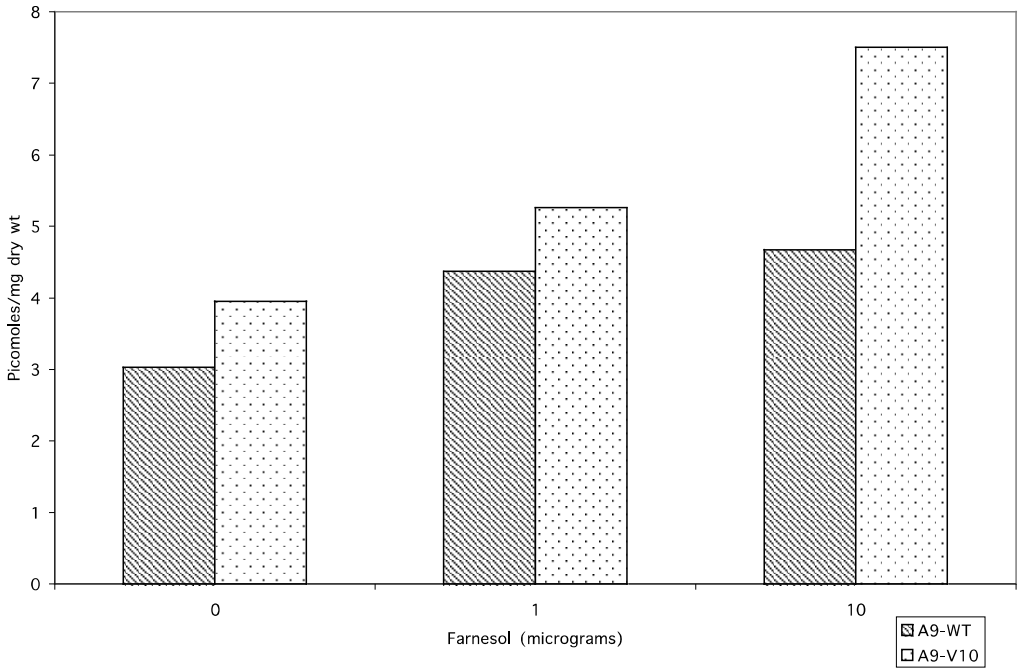

Histidine
Picomoles/mg dry wt
0
1
2
3
4
5
6
7
0
1
10
Farnesol (micrograms)
A9-WT
A9-V10

Figure 2

Leucine


8
7
6
5
4
3
2
1
0
Picomoles/mg dry wt
0
1
10
Farnesol (micrograms)
A9-WT
A9-V10

Serine



Legend

Figs. 1-2:

Incorporation of four different L-[^{3}H] amino acids by pretreated farnesol *C. albicans* strains A9-WT(hydrophilic) and A9-V10 (hydrophobic) was determined after a 1 h incubation at 37°C. Values represent the SEM of triplicate samples from several experiments (n=6). Student's *t* test was used to analyze the data. When incorporation comparisons between cell strains for a particular amino acid was determined, a $P \leq 0.05$ was considered to indicate a significant difference between groups.